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**THE MEASUREMENT OF NOCICEPTIVE PEPTIDES FOR
THE ASSESSMENT OF ACUTE AND CHRONIC PAIN IN
THE MEDICOLEGAL INVESTIGATION OF VIOLENT
DEATH**

by

Lawrence Quarino, M.S., M.Phil.

**A dissertation submitted to the Graduate Faculty in Criminal
Justice in partial fulfillment of the requirements for the degree
of Doctor of Philosophy, The City University of New York**

2000

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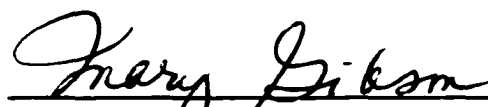
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ABSTRACT

THE MEASUREMENT OF NOCICEPTIVE PEPTIDES FOR THE ASSESSMENT OF ACUTE AND CHRONIC PAIN IN THE MEDICOLEGAL INVESTIGATION OF VIOLENT DEATH

by

Lawrence Quarino

Adviser: Dr. Peter De Forest

Often during the sentencing phase of a homicide trial, juries are asked to determine the allocation of punitive damages and if aggravating factors exist. Very often, these determinations are made on a subjective assessment of the amount of pain inflicted upon a victim. The current study was designed to provide objective and qualitative criteria for these tasks by quantitatively measuring nociceptive peptides in post-mortem serum and brain tissue.

Three peptides (substance P, bradykinin, and met-enkephalin) were chosen for quantitative measurement by radioimmunoassay. These three peptides were chosen because of central but varied roles in the transmission and modulation of

nociception.

Peptide levels differ in acutely and chronically painful death. Peptide levels indicative of acute pain and nociception are apparent in violent death when the incident-death interval is of short duration (minutes). Conversely, peptide levels reflective of chronic pain and antinociception become dominant in violent death when the incident-death time interval is large (months).

The mode of violent death affects peptide levels at certain incident-death time intervals. The variable that appears to affect the nociceptive response is the level of tissue damage typically associated with a particular mode of death. Those modes of death which tend to be characterized by extensive tissue trauma (i.e., thermal burns), show an accelerated nociceptive response.

Peptide levels in infant and child violent deaths do not differ from adult violent death values regardless of the incident-death time interval. Peptide levels in violent infant/child deaths, however, do significantly differ from those in SIDS and other clinical infant/child deaths.

Peptide levels in respective non-violent deaths differ due to the nature (acute and chronic) and extent of pain in each particular non-violent death. Ratios of nociceptive to antinociceptive serum and brain peptides in various non-violent deaths demonstrate the extent of pre-mortem acute and chronic pain in each type of death. By being able to equate the ratio of nociceptive to antinociceptive peptide levels

in individuals who were violently killed with a particular type of non-violent clinical death. a qualitative and objective assessment can be made of the level of pain experienced by the victim. A clearer picture of the level of pain can be presented if peptide levels found in a victim of a violent death can, for example, be equated with death due to renal disease. embolism. or cancer.

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TABLE OF CONTENTS

I. Introduction.....	p. 1
II. Theories of Pain.....	p. 3
III. Defining Pain.....	p. 6
IV. Anatomy of Pain Centers.....	p. 8
V. The Process of Nociceptive Transmission.....	p. 11
VI. Substance P and Nociceptive Transmission.....	p. 12
VII. Met-Enkephalin and the Modulation of Nociceptive Transmission.....	p. 17
VIII. Neuropeptides in Blood During Tissue Injury.....	p. 21
IX. Other Neuropeptides Involved in Nociceptive Transmission and Modulation...	p. 25
A. Beta-Endorphin and Dynorphin.....	p. 26
B. Non-Opioid Modulators - Neurotensin, Calcitonin, Vasopressin, and Cholecystokinin.....	p.27
C. Other Nociceptive Transmitting Neuropeptides - Somatostatin and Bombesin.....	p. 29
X. Substance P and Other Nociceptive Peptides and Their Potential Applications to Medicolegal Investigation.....	p. 30
A. Endogenous Stability and Degradation of Substance P, Bradykinin, and Met-Enkephalin.....	p. 32
B. Clinical Manifestations.....	p. 35
XI. Pain, Homicide, and the Law.....	p. 39
XII. Case Studies.....	p. 43

XIII. Materials and Methods.....	p. 48
A. Extraction of Peptides from Blood.....	p. 48
B. Extraction of Peptides from Brain Tissue.....	p. 51
C. Radioimmunoassay.....	p. 52
D. Preparation of Standard Curve and Data Calculation.....	p. 55
E. Statistical Evaluation of Data.....	p. 56
XIV. Hypotheses.....	p. 57
XV. Results.....	p. 60
XVI. Discussion of Results.....	p. 61
A. Peptide Serum Levels in Alive Subjects with Varying Levels of Pain.....	p. 61
B. Peptide Levels In Violent Deaths Having Incident-Death Time Intervals.....	p. 64
1. Serum study.....	p. 64
2. Serum/Brain study.....	p. 71
C. Comparison of Peptide Levels at Various Incident-Death Time Intervals in all Violent Deaths with Peptide Levels of Alive Adult Subjects with Varying Levels of Pain - Determination of Time Interval for Onset of Acute and Chronic Pain After Violent Episode in Post-Mortem Serum.....	p. 79
D. Comparison of Serum Peptide Levels in Various Types of Instantaneous Death with Serum Peptide Levels in Healthy Adults - Determination of Death-Induced Nociception.....	p. 81

E. Comparison of Serum Peptide Levels with Midbrain Peptide Levels in Various Types of Violent Instantaneous Death - An Indication of the Relationship Between Trauma and Nociceptive Induction.....	p. 91
F. Comparison of Peptide Levels in Accidental, Homicidal, and Suicidal Violent Death - An Assessment of the Effect of Manner of Violent Death and Psychological Factors on Substance P, Bradykinin, and Met-Enkephalin Levels.....	p. 101
1. Serum study.....	p. 101
2. Serum/Brain study.....	p. 112
G. Comparison of Peptide Levels in Various Modes (Blunt Trauma, Gunshot, Stabbing, and Thermal Burns) of Violent Death - Possibility of a Bimodal Nociceptive Response.....	p. 125
1. Blunt Trauma Deaths - Serum study.....	p. 125
2. Gunshot, Stabbing, and Thermal Deaths - Serum study.....	p. 129
3. Blunt Trauma Deaths - Serum/Brain study.....	p. 135
4. Gunshot, Stabbing, and Thermal Burn Deaths - Serum/ Brain study.....	p. 137
H. Peptide Levels in all Infant-Child Deaths - Comparisons Between SIDS Deaths and Deaths Caused By Violent Trauma, Infection, and Other Clinical Etiologies.....	p. 148
1. Serum study.....	p. 149
2. Serum/Brain study.....	p. 158
I. Comparison of Mean Peptide Serum Levels at Similar Incident-Death Time Intervals in all Adult and Infant/Child Deaths.....	p. 163

J. Peptide Levels in Clinical Non-Violent Deaths.....	p. 168
1. Serum study.....	p. 168
2. Insight Into Chronic and Acute Pain - t Testing of Mean Peptide Serum Levels in Non-Violent Deaths with all Instantaneous Violent Deaths and all Violent Deaths with a 1-8 Month Incident-Death Time Interval.....	p. 174
3. Serum/Brain study.....	p. 180
K. Nociceptive/Antinociceptive Peptide Ratios in Non-Violent Deaths - Providing Objective Criteria for the Assessment of Pain in Medicolegal Investigation.....	p. 194
XVII. Review of Hypotheses.....	p. 199
XVIII. The Problem of Defining Negative Controls.....	p. 208
XIX. Significance of Study and Future Considerations.....	p. 214
XX. Appendix A - Diagrams.....	p. 223
XXI. Appendix B - Case Summaries.....	p. 230
XXII. References.....	p. 316

LIST OF TABLES

Table 1a -	Substance P Serum Levels In Living Adult Subjects With Varying Levels Of Pain.....	p. 62
Table 1b -	Bradykinin Serum Levels In Living Adult Subjects With Varying Levels Of Pain.....	p. 62
Table 1c -	Met-Enkephalin Serum Levels In Living Adult Subjects With Varying Levels Of Pain.....	p. 62
Table 2a -	Substance P Serum Levels In All Violent Deaths.....	p. 65
Table 2b -	Bradykinin Serum Levels In All Violent Deaths.....	p. 66
Table 2c -	Met-Enkephalin Serum Levels In All Violent Deaths.....	p. 66
Table 2d -	t Testing Comparisons Of Mean Substance P Serum Levels At Various Incident-Death Time Intervals In All Violent Deaths.....	p. 68
Table 2e -	t Testing Comparisons Of Mean Bradykinin Serum Levels At Various Incident-Death Time Intervals In All Violent Deaths.....	p. 69
Table 2f -	t Testing Comparisons Of Mean Met-Enkephalin Serum Levels At Various Incident-Death Time Intervals In All Violent Deaths.....	p. 70
Table 2g -	Substance P Serum And Midbrain Levels In All Violent Death.....	p. 73
Table 2h -	Bradykinin Serum And Midbrain Levels In All Violent Death.....	p. 74
Table 2i -	Met-Enkephalin Serum And Midbrain Levels In All Violent Death.....	p. 75
Table 2j -	t Testing Comparisons Of Mean Met-Enkephalin Serum And Midbrain Levels At Various Incident-Death Time Intervals In All Violent Deaths.....	p. 76
Table 2k -	Bradykinin Serum And Midbrain Levels In All Violent Death.....	p. 77

Table 2l -	t Testing Comparisons Of Mean Bradykinin Serum And Midbrain Levels At Various Incident-Death Time Intervals In All Violent Death.....	p.78
Table 3a -	t Testing Comparisons Of Mean Substance P Serum Levels At Various Incident-Death Time Intervals In All Violent Deaths With Those Of Living Adult Subjects With Varying Levels Of Pain.....	p. 82
Table 3b -	t Testing Comparisons Of Mean Bradykinin Serum Levels At Various Incident-Death Time Intervals In All Violent Deaths With Those Of Living Adult Subjects With Varying Levels Of Pain.....	p. 83
Table 3c -	t Testing Comparisons Of Mean Met-Enkephalin Serum Levels At Various Incident-Death Time Intervals In All Violent Deaths With Those Of Living Adult Subjects With Varying Levels Of Pain.....	p. 84
Table 4a -	Substance P Serum Levels In Various Types Of Violent Instantaneous Death.....	p. 86
Table 4b -	Bradykinin Serum Levels In Various Types Of Violent Instantaneous Death.....	p. 86
Table 4c -	Met-Enkephalin Serum Levels In Various Types Of Violent Instantaneous Death.....	p. 87
Table 4d -	t Testing Comparisons Of Mean Substance P Serum Levels In Instantaneous Death With Healthy Adult Levels.....	p. 88
Table 4e -	t Testing Comparisons Of Mean Bradykinin Serum Levels In Instantaneous Death With Healthy Adult Levels.....	p. 89
Table 4f -	t Testing Comparisons Of Mean Met-Enkephalin Serum Levels In Instantaneous Death With Healthy Adult Levels.....	p. 90
Table 5a -	Substance P Serum And Midbrain Levels In Various Types Of Violent Instantaneous Death.....	p. 93
Table 5b -	Bradykinin Serum And Midbrain Levels In Various Types Of Violent Instantaneous Death.....	p. 94

Table 5c -	Met-Enkephalin Serum And Midbrain Levels In Various Types Of Violent Instantaneous Death.....	p. 95
Table 5d -	t Testing Comparisons Of Mean Substance P Serum And Midbrain Levels In Various Types Of Violent Death.....	p. 99
Table 5e -	t Testing Comparisons Of Mean Bradykinin Serum And Midbrain Levels In Various Types Of Violent Death.....	p. 100
Table 5f -	t Testing Comparisons Of Mean Met-Enkephalin Serum And Midbrain Levels In Various Types Of Violent Death.....	p. 100
Table 6a -	Substance P Serum Levels In All Accidental Violent Deaths.....	p. 103
Table 6b -	Substance P Serum Levels In All Homicides.....	p. 103
Table 6c -	Substance P Serum Levels In All Suicides.....	p. 104
Table 6d -	t Testing Comparisons Of Serum Substance P Levels In Accidental, Homicidal, And Suicidal Violent Deaths At Varying Incident-Death Time Intervals.....	p. 105
Table 6e -	Bradykinin Serum Levels In All Accidental Violent Deaths.....	p. 106
Table 6f -	Bradykinin Serum Levels In All Homicides.....	p. 106
Table 6g -	Bradykinin Serum Levels In All Suicides.....	p. 107
Table 6h -	t Testing Comparisons Of Serum Bradykinin Levels In Accidental, Homicidal, And Suicidal Violent Deaths At Varying Incident-Death Time Intervals.....	p. 108
Table 6i -	Met-Enkephalin Serum Levels In All Accidental Violent Deaths.....	p. 110
Table 6j -	Met-Enkephalin Serum Levels In All Homicides.....	p. 110
Table 6k -	Met-Enkephalin Serum Levels In All Suicides.....	p. 111

Table 6l -	t Testing Comparisons Of Serum Met-Enkephalin Levels In Accidental, Homicidal, And Suicidal Violent Deaths At Varying Incident-Death Time Intervals.....	p. 114
Table 6m -	Substance P Serum And Midbrain Levels In All Accidental Violent Deaths.....	p. 118
Table 6n -	Bradykinin Serum And Midbrain Levels In All Accidental Violent Deaths.....	p. 119
Table 6n -	Met-Enkephalin Serum And Midbrain Levels In All Accidental Violent Deaths.....	p. 120
Table 6p -	Substance P Serum And Midbrain Levels In All Homicides.....	p. 121
Table 6q -	Bradykinin Serum And Midbrain Levels In All Homicides.....	p. 122
Table 6r -	Met-Enkephalin Serum And Midbrain Levels In All Homicides.....	p. 123
Table 6s -	Substance P Serum And Midbrain Levels In All Suicides.....	p. 123
Table 6t -	Bradykinin Serum And Midbrain Levels In All Suicides.....	p. 124
Table 6u -	Met-Enkephalin Serum And Midbrain Levels In All Suicides.....	p. 124
Table 7a -	Substance P Serum Levels In Accidental, Homicidal, And Suicidal Blunt Trauma Deaths.....	p. 126
Table 7b -	Bradykinin Serum Levels In Accidental, Homicidal, And Suicidal Blunt Trauma Deaths.....	p. 127
Table 7c -	Met-Enkephalin Serum Levels In Accidental, Homicidal, And Suicidal Blunt Trauma Deaths.....	p. 128
Table 7d -	Substance P Serum Levels In Homicidal And Suicidal Gunshot Deaths.....	p. 131
Table 7e -	Bradykinin Serum Levels In Homicidal And Suicidal Gunshot Deaths.....	p. 132

Table 7f -	Met-Enkephalin Serum Levels In Homicidal And Suicidal Gunshot Deaths.....	p. 132
Table 7g -	Substance P Serum Levels In Homicidal And Suicidal Stabbing Deaths.....	p. 133
Table 7h -	Bradykinin Serum Levels In Homicidal And Suicidal Stabbing Deaths.....	p. 133
Table 7i -	Met-Enkephalin Serum Levels In Homicidal And Suicidal Stabbing Deaths.....	p. 134
Table 7j -	Substance P Serum Levels In Accidental And Suicidal Deaths Due To Thermal Burns.....	p. 134
Table 7k -	Bradykinin Serum Levels In Accidental And Suicidal Deaths Due To Thermal Burns.....	p. 135
Table 7l -	Met-Enkephalin Serum Levels In Accidental And Suicidal Deaths Due To Thermal Burns.....	p. 135
Table 7m -	Substance P Serum And Midbrain Levels In Blunt Trauma Deaths Classified As Accidents, Homicides, and Suicides.....	p. 138
Table 7n -	Bradykinin Serum And Midbrain Levels In Blunt Trauma Deaths Classified As Accidents, Homicides, and Suicides.....	p. 139
Table 7o -	Met-Enkephalin Serum And Midbrain Levels In Blunt Trauma Deaths Classified As Accidents, Homicides, and Suicides.....	p. 140
Table 7p -	Substance P Serum And Midbrain Levels In Homicidal And Suicidal Gunshot Deaths.....	p. 143
Table 7q -	Bradykinin Serum And Midbrain Levels In Homicidal And Suicidal Gunshot Deaths.....	p. 143
Table 7r -	Met-Enkephalin Serum And Midbrain Levels In Homicidal And Suicidal Gunshot Deaths.....	p. 144

Table 7s -	Substance P Serum And Midbrain Levels In Accidental Deaths Due To Thermal Burns.....	p. 144
Table 7t -	Bradykinin Serum And Midbrain Levels In Accidental Deaths Due To Thermal Burns.....	p. 145
Table 7u -	Met-Enkephalin Serum And Midbrain Levels In Accidental Deaths Due To Thermal Burns.....	p. 145
Table 7v -	Substance P Serum And Midbrain Levels In Homicidal Stabbing Deaths.....	p. 146
Table 7w -	Bradykinin Serum And Midbrain Levels In Homicidal Stabbing Deaths.....	p. 146
Table 7x -	Met-Enkephalin Serum And Midbrain Levels In Homicidal Stabbing Deaths.....	p. 147
Table 8a -	Substance P Serum Levels In All Infant-Child Deaths.....	p. 149
Table 8b -	Bradykinin Serum Levels In All Infant-Child Deaths.....	p. 150
Table 8c -	Met-Enkephalin Serum Levels In All Infant-Child Deaths.....	p. 150
Table 8d -	t Testing Comparisons Of Mean Substance P Serum Levels In SIDS Deaths With Other Infant-Child Deaths.....	p. 153
Table 8e -	t Testing Comparisons Of Mean Bradykinin Serum Levels In SIDS Deaths With Other Infant-Child Deaths.....	p. 154
Table 8f -	t Testing Comparisons Of Mean Met-Enkephalin Serum Levels In SIDS Deaths With Other Infant-Child Deaths.....	p. 155
Table 8g -	Substance P Serum And Midbrain Levels In All Infant-Child Deaths.....	p. 159
Table 8h -	Bradykinin Serum And Midbrain Levels In All Infant-Child Deaths.....	p. 160

Table 8i -	Met-Enkephalin Serum And Midbrain Levels In All Infant-Child Deaths.....	p. 161
Table 9a -	t Testing Comparisons Of Mean Substance P Serum Levels At Similar Incident-Death Time Intervals In All Adult And Infant-Child Violent Deaths.....	p. 165
Table 9b -	t Testing Comparisons Of Mean Bradykinin Serum Levels At Similar Incident-Death Time Intervals In All Adult And Infant-Child Violent Deaths.....	p. 166
Table 9c -	t Testing Comparisons Of Mean Met-Enkephalin Serum Levels At Similar Incident-Death Time Intervals In All Adult And Infant-Child Violent Deaths.....	p. 167
Table 10a -	Substance P Serum Levels In Clinical Non-Violent Deaths.....	p. 170
Table 10b -	Bradykinin Serum Levels In Clinical Non-Violent Deaths.....	p. 171
Table 10c -	Met-Enkephalin Serum Levels In Clinical Non-Violent Deaths.....	p. 172
Table 10d -	t Testing Comparisons Of Mean Substance P Serum Levels In Various Non-Violent Deaths With All Instantaneous Violent Deaths And All Violent Deaths With A 1-8 Month Incident-Death Time Interval.....	p. 175
Table 10e -	t Testing Comparisons Of Mean Bradykinin Serum Levels In Various Non-Violent Deaths With All Instantaneous Violent Deaths And All Violent Deaths With A 1-8 Month Incident-Death Time Interval.....	p. 176
Table 10f -	t Testing Comparisons Of Mean Met-Enkephalin Serum Levels In Various Non-Violent Deaths With All Instantaneous Violent Deaths And All Violent Deaths With A 1-8 Month Incident-Death Time Interval.....	p. 177
Table 10g -	Substance P Serum And Midbrain Levels In Clinical Non-Violent Deaths.....	p. 183

Table 10h -	t Testing Comparisons Of Mean Substance P Serum And Midbrain Levels In Various Types Of Clinical Non-Violent Death.....	p. 184
Table 10i -	Met-Enkephalin Serum And Midbrain Levels In Clinical Non-Violent Deaths.....	p. 187
Table 10j -	t Testing Comparisons Of Mean Met-Enkephalin Serum And Midbrain Levels In Various Types Of Clinical Non-Violent Death.....	p. 189
Table 10k -	Bradykinin Serum And Midbrain Levels In Clinical Non-Violent Deaths.....	p. 192
Table 10l -	t Testing Comparisons Of Mean Bradykinin Serum And Midbrain Levels In Various Types Of Clinical Non-Violent Death.....	p. 193
Table 11 -	Nociceptive/Antinociceptive Peptide Ratios In Clinical Non-Violent Deaths (Temporal Pain Scale).....	p. 197

LIST OF DIAGRAMS

- Diagram I - Schematic indicating the locations of laminae in cervical (C5), thoracic (T4), and lumbar (L7) segments of the spinal cord.....p. 224
- Diagram II - The peripheral activation of primary afferent fibers after tissue injury.....p. 225
- Diagram III - Summary of processing and modulation of nociceptive information..p. 226
- Diagram IV - Effects of Neuropeptides on inflammation and tissue repair.....p. 227
- Diagram V - Theoretical Serum Peptide Levels v. Duration Between Incident And Death.....p. 228
- Diagram VI - Theoretical Brain Peptide Levels v. Duration Between Incident And Death.....p. 229

I. Introduction

With the universal acceptance of forensic DNA technology, forensic science may have reached the practical limits of biological individualization. Routinely, forensic scientists specializing in DNA typing methods report out statistical matches in the magnitude of one in several billions or even trillions. With technical improvements made in throughput by developments in fluorescent labeling detection and multiplexing in STR technology, future research in biological methods of individualization may often be redundant and contribute little to the field. Furthermore, the forensic application of mitochondrial DNA sequencing allows for typing of biological materials where genomic DNA is either missing (e.g., hairs devoid of roots) or badly degraded. This does not mean, however, that forensic science cannot utilize the expertise of practitioners of biochemistry, immunology, and molecular biology to make further contributions. Several problems remain that the biological sciences may help to solve.

One area of potential inquiry is whether biochemical characterization of tissue or blood can be of use in the medicolegal investigation of death. Specifically, can the types and/or quantity of certain biochemicals in post-mortem specimens be indicative of certain kinds of death? Currently, biochemical characterization as a way to determine cause of death is limited to forensic laboratories in medical examiners' offices which utilize isoelectric focusing to detect hemoglobin variants such as

hemoglobin S [14]. In cases where the mode of death (natural or sinister) is not clear (for instance, distinguishing infant death by suffocation or SIDS), can such measurable parameters be of value? If an individual suffers pain greatly before death, are there biochemical changes that occur that would otherwise be non-existent if the individual died suddenly and without pain? Similarly, can quantities of certain biochemicals be useful in determining episodes of child abuse when either the episodes are not evident or if the child is not communicative?

The answers to these questions may lie not in what happens at the time of death, but rather what happens prior to death. If it is assumed that the mechanisms of acute pain are activated if tissue trauma occurs before death and not if tissue trauma does not occur, then the measure of acute pain may provide information about events that happened before death. If biochemical parameters of acute pain accumulate over time, then the time interval between incident and death and the amount of pain suffered may be correlated. If this time interval becomes so long that acute pain turns into chronic pain, then the measure of biochemical events related to chronic pain may be informative.

The detection of biochemical factors related to either acute or chronic pain may also be utilized to determine the existence of aggravating circumstances which are often considered in the sentencing of an individual convicted of violent crime. If, for sentencing purposes of someone convicted of homicide, it is necessary to know how

much pain was suffered by the victim prior to death, the measurement of biochemicals expressed during pain transmission may be useful. Thus, if such factors can be isolated, it may be possible to distinguish between the person tortured before death and the person who is suddenly and unknowingly killed by a bullet to the back of the head. The potential of using scientific technology in the sentencing phase of a criminal trial may bring forensic science into a facet of the criminal justice system previously untouched by scientific inquiry. Additionally, a biochemical assessment of pain may be useful in assigning punitive damages (which are typically arbitrary) for pain and suffering in wrongful death actions in homicide cases.

The following study will attempt to address these issues along with others such as the potential use of pain biochemical markers in the determination of SIDS or death by child abuse syndrome. Before the study is presented, the anatomy of pain centers and physiology of pain will be discussed. After the results of the study are presented, a rationale for its use in the medicolegal investigation of death will be offered.

II. Theories of Pain

In 1664, Descartes described nerves as tubes composed of large numbers of fine threads that function to connect the brain with end organs in the skin and other

tissues [35]. This idea was expanded by Muller in 1840 who proposed that the brain receives information about external objects and body structures through sensory nerves. Muller described five categories of sensory nerves, each transmitting an energy specific for each of the five senses (sight, smell, taste, hearing, and touch). Pain was not considered a distinct sensation but rather a component of the sense of touch [111]. In 1884, Blix experimentally showed that the sensations of touch, hot, cold, and pain could be produced by stimulating particular points on the skin. For instance, a hot spot could only be stimulated by heat. Even if stimulated electrically, the sensation produced was of heat [15].

The discovery by Blix of distinct mechanical and thermal areas of the skin suggested that each area is associated with a specific sensory end organ and that pain is a distinct sensation with its own set of sensory equipment. To help confirm this observation, von Frey examined each of these areas histologically and identified specific and separate nerve endings [165]. These nerve endings were assumed to be responsible for sensations.

Around the time of Blix and von Frey, an alternate theory of pain transmission was formed. The intensity theory, as it became known, states that pain results when sensory receptors are excessively stimulated regardless of the type of stimulation. According to this theory, pain results when excessive stimulation of sensory receptors normally activated by benign thermal or mechanical stimuli occurs or by pathological

conditions that enhance impulses from benign stimuli [18].

The intensity theory does not account for several experimental and clinical findings. First, intense stimulation of some sensory nerve endings never produces pain. Second, pathological conditions of the spinal cord often eliminate pain sensations without losing the ability to feel touch or extremes in temperatures. Third, surgical procedures which remove pain pathways in an attempt to alleviate chronic pain often do not remove pain sensations. Fourth, the theory also does not explain the phenomena of hyperalgesia (where nonpainful stimuli becomes painful) and the ability of cognitive processes to influence pain [18].

Noting these observations, Melzack and Wall proposed the gate control theory in 1965 [106]. This theory states that the sensation of pain is mediated through the dorsal horn of the spinal cord. Specifically, information from painful stimuli are transmitted to the superficial laminae of the dorsal horn (substantia gelatinosa) by small diameter afferent nerve fibers (noxious afferents) leading from the skin and internal organs. The superficial laminae of the dorsal horn then serves as a “gate” where the afferent input is processed before being conveyed via transmission fibers deep into the inner laminae of the dorsal horn where the sensation of pain originates. Conversely, stimuli processed by large diameter afferent fibers (non-noxious afferents) “closes the gate” and, therefore, transmission does not occur. Melzak and Wall also postulated that descending nerve fibers leading from the brain to the spinal

cord and cognitive factors influence the gating process [106].

Although clinical and experimental evidence support the concept that pain transmission is mediated in the spinal cord, several physiological findings are not explained by the “gating” theory. Whitehorn and Burgess found that the inhibition of pain transmission only occurred when large and small afferents were stimulated respectively [171]. Furthermore, several investigators have identified nerve fibers in the substantia gelatinosa and transmission fibers that selectively respond to different types of noxious stimuli [28, 84, 173]. Since not all areas of the substantia gelatinosa receive noxious and/or non-noxious input, the role that this part of the spinal cord plays in the “gating” of afferent inputs to pain transmission is not entirely understood.

III. Defining Pain

Pain may be defined as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage [19].” The emotional component of pain in this definition clearly refers to the human experience of pain. Thus, the emotional aspect of pain can only be studied in humans. Since pain, however, is studied predominantly in animals, the term nociception is usually used for the study of pain. The term nociception was originally proposed by Sherrington in 1906 [147]. Sherrington observed that stimuli which cause pain are often accompanied by tissue

damage. He labeled such stimuli as “noxious” and the resulting pain as “nociception.” The term “nociceptors” was given to afferent receptors which carry and transmit noxious stimuli. Sherrington was the first to believe that the production of pain is protective in nature and occurs before tissue damage is commenced and thus serves as a warning signal to remove or avoid the noxious stimulus.

There are two separate pain phenomena. The first, physiological pain, results only from the activation of nociceptors by intense or potentially damaging noxious stimuli. Activated nociceptors cause the transmission of impulses down specialized nociceptor afferent fibres to the spinal cord. Physiological pain is highly localized and is transient if no tissue damage occurs. This pain informs the body of potential danger as in the case with the exposure to excessive heat or cold, chemical irritants, and mechanical abrasives. Physiological pain has distinct mechanical, thermal, and chemical thresholds and its amplitude is related to the intensity of the stimulus [174].

In contrast, pathological pain, arises from tissue or nerve damage. It is characterized by the disruption of normal sensory mechanisms so that pain can occur in the absence of a clear stimulus, in response to innocuous stimuli, and in an exaggerated and prolonged fashion to noxious stimuli. These examples represent an increase in the sensitivity of sensory function leading to a reduction in pain thresholds, hyperalgesia, and prolonged post-stimulus sensations, respectively. The pain when occurring acutely and associated with inflammation has a protective function. The

increased sensitivity promotes recuperation and tissue repair by minimizing further damage by discouraging both innocuous and noxious stimuli from coming into contact with the inflamed area. Conversely, chronic pathological pain which is typically associated with injury to the peripheral or central nervous system, appears to have lost any protective function and is, therefore, truly pathological [174].

In chronic pain states, there is a loss of the ability to modulate pain due to changes in the neurochemistry of pain transmission. These changes help produce hypersensitivity that exaggerates and prolongs non-noxious stimuli and results in pain perception. Additionally, structural changes caused by injury to the peripheral and central nervous system (such as loss of spinal interneurons and inappropriate rearrangement of afferent nerve processes in the spinal cord) causes diminished pain modulation [54].

IV. Anatomy of Pain Centers

Most of the pain processing activity occurs in the dorsal horn of the spinal cord. It was once believed that there is a rigid separation of sensory and motor neurons into the dorsal and ventral horns of the spinal cord, respectively. Recent evidence shows that this is not entirely true [22]. Although most sensory processing occurs in the dorsal horn, some occurs in the ventral horn. Conversely, some motor

activity can be found in the dorsal horn.

Dorsal horn neurons are usually classified according to their primary afferent input sent from a variety of peripheral sensory receptors [22]. Three major types of receptors have been identified: cutaneous mechanoreceptors, cutaneous nociceptors, and cutaneous thermoreceptors. Fibers which lead from each of these receptors terminate in particular laminae or columns of the dorsal horn of the spinal cord. Ten laminae have been identified and named I-X (Appendix A. Diagram I).

Cutaneous mechanoreceptors respond to low-threshold, non-painful stimuli [22]. Terminations of cutaneous mechanoreceptors have been located in all laminae (particularly laminae II, III, IV, and V), except for lamina I.

Cutaneous nociceptors respond to painful thermal, mechanical, and irritating stimuli [22]. Terminations of these receptors are located within laminae I, II, V, and X. Not surprisingly, these laminae are believed to be the regions of the dorsal horn which are involved in the transmission of nociceptive information.

Cutaneous thermoreceptors relay cold and warm stimuli to the dorsal horn [22]. Terminations of these receptors are believed to be found in laminae I-III.

Areas of the brain are also involved in the transmission of nociceptive information [172]. Sensory transmitting fibers leading from the dorsal horn have been found to terminate in the thalamus, medullary and pontine reticular formations, caudal midbrain, and periaqueductal gray.

Fibers projecting to the thalamus are predominantly found in laminae I and V-IX. Spinothalamic fibers respond to thermal, mechanical, and chemical painful stimuli which may originate cutaneously, muscularly, or viscerally [172].

The medullary and pontine reticular formations are believed to be responsible for alerting and arousal reactions associated with pain. Fibers leading to these regions of the brain reside predominantly in laminae VII and VIII. Smaller quantities can be found in laminae I, V, and X. Spinoreticular fibers respond to non-noxious and noxious mechanical cutaneous stimuli, noxious heat, and light tactile stimuli [42, 43, 172].

Spinomesencephalic fibers projecting to the caudal midbrain process exclusively nociceptive stimuli. They are also involved in behavioral responses to pain [69]. These fibers are found in laminae I, V, VII, and X.

Spinothalamic, spinoreticular, and spinomesencephalic fibers are believed to be involved in pain transmission due to two experimental findings [172]. First, chordotomy or disease processes of these fibers produce relief of nociception. Second, stimulation of these fibers of sufficient intensity causes pain.

Certain nerve fibers which run from specific areas of the brain to the dorsal horn have been found to play a role in the modulation of spinal cord nociceptive transmission. These fibers originate from the cortex, hypothalamus, periaqueductal gray, nucleus locus coeruleus and subcoeruleus, nucleus raphe magnus, lateral

reticular nucleus, nucleus tractus solitarii, and the A5 cell group [18].

V. The Process of Nociceptive Transmission

The process of nociceptive transmission begins with the activation of nociceptors on peripheral sensory afferents following pain of injury. The activation of these nociceptors is due to the action of bradykinin in combination with prostaglandins and leukotrienes. During tissue injury, prostaglandins and leukotrienes are released from tissue as a result of the activation of the arachidonic acid cascade. Also, bradykinin is enzymatically converted from kinogen as a result of the activated cascade [31].

Once activated, peripheral afferents release certain neurochemicals which are believed to be primarily responsible for the transmission of nociceptive information [96]. These neurochemicals are either neuropeptides, such as substance P and somatostatin, or excitatory amino acids, such as glutamate and aspartate (Appendix A. Diagram II).

It was once believed that excitatory amino acids mediate fast nociceptive transmission and that neuropeptides mediate slow nociceptive transmission [143]. Considerable evidence, however, shows an interaction between the two exists during all types of nociceptive transmission [11, 34]. Both glutamate and substance P exist

in dorsal root ganglia cells and in peripheral afferent fibers. Additionally, noxious stimuli have been shown to promote the release of excitatory amino acids and substance P from the spinal cord [104, 148]. Kangra *et al.* postulate that substance P and calcitonin gene-related peptide may promote the release of excitatory amino acids leading to enhanced nociceptive transmission [78].

The release of neurochemicals such as substance P from peripheral afferents triggers the release of the same neurochemicals from the dorsal horn [13]. The release of neurochemicals in the dorsal horn, in turn, causes nociceptive transmission to move from the spinal cord to sites in the brain where modulation networks are activated.

VI. Substance P and Nociceptive Transmission

The eleven amino acid peptide known as substance P was isolated by von Euler and Gaddum in equine intestine in 1931 [164]. The newly discovered peptide was believed to play a role in intestinal smooth muscle contraction and in lowering blood pressure [164]. The term substance P derives from the use of early crude “preparations” of the bioactive compound [164]. Subsequent bioassay methods used by Gaddum found substance P in the brain tissue of all vertebrate species tested [47].

The amino acid sequence of substance P was determined by Chang and

Leeman in 1970 [26]. These researchers used synthetic substance P as an immunogen to raise antisera for radioimmunoassay and immunochemical studies which have contributed greatly to the knowledge of substance P [26]. Further work by Erspamer determined that substance P is a member of the family called the tachykinins [39]. All tachykinins share an array of biological functions and share an amino acid sequence at the carboxyl terminus.

Substance P is believed to be synthesized as part of a larger polypeptide precursor. Subsequent proteolytic cleavage of this large polypeptide forms substance P [61].

Although substance P was isolated from the intestine, found in several organs, and appears to have many functions, the majority of scientific inquiry has focused on its role in nociceptive transmission.

The first suggestion that substance P has a role in nociceptive transmission was provided by Lembeck in 1953, who by using bioassay techniques, found substance P-like material in large quantities in spinal dorsal roots and in lesser quantities in spinal ventral roots [126]. The identity of the material was later confirmed as substance P by Otsuka and Konishi in 1975 [120]. Furthermore, the development and use of radioimmunoassay and immunohistochemical techniques have found high levels of substance P in certain sensory neural pathways (about 10-20% of all sensory neurons in spinal ganglia contain substance P [63]) found in the periphery. The terminals of

these pathways in the spinal cord were also found to contain large concentrations of substance P [66].

Using immunohistochemical techniques, fibers containing substance P have been found in the spinal cord and brain [65]. In the spinal cord, the greatest concentration of substance P is found in laminae I and II of the dorsal horn with smaller quantities found in deeper layers [65]. Substance P containing neural pathways run to and from the spinal cord and the brain. Areas of the brain containing high levels of substance P terminals include the substantia nigra, periaqueductal gray, and hypothalamus [65].

The idea that substance P plays a role in nociceptive transmission was advanced by Otsuka et al., who showed that substance P is released in vitro upon electrical stimulation of dorsal roots in spinal cord preparations [120]. This release is contingent on calcium being present in the bathing medium. They were also the first to show that substance P exerts excitatory effects on spinal cord neurons.

To support the work of Otsuka et al., several researchers have shown that dorsal horn nerve fibers which respond to the most to painful stimuli are the ones which are the most excited by applied substance P [60]. These findings helped develop the hypothesis that substance P was associated with pain transmission and that it may even be a pain transmitter.

The most significant body of work which addresses the question of the

relationship of substance P to pain transmission are studies involving capsaicin. Capsaicin is a naturally occurring alkaloid found in some species of plants that has antinociceptive qualities [12]. It has been observed, for instance, that adult rats pretreated systemically with capsaicin were temporarily insensitive to painful chemical irritants, but neonatal systemic treatment with capsaicin caused irreversible impairment of chemosensation [72].

It has also been noted that erythema produced by stimulation of sensory nerves is inhibited by capsaicin [126].

These studies led to the hypothesis that capsaicin interferes with the production, storage, or release of one or more vasoactive neurochemicals involved in nociceptive transmission [13].

Through work done by Theriault et al. and Lembeck et al., it was demonstrated that this neurochemical is substance P [126]. Capsaicin appears to deplete substance P in peripheral sensory nerves blocking axonal transmission.

Kuraishi et al. found that certain types of noxious stimuli increase substance P release from the lumbar dorsal horn in situ in decerebrated rats [85]. Among the types of stimuli which increased substance P release include noxious pinch, subcutaneous injections of formaldehyde, and very intense heat. Other types of stimuli, however, such as ice cold, moderate heat, and innocuous mechanical stimuli produced no such increase.

These results which suggest that mechanical and inflammatory, but not thermal stimuli, increase substance P release confirm earlier work [86]. The same researchers postulate that another neuropeptide, somatostatin, is responsible for transmitting thermal information [86].

Elevated levels of substance P have been found in the dorsal horn of individuals who suffer from chronically painful conditions such as syringomyelia [108], sciatica [74], and limb amputation [68]. Conversely, substance P containing neurons in the dorsal horn have been found to be depleted in individuals with decreased pain sensitivity, such as in the cases of Riley-Day syndrome and peripheral nerve injury [74].

Jessell and Iverson showed in vitro that morphine and related analgesics can inhibit the release of substance P from sensory neurons [75]. This observation may explain the analgesic properties of morphine on the spinal cord.

Some researchers have attributed an antinociceptive role for substance P [56]. It seems likely, however, that any functional significance that substance P has in the modulation of pain is related to its influence on the release of other neuropeptides. The release of substance P has been found to cause the release of serotonin from basal ganglia [134], acetylcholine from the intestine [179], beta-endorphin from the pituitary [101], and met-enkephalin from the brain and spinal cord [159]. These neuropeptides all have pain modulating qualities, but unlike the others, met-

enkephalin's relationship with substance P has been extensively studied.

VII. Met-Enkephalin and the Modulation of Nociceptive Transmission

Once nociceptive transmission reaches the spinal cord, endogenous mechanisms for the modulation or inhibition of transmission are triggered. Modulation can occur at the spinal cord level or at supraspinal sites within the brain (Appendix A, Diagram III). Inhibition may occur by preventing either the release of neurochemicals from afferent fibers or the activation of dorsal horn neurons.

Inhibitory neuropeptides are released into the spinal cord after painful stimuli. Included among these inhibitory neuropeptides are the opioids [95], such as the endorphins and enkephalins, norepinephrine [175], acetylcholine [176], neurotensin [177], serotonin [166], and somatostatin [50]. Some of these neuropeptides work presynaptically by inhibiting the release of nociceptive transmitting neurochemicals [181]. One inhibitory neuropeptide, gamma-aminobutyric acid or GABA, works postsynaptically thus blocking nociceptive transmission to the brain [121]. In addition to GABA, spinal opioids have postsynaptic inhibitory ability [70].

Supraspinal networks involved in modulation are activated through the release of excitatory amino acids from fibers leading from the spinal cord to the brain [73]. It is not known whether different nociceptive stimuli activate different areas of the

brain involved in the modulation process. Each region of the brain may act independently by sending nerve fibers directly to the spinal cord or may send fibers to an interdependent network where fibers from one region may influence fibers from others [7]. The release of two neurotransmitters, serotonin and norepinephrine, by these fibers causes inhibition of dorsal horn transmission [2].

The release of enkephalins into the dorsal horn during episodes of pain was observed by Jessell in 1982 [74]. Evoked release of enkephalins in the spinal cord have been observed as a response to noxious mechanical [89], chemical [24], and noxious thermal stimuli [23]. Enkephalin-containing neurons have been located in laminae I, II, and III of the dorsal horn [74].

Tang *et al.* found that met-enkephalin is released in the rat spinal cord in both *in vitro* and *in vivo* studies when substance P is injected in the subarachnoid space [159]. This effect is calcium dependent and can be blocked by substance P antagonists.

A hypothesis has been proposed for the mechanism of pain modulation by met-enkephalin [81]. It has been suggested that in the spinal cord, neurons containing stores of met-enkephalin interact with those of substance P via a transsynaptic mechanism during episodes of nociceptive transmission leading to a presynaptic inhibition of substance P release. Experimental evidence supporting this hypothesis is suggestive but not conclusive.

Leander et al. found that substance P release from the spinal cord following chemical or electrical stimulation of sensory neurons is completely or partially inhibited by local application of met-enkephalin to sensory terminals [88]. Substance P release resumed after the opiate antagonist, naloxone, was administered demonstrating the specificity of met-enkephalin effects.

Studies on substance P containing sensory neurons grown in cell culture have suggested an ionic mechanism by which met-enkephalin may inhibit substance P release [159]. Adding met-enkephalin to sensory neurons in culture greatly reduces the action potential recorded intracellularly from the sensory neurons. The decrease in action potential appears to be caused by a reduction in the flux of calcium ions into sensory neurons. Since substance P release is dependent on the calcium concentration in neuron terminals, the ability of met-enkephalin to decrease calcium flow into the neurons might be expected to cause a reduction in substance P release, thus slowing pain transmission.

The immunohistochemical-anatomical findings of Hokfelt et al. also supports this hypothesis [67]. They studied the distribution of met-enkephalin and substance P immunoreactive neurons by indirect immunofluorescence in areas of the brain and spinal cord related to pain and analgesia. In addition to the spinal cord dorsal horn, met-enkephalin and substance P positive neurons and terminals were both observed in the periaqueductal central gray, the nucleus raphe magnus, and the marginal layer

and substantia gelatinosa of the spinal trigeminal nucleus. Experiments dealing with the transection of the spinal cord and dorsal rhizotomy showed that met-enkephalin neurons in the dorsal horn and possibly in the spinal trigeminal nucleus are interneurons forming axonic synapses with substance P containing afferents.

Electron microscopy studies by Barber et al. showed that substance P containing neurons contact other axons both presynaptically and postsynaptically in the marginal zone and the substantia gelatinosa of the rat spinal cord [9]. The presence of met-enkephalin, however, was not observed in any of these axons using electron microscopy.

Some researchers contend that the inhibition of substance P release by met-enkephalin does not occur by presynaptic contact of axons, but rather axodendritically. Pickel et al., for instance, found the majority of enkephalin-positive terminals in the rat neostriatum make these sort of contacts with other neurons in the spinal marginal zone and lamina V [127].

Regardless of the mechanism that met-enkephalin modulates nociceptive transmission by inhibiting substance P release, it seems obvious that it is released in the central nervous system as a response to substance P-generated axonal transmission. It is expected, therefore, that as the duration of pain transmission becomes longer, the ratio of substance P concentration to met-enkephalin concentration should become smaller, at least until a steady state is reached. At the

onset of pain transmission, however, this ratio should be at its highest value since it seems likely that at this point substance P release is maximal and has yet to be affected by met-enkephalin.

VIII. Neuropeptides in Blood During Tissue Injury

Nociceptive transmission is usually accompanied by tissue injury and inflammation at the site where the painful stimuli originated. Neuropeptides released from the central and peripheral nervous systems into the blood help produce neurogenic inflammation which is manifested by redness, edema, and hyperthermia [96]. These clinical indicators of inflammation develop due to the influence of neuropeptides or mediators on cellular elements of inflammatory responses and tissue repair.

During the inflammatory process, substance P and another neuropeptide, neurokinin A, produce vasodilation and plasma extravasation [36]. Plasma extravasation contributes to the production of edema, a common feature of inflammation, and the continued release of vasoactive substances [36].

One of these substances is bradykinin which works synergically with substance P to promote the release of histamine from mast cells [145]. Histamine also produces plasma extravasation and stimulates peripheral nerve fibers [91].

Bradykinin is a nonapeptide cleaved from α_2 -globulins by kallikreins circulating in the plasma and activated at sites of tissue injury [49]. Bradykinin is a very potent pain producing substance. When administered intradermally, intraarterially, or intraperitoneally, bradykinin has been found to cause pain, itching, heat, and redness [41]. The effects of bradykinin are mediated by B_2 -bradykinin receptors which are located on the terminals of primary afferent neurons which when activated will transmit painful stimuli to the spinal cord [153]. Activation of these receptors involves the generation of diacylglycerol and activation of protein kinase C causing an increase in sodium conductance [146].

In addition to pain production, bradykinin also sensitizes nociceptors to produce hyperalgesia [97] and has been shown to lower nociceptive thresholds in animal behavioral studies [157]. Bradykinin-induced sensitization of primary afferent neurons and hyperalgesia is dependent on sympathetic postganglionic neuron production of prostaglandins [158].

Bradykinin stimulates the generation of prostaglandins. Bradykinin-activated B_2 receptors is coupled to phospholipase A_2 which in turn generates arachidonic acid from membrane phospholipids [27]. The inhibition of peripheral prostaglandin production by non-steroidal anti-inflammatory drugs prevents the sensitization of primary afferent neurons and decreases hyperalgesia [27].

Bradykinin has also been shown to be involved in thermal sensitization in sites

of imflamed tissue [83].

Within the central nervous system, bradykinin receptors have been found in the periaqueductal gray and other supraspinal areas [32] and evidence exists that they are present in the spinal cord as well. The role of bradykinin in the central nervous system appears to be antinociceptive. Behavioral studies have indicated that intrathecally administered bradykinin causes modulation of painful stimuli [135]. Thus, the role of bradykinin in the peripheral and central nervous systems appears to be reversed.

Once vasodilation and plasma extravasation takes place, substance P begins to stimulate leukocyte and macrophage activity to remove damaged tissue. Leukocyte chemotaxis is initiated by the recognition of neuropeptides by stereospecific receptors on the surface of leukocytes [162]. Met-enkephalin released from activated macrophage and substance P released from peripheral afferents during tissue injury stimulates human mononuclear leukocyte chemotaxis [136, 162]. Chemotactic response by polynuclear leukocytes is attributed to the binding of substance P to N-formyl-methionyl-leucyl-phenylalanine (fMLP) receptors [99]. The binding of substance P to fMLP receptors on polynuclear leukocytes causes the release of lysosomal enzymes [99] and stimulates phagocytic activity [8].

The various effects of substance P on leukocytic activity seems to be related to certain structural moieties on the peptide. The carboxy-terminal end of substance

P seems to initiate chemotaxis whereas the N-terminal end is responsible for phagocytosis-enhancing activity [8].

Substance P elicits several macrophage activities necessary for proper response to tissue injury. These activities include the stimulation of synthesis and release of metabolites of arachidonic acid, the down-regulation of membrane-associated nucleotidase, and the release of thromboxane A_2 , O_2 , H_2O_2 [59].

Once damaged tissue is removed, substance P then helps to elicit the proliferation of smooth muscle cells and fibroblasts needed for tissue repair [114]. Nilsson et al. found the proliferative effects of substance P on smooth muscle cells is diminished by preincubation with substance P antagonists or simultaneous incubation with suboptimal concentrations of other growth factors, suggesting a receptor mediated response [114].

Substance P stimulated fibroblast proliferation is accompanied by an increase in cytosolic Ca^{2+} , implying an activation of the phosphatidylinositol pathway [125]. It has been observed that the amino acid sequence of substance P is very similar to that of known fibroblast growth factors [52].

It appears that the initiation of nociception in the periphery and the appropriate inflammatory and immune response to tissue repair are interrelated. Neuropeptides such as bradykinin, substance P, and met-enkephalin have roles in both processes. To reiterate, bradykinin works synergistically with prostaglandins to activate peripheral

afferents upon noxious stimulation to begin nociception. Nociceptive transmission moves to the dorsal horn of the spinal cord primarily because of the release of substance P and other neurochemicals in the peripheral afferents. At the same time, substance P is released from afferent terminals into the circulation to work with other neuropeptides such as bradykinin to begin the inflammatory and immune response to tissue injury. Once damaged tissue is removed, the continued release of substance P at the site of inflammation is directed toward promoting tissue growth and mitogenesis. Met-enkephalin, whose primary role is to centrally modulate nociception, also plays a role in the immune response to tissue injury by stimulating leukocyte chemotaxis. Met-enkephalin is released into the circulation by activated macrophages which gather at the site of injury. A schematic of the effects of neuropeptides on inflammation and tissue repair is given in Appendix A, Diagram IV.

IX. Other Neuropeptides Involved in Nociceptive Transmission and Modulation

Many other neuropeptides are involved in the transmission and modulation of nociception. Since relationships exist between many of them with neuropeptides extensively described in the foregoing and to further demonstrate the complexity of the system, a brief discussion of some of them is presented.

A. Beta-Endorphin and Dynorphin

Soon after the discovery of the enkephalins, a distinct peptide was isolated from the pituitary that also had high affinity for opiate receptors. The peptide was named beta-endorphin and is derived from the C-fragment of the larger peptide beta-lipotropin. It consists of 31 amino acids including the sequence of met-enkephalin at its carboxyl terminal [94]. Although there was initial debate whether beta-endorphin represented a precursor form of the enkephalins, it soon became apparent that they are two distinct systems. Similar to met-enkephalin, beta-endorphin inhibits the neuron release of nociceptive transmitting neuropeptides in areas of the brain and spinal cord [169]. Unlike the smaller met-enkephalin, however, beta-endorphin has been shown to be a potent analgesic agent when administered either intravenously [161] or directly into the periaqueductal gray [170].

The observation that beta-endorphin made up only a small fraction of total opioid activity in pituitary extracts led to the discovery of dynorphin and dynorphin-related compounds [77]. The dynorphins have their own unique precursor molecule and contain the sequence of leucine-enkephalin. Immunohistochemical studies using specific antisera show that the dynorphins have an unique distribution in the central nervous system compared with the other opioid peptides [168].

Additionally, the behavioral effects of dynorphins are different as well. Dynorphin is not analgesic when given intravenously, but is when administered

intrathecally [58]. The analgesic effects of dynorphin is not completely reversed by naloxone but intrathecal administration of anti-dynorphin antibodies inhibits the effects of electroanalgesia in experimental animals [58]. Furthermore, dynorphin, unlike met-enkephalin, does not inhibit noxious stimulus-induced substance P release from the dorsal horn in situ [63].

B. Non-Opioid Modulators - Neurotensin, Calcitonin, Vasopressin, and Cholecystokinin

Evidence exists that there are non-opioid mechanisms of endogenous antinociception. It has been observed, for example, that analgesia produced by heat-shock stress in experimental animals may not be reversible by naloxone depending on temporal characteristics of the applied shock [93].

One such non-opioid analgesic neuropeptide is the tridecapeptide, neurotensin, which is widely distributed in areas of the brain involved in both transmission and modulation of nociception and in the spinal dorsal horn. Unlike met-enkephalin receptors, however, neurotensin receptors are not altered by dorsal rhizotomy suggesting that the terminals of these receptors do not end in the dorsal horn [115].

Neurotensin is a potent analgesic and hypothermic agent when given intracisternally to experimental animals. The effects of intracisternal neurotensin are not reversed by naloxone but are antagonized endogenously by thyrotropin-releasing hormone [119].

Calcitonin is a 32 amino acid peptide with strong concentration in the hypothalamus, midbrain, and pons which when infused into the cerebral ventricles of experimental animals produces antinociception. The effect is reversed by calcium but not naloxone, suggesting an opiate-independent analgesic mechanism [140]. Calcitonin has been shown to be an effective analgesic when administered intrathecally to people with severe cancer pain [44].

Vasopressin is an octapeptide localized in the supraoptic and paraventricular nuclei of the hypothalamus and is part of the descending modulation system [156]. Vasopressin-deficient animals have been shown to have a lower threshold to nociceptive stimuli, which is normalized when vasopressin is administered [17]. Vasopressin effects are not reversed by naloxone suggesting a non-opiate mechanism of antinociception, although beta-endorphin immunoreactivity in blood increases after the administration of vasopressin [5].

Another octapeptide, cholecystokinin, is found in the periaqueductal gray, dorsomedial hypothalamus, and dorsal root ganglia. Antinociception is produced by cholecystokinin when it is administered subcutaneously [180] or into the periaqueductal gray in experimental animals [76]. Cholecystokinin may produce antinociception effects by stimulating opioid activity or perhaps by inhibiting enkephalinase and other opioid degradative enzymes [10].

C. Other Nociceptive Transmitting Neuropeptides - Somatostatin and Bombesin

Somatostatin is a tetradecapeptide that is widely distributed in the central nervous system but found in highest concentration in the hypothalamus and dorsal root ganglia [64]. Studies show that somatostatin immunoreactivity in cerebral spinal fluid is increased compared with substance P when certain noxious stimuli are applied (primarily thermal), suggesting a role in nociceptive transmission [86].

Conversely, somatostatin has been found to be a potent analgesic agent when administered intrathecally to people with intractable pain [107]. The analgesic actions of somatostatin may be explained by its ability to bind opiate receptors and inhibit aminopeptidases and enkephalinase [10].

Bombesin is a tetradecapeptide widely distributed in the brain and spinal cord and is detected in the cerebrospinal fluid [178]. Evidence exists that suggest that bombesin plays a role in transmitting noxious stimuli. First, the concentration of bombesin is much higher in the dorsal than the ventral spinal horn [100]. Secondly, bombesin immunoreactivity is decreased by dorsal rhizotomy [46]. Thirdly, bombesin is 100 times more potent than substance P in eliciting a “bite-scratch” response in behavioral experiments [117].

Similar to somatostatin, bombesin may modulate nociception at several central nervous system levels. For example, microiontophoretic application of bombesin into the periaqueductal gray has been shown to produce analgesia that is not naloxone

reversible [135].

X. Substance P and Other Nociceptive Peptides and Their Potential Application to Medicolegal Investigation

Any study designed to measure the amount of pain an individual experienced prior to death must evolve around substance P. Many neurological, hormonal, and immunological substances are implicated in the mediation and transmission of pain, but none as central to the process and been as extensively studied as substance P. Since substance P release in the blood is increased during and after the onset of pain and tissue injury, it is an ideal parameter to be used as a nociceptive marker because blood is routinely taken from autopsy victims and sent to forensic biology laboratories. Although less commonly sent to forensic biology laboratories, central nervous system tissue could additionally be used to measure substance P.

The evaluation of substance P as a marker for the measurement of pain must be accompanied by the measurement of other substances which either work synergistically with substance P or are released as a response to substance P release. The measurement of other substances involved in pain transmission may better gauge the amount of pain an individual suffers prior to death since clinical manifestations of certain diseases may alter the levels of certain pain mediators but not others. It is assumed that the experience of acute pain will raise the levels of all mediators

involved in nociceptive transmission.

Two substances which may be measured along with substance P to gauge acute pain or chronic pain are bradykinin and met-enkephalin. As mentioned in the foregoing, bradykinin and substance P are directly involved in the transmission of pain. Additionally, substance P, bradykinin, and met-enkephalin all perform roles in the inflammatory and immunological response to tissue injury. To reiterate, bradykinin works synergistically with substance P to produce plasma extravasation and met-enkephalin helps stimulate leukocytic chemotaxis. Met-enkephalin also is released in the spinal dorsal horn and other central nervous system tissue to modulate substance P mediated nociceptive transmission. The ratio of substance P to met-enkephalin in the dorsal horn, other central nervous system tissue, and in the circulation may not only provide insight into the level of pain but its duration as well.

Looking at substance P levels in relation to those of bradykinin and met-enkephalin also may help to account for the effects of degradation in post-mortem tissues and fluids if the effects of degradation on peptide levels are reasonably relative. Enzymatic degradation of peptides would be expected to occur to some degree in post-mortem specimens; the extent of which will determine its usefulness in medicolegal investigation.

A. Endogenous Stability and Degradation of Substance P, Bradykinin, and Met-Enkephalin

Under non-disease and non-trauma conditions, various organs have been found to have the capacity to inactivate circulating substance P. Lembeck *et al.* have shown that the highest degree of substance P inactivation occurs in the liver followed by the kidney [126]. It has been shown experimentally that more than 80% of substance P infused into the portal vein is degraded by the liver [57]. *In vivo*, the kidney has been observed to completely metabolize systemically administered substance P, probably by both vascular and tubular elements [21].

Substance P is inactivated by proteolytic degradation in the brain, peripheral tissues, and blood [71]. This appears to be the mechanism of degradation of substance P following its release from nerve terminals.

By virtue of its structure, substance P is protected from enzymatic cleavage by exopeptidases [71]. Nerve and other tissue, however, contain numerous endopeptidases capable of degrading substance P, although most of these are intracellular and would not have access to substance P. Two endopeptidases which have been observed to degrade substance P are described by Blumberg *et al.* [16] and Lee *et al.* [90].

The first of these is a neutral endopeptidase called “post-proline cleaving enzyme” which cleaves substance P between Pro⁴ and Gln⁵ to yield a C-terminal heptapeptide and amino (N) terminal tetrapeptide fragments. This alone does not

inactivate substance P as the C-terminal heptapeptide fragment retains full biological activity. Further degradation by aminopeptidases is needed for inactivation.

The second of these is a neutral metalloendopeptidase called “substance P degrading enzyme.” This enzyme completely inactivates substance P by cleaving at three alternative sites in the region of phenylalanine residues at positions 7 and 8. This enzyme may represent the dominant mechanism of substance P degradation. Eison et al. found that a synthetic analogue of substance P, structurally protected from degradation by the substance P degrading enzyme, was resistant to metabolism when injected into rat brain in vivo causing prolonged behavioral excitation [38].

Substance P appears to have good post-mortem stability. Gale and Iverson et al. found no significant changes in substance P concentration in post-mortem human brain kept at 4°C between 4 and 66 hours after death [48].

Conversely, McGregor and Bloom found a 30% loss in immunoreactive substance P in mouse brain during post-mortem storage for 8 hours at both 4°C and 28°C [103]. During the first 2 hours, however, an increase in substance P was observed at 28°C but not at 4°C. At 4°C, a significant and rapid decrease in substance P concentration was observed in the first 2 hour period. McGregor and Bloom postulate that the increase at 28°C may be due to the generation of a more immunoreactive fragment of substance P, caused by enzymatic degradation, which is more apparent at higher temperatures. This would be consistent with the observation

that enzymatic activity is normally greater at 28°C than at 4°C. However, there is no evidence that such a fragment exists.

Alternatively, the rise in substance P may be caused by the production of substance P. post-mortem, from a previously produced post-translational precursor [103]. This may be occurring at the same time existing substance P is being enzymatically degraded. If production outweighs degradation, which would be expected at higher temperatures, then a net increase in substance P concentration would be observed.

McGregor and Bloom also found that no further decrease in substance P was noted from 8 to 48 hours post-mortem at both 4°C and 28°C [103]. This labile pool of substance P may be that which is present in cellular vesicular components and thus protected from enzymatic degradation.

Post-mortem rat brain stored frozen and then thawed prior to analysis was found to contain about half the substance P immunoreactivity as post-mortem samples analyzed frozen [103]. Apparently, thawing facilitates the release of degradative lysosomal enzymes. It appears that either immediate extraction or snap-freezing of freshly dissected tissue is best suited for accurate quantitative determination of endogenous substance P.

The post-mortem stability of met-enkephalin and bradykinin appears not to have been studied. It is known that met-enkephalin inactivation occurs rapidly in the

brain and in the blood through the enzymatic action of enkephalinase, a dipeptidyl carboxypeptidase [81]. Enkephalinase activity in the brain and blood varies with changes in opioid release, suggesting specificity. Met-enkephalin, as well as all opioids, are resistant to aminopeptidase cleavage [81].

Bradykinin has been reported to have a relatively long half-life (17 seconds) [138]. The vast majority of bradykinin degradation occurs through the hydrolysis of peptide bonds by enzymes in or near the vascular endothelium of the lungs [138]. The remainder is degraded by the serine proteinase kininase II in blood [141].

B. Clinical Manifestations

Before the results of the study can be properly interpreted to determine whether levels of substance P, bradykinin, and met-enkephalin can be used to gauge the level of pain prior to death, the effects of clinical disease on the levels of these peptides must be ascertained. Without this knowledge and subsequent value normalization, erroneous assumptions about the source of pain (disease or injury) can be made.

It has been reported that many clinical conditions affecting the brain and spinal cord cause changes in the level of substance P and met-enkephalin. Researchers have found that substance P and met-enkephalin are significantly decreased in the substantia nigra and globus pallidus of post-mortem brain specimens of individuals who have died of Huntington's chorea [154]. Decreased levels of substance P are

also observed in the spinal cord of individuals with Riley-Day syndrome [74]. Individuals with amputated limbs also demonstrate a loss of substance P in the area of the spinal cord corresponding to the amputated limb [68].

Conversely, Nishimura et al. have found that infants with brainstem or cerebellar infarctions that cause respiratory abnormalities have an increased amount of substance P immunoreactive fibers in and around the area of the infarction [116]. Similarly, Fukumizu et al. found that brainstems of children with aqueductal stenosis also display an increased amount of substance P immunoreactive fibers in the brainstem [45]. Furthermore, Milhorat found spinal cord levels of substance P to be higher in deceased individuals with syringomyelia, spinal cord injuries, and spinal cord tumors than in other deceased individuals [108].

Levels of substance P and met-enkephalin are increased in the medial globus pallidus of the brain in Parkinson's disease [33]. However, it has been reported that substance P levels in the basal ganglia of individuals afflicted with Parkinson's disease are greatly reduced [33].

Met-enkephalin immunoreactivity has been shown to be significantly greater in post-mortem hippocampi specimens of epileptic individuals than in non-epileptic controls [132].

Elevated levels of substance P and met-enkephalin have been observed in the cerebrospinal fluid of individuals who suffer from fibromyalgia [137] and painful late

stage malignancy [139].

Afflictions of peripheral nerves also seem to alter substance P levels. For instance, sympathectomy of the iris causes the amount of substance P in nerve endings of the iris to increase [144]. Furthermore, it has been reported that damaged optic nerves have significantly more substance P than normal optic nerves [98].

Substance P levels are also altered in diseased and injured tissue. Surgically removed tissue from individuals with inflammatory bowel disease have displayed high levels of substance P [98]. Similarly, inflamed respiratory tissue from asthmatics and those with chronic rhinitis also show levels of substance P that are significantly higher than levels in non-diseased respiratory tissue [105]. Psoriatic lesions of the skin also have been reported to contain a very high concentration of substance P [37].

Nicotine causes the concentration of met-enkephalin in peripheral tissues to rise [128]. Included among these tissues are the adrenal medulla, spleen, lung, salivary gland, vas deferens, heart, duodenum, and jejunum.

How any of these conditions affect peptide levels in blood is a matter of conjecture. Schiller et al. have reported that plasma substance P levels are elevated in individuals suffering from chronic diarrhea [142]. Similarly, Kuis et al. have found that rheumatoid arthritis and juvenile chronic arthritis cause substance P plasma levels to rise [105]. Conversely, plasma levels of substance P are lower than normal in patients with chronic back pain syndromes [124].

The effect of autonomic responses due to the fear of impending death or injury on nociceptive peptide levels also must be considered. It has been reported that individuals inflicted with panic disorder do not have elevated levels of substance P in blood [1].

Blood levels of bradykinin have been reported to be elevated in individuals who have susceptibility or have experienced anaphylactoid reactions [163]. Inflammation of the nasal and/or oropharyngeal cavity also causes levels of bradykinin to rise in blood [132]. Furthermore, bradykinin plasma levels are also particularly high in asthmatics [130]. Hypertension also appears to elevate bradykinin levels in blood leading to renal permeability disorders [129].

Malarial infections which activate the coagulation cascade cause bradykinin plasma levels to rise [30]. Similarly, increased bradykinin levels in blood seems to be a consequence of many bacterial infections [3].

Cancer also appears to create higher than normal bradykinin levels in blood. Fluid accumulation typically seen in pleural and ascitic carcinomatoses is largely due to activated bradykinin-generating systems [3]. Similarly, it has been found that bradykinin B2 receptors are expressed in cancerous prostate and endometrial tissue [29].

Plasma levels of met-enkephalin increase in liver disease [155]. Liver disease causes met-enkephalin to be released from sympathetic nerves into the blood.

XI. Pain, Homicide, and the Law

The biochemical measurement of pain in homicide victims has several legal implications. First, such a quantitative measurements may help juries determine the amount of punitive damages awarded to a homicide victim's family for the pain and suffering endured by the victim prior to death.

Sixteen states have explicit case law allowing for the civil appropriation of punitive damages [109]. For example, the Alaska Supreme Court held in *Tommy's Elbow Room, Inc. v. Kavorkian* that punitive damages may be awarded "when there is clear evidence that the wrongdoer acted maliciously, fraudulently, or with a wanton disregard for the decedent's safety" [160]. In *Gavica v. Hanson*, the Idaho Supreme Court rejected as "illogical the argument that punitive damages for pain and suffering should be available when a victim is injured, but denied when the victim is killed" [51]. Furthermore, the New Jersey Supreme Court extended the Executors and Administrators Act to allow a homicide victim's estate to seek punitive damages for pain and suffering prior to death in *Kern v. Hogan* [80].

Several state supreme courts, however, have ruled that the granting of punitive damages for wrongful death through case law is not permissible. The case against punitive damages in wrongful death actions was addressed by the Indiana Supreme Court in *Andis v. Hawkins*. The Andis Court held that "actions for wrongful death

were not allowed at common law, but are of statutory origin. Such statutes being in derogation of common law must be strictly construed and only those damages specified in the statutes may be recovered. Since the wrongful death statutes are intended to compensate the decedent's family only for the pecuniary loss sustained by reason of death, punitive damages being in excess of such compensation are not recoverable." Furthermore, the Indiana Court reasoned that the "purpose of wrongful death statutes is not to compensate for injury to the decedent or to punish someone who has caused a wrongful death. Rather, the purpose is to provide a means whereby the decedent's spouse or dependents may be compensated for the loss which they have sustained by reason of the death" [4].

Punitive damages for pain and suffering are allowed in several states under wrongful death and survival statutes. These statutes often differ in the scope and magnitude of the type and amount of damages that can be awarded. The Ohio survival statute allows for recovery of damages for pain and suffering to the decedent's estate but makes it clear that the plaintiff's family must prove the victim's pain and suffering prior to death [118]. Under current technology, this is often not easy to prove. A similar statute in Kansas does not allow for punitive damages, in part, because of difficulty in quantifying pain and suffering [79].

The disparity in the awarding of punitive damages in wrongful death actions has also been observed in the federal courts. In 1977, the United States District Court

of the Central District of California ruled that a California statute denying punitive damages in wrongful death actions violated the equal protection clause of the United States and California Constitutions [122]. The Ninth Circuit, however, overruled the District Court stating that the California statute was constitutional [123].

Technologies which can biochemically measure the amount of pain a homicide victim suffers just prior to death may help lessen disparities in punitive damage claims in homicide cases from court to court and state to state. Perhaps such technologies may provide the impetus for the standardization of laws and statutes governing wrongful death action.

Secondly, the determination of the amount of pain a homicide victim suffered prior to death may be helpful in the consideration of aggravating circumstances during sentencing. For instance, in the New Jersey statute governing sentencing, harsher sentences may be imposed if the offense was committed in a heinous, cruel, or depraved manner [113]. Additionally, the New Jersey statute governing homicide states that aggravating circumstances exist if the murder was committed in a vile, horrible, or inhumane manner [112]. It stands to reason that the quantitation of pain will be influential in determining whether the homicide was committed in such a manner.

However, the United States Supreme Court ruled that an Oklahoma statute using language such as "heinous, atrocious, and cruel" was too vague to offer

sufficient guidance to a jury when assessing aggravating circumstances in a homicide and therefore violated the Eighth Amendment to the Federal Constitution [102]. This decision upheld an earlier one by the Court which found a similar Georgia statute unconstitutional because of language such as “outrageously or wantonly vile, horrible, or inhumane” [53]. The existence of a quantitative pain scale may offer an objective criteria for determining the cruel nature of a homicide and make such vague statutory language unnecessary.

New Jersey Courts have directly dealt with the issue of pain of the homicide victim in assessing aggravating factors in sentencing. In *State v. Moore*, the New Jersey Supreme Court ruled that the intention to inflict pain on a homicide victim before death can be used as an aggravating factor in sentencing [151]. Additionally, the New Jersey Court has also ruled in two separate cases that physical pain can be used as a factor in imposing the death penalty on individuals convicted of first-degree murder [150, 152].

Finally, a measurement of acute pain prior to homicidal death may be helpful in determining the degree of homicide a perpetrator should be charged with. For instance, the New Jersey Code of Criminal Justice states that in order to charge an individual with first-degree felony murder in the State of New Jersey, several criteria need to be fulfilled [112]. Among these criteria are the intent to kill, the intent to inflict serious bodily injury, and malice. The level of inflicted pain on the victim.

although not currently statutorily mandated, may be useful in satisfying these criteria.

XII. Case Studies

The evaluation of substance P, bradykinin, and met-enkephalin as a quantitative and qualitative marker for pain just prior to death will be accomplished by examining the levels of these peptides in the blood and brain of deceased individuals who have died from confirmed manners of death. Studies conducted in both blood and brain tissue will be performed in an attempt to accurately establish when peptide levels begin to deviate from normal after violent assault or clinical incident (e.g. heart attack). Since nociception begins in the peripheral nervous system and is processed centrally, it is expected that peptide levels will change in brain before they will in the general circulation. Having values in both blood and brain tissue may allow for the creation of a time line showing where pain begins in the interval between incident and death for different types of death.

Since blood is more accessible than brain to laboratory personnel (autopsy blood is routinely sent to forensic biology laboratories), initial studies will be performed in serum. If informative data are observed in blood serum and/or serum data need clarification, subsequent studies will be conducted in both brain tissue and serum. Brain tissue will be sampled from the periaqueductal gray region of the

midbrain. This is the region of choice because it is the only region of the central nervous system that collectively has measurable quantities of substance P, bradykinin, and met-enkephalin containing fibers.

Before the effect of various types of traumatic and clinical deaths can be ascertained, the role that death itself plays in nociceptive peptide levels must be determined. To investigate this, serum peptide levels of several living subjects in varying degrees of health are compared with serum peptide levels from deceased individuals who, at best it can be ascertained, died with little or no pain (e.g. instantaneous deaths with no prior clinical manifestations). The results from these two groups will then be compared for statistical significance.

Once the role that death plays on peptide levels has been established, an assessment is made of the effect of both chronic and acute pain on peptide levels in deceased individuals. Since the mechanisms of chronic and acute pain are different, it is likely that peptide concentrations and ratios are different in the two states. Determining these differences may help to differentiate between cases where the interval between incident and death is long (e.g. weeks, months) and cases where the interval is short (e.g. hours, days).

In determining the range of peptide values for acutely and chronically painful death, a comparison of peptide values between various types of violent death will be undertaken. Test cases will have a range of time intervals between incident and death.

The time interval where acute pain turns into chronic pain can then be determined experimentally if peptide levels are disparate in the two pain states.

Violent deaths will be classified into homicides, suicides, and accidents. Each classification will include various modes of death. Homicides will include stabbing, gunshot, strangulation, and blunt trauma deaths. Suicides will include stabbing, gunshot, drug overdose, hanging, blunt trauma, and fire deaths. Accidents will be broken down into drug overdose, blunt trauma, asphyxiation, drowning, smoke inhalation, hanging, fire, and electrocution deaths. The presence of the same mode of death in two classifications of death is unfortunate but unavoidable. Although the values between an individual who drowns by accident or on purpose are not expected to differ according to data previously cited [1], it may give some insight into the effect, if any, psychological factors have on peptide levels. If it is assumed that factors such as depression and mental illness play a role in suicidal death and not in accidental death, it may be best to separate suicidal from homicidal and accidental deaths of the same mode. Tests of statistical significance will be conducted on test samples with different time intervals between incident and death.

The results of violent death will be compared with peptide levels from individuals who die from non-violent or clinical means. Several non-violent or clinical deaths are investigated. These will be classified into death by cardiovascular disease, embolism, hepatic disease, malignancy, AIDS, non-AIDS infectious disease,

gastrointestinal disease, renal disease, diabetes mellitus and other diseases of the pancreas, respiratory disease, disorders of the central nervous system (primarily the brain), immunological and hematological diseases, and non-cerebral hemorrhage. Comparisons of each non-violent death will be made with both instantaneous violent death and violent death with a long incident-death time interval using statistical tests for significance. Using the premise that all non-violent deaths must exhibit some level of pain, such comparisons may help gauge the level of pain (none, acute, or chronic) exhibited in violent death.

The results from the different classifications of non-violent death will be used to give objective meaning to peptide levels in examples of violent death. If, for instance, the pain experienced by a homicide victim can be equated with pain suffered by an individual who died of AIDS, cardiovascular disease, or cancer, a tangible meaning may be attached to the pain of the homicide victim. This certainly will be of value to juries and judges in assessing the pain suffered by an accident or homicide victim. If peptide levels differ substantially in several of the non-violent or clinical deaths, then a pain scale may be constructed to which pain from violent deaths may be compared.

Knowing the expected range of values of peptide concentration for tested diseases may also help to control for chronically painful conditions in individuals who die from traumatic or violent means (especially in cases where peptide values which

reflect acute pain are expected).

Infant and child deaths will be treated separately. This group, loosely defined, will include all cases where the victim is under 13 years of age. Since the central nervous system may be immature and not completely developed in infants and children [18, 172], the release of nociceptive peptides under similar conditions may differ in comparison with adults. Thus, mixing data from adult subjects with that from infants and children may not be scientifically valid. Additionally, the existence of causes of death specific to infants (SIDS, complications due to prematurity) renders separate categorization necessary. Furthermore, blunt trauma homicides in children and infants are often distinguished from adult blunt trauma homicides by being designated by medical examiners as fatal child abuse syndrome. Very often, factors such as malnutrition and psychological abuse play a role in fatal child abuse syndrome which do not in adult blunt trauma homicides. Comparisons between adult and infant-child violent deaths will be made using statistical tests for significance for similar incident-death intervals.

Peptide levels in clinical or non-violent infant and child deaths due to such causes as SIDS, infections, and other clinical manifestations such as hypoglycemia and intestinal obstruction are compared to peptide levels in violent death. In addition to cases of child abuse syndrome, infant and child deaths caused by choking on food will be studied and grouped in the violent death category as well.

Special attention is paid to SIDS cases to see if particular cases show deviations from apparent normative peptide levels. Since SIDS is typically hard to diagnose (often determined after all other possible causes of death are eliminated), the possibility always exists that death occurred as a result of an unknown occult cause. Deviations from normative SIDS peptide levels in a suspected SIDS case may point to another cause of death. This may be helpful, for instance, in cases where the possibility of child abuse is considered but no physical manifestation exists. Peptide values in SIDS deaths will be compared using statistical tests for significance with both clinical and violent infant-child deaths.

The total number of all cases that will be examined for peptide levels in blood is five hundred and two, one hundred and thirty nine of which will include studies in brain tissue.

XIII. Materials and Methods

A. Extraction of Peptides from Blood

Blood samples are collected and prepared for extraction using the following procedure [131]:

1. Blood is collected from autopsy subjects and placed in a 10ml evacuated glass tube

containing EDTA (1.5mg/ml blood) as a coagulant and stored in a refrigerator (2-5°C).

2. Within 24 hours the blood is centrifuged at 1,600 x g for 15 minutes at 4°C.

3. Remove 1ml of serum and place in a 1.5ml plastic microcentrifuge tube containing Aprotinin (500 KIU/ml serum; Sigma, catalog number A-6279). Aprotinin serves as a protease inhibitor.

4. Freeze the samples at -15°C or lower if not extracted immediately.

Prepared blood samples are extracted using the following procedure [131]:

1. Equilibrate a SEP-Column containing 200mg of C18 (Peninsula Laboratories, catalog number RIK-SEPCOL-1) by first washing with 1ml of 60% HPLC grade acetonitrile (Fisher, catalog number A998^{SK}-8) in 0.1% HPLC grade trifluoroacetic acid (JTBaker, catalog number 9470-02). The wash solution (solution A) is added by using pressure from a 10cc syringe (Becton-Dickinson, catalog number 9604) attached to the open end of the column. Pressure should be applied that will allow the wash solution to elute through the column in a dropwise fashion. The column is then washed three times with 3ml of 0.1% HPLC grade trifluoroacetic acid in water

(solution B). Solution B is washed through the column without the use of additional pressure from a syringe.

2. Defrost the 1ml serum sample, if necessary, and mix with 2ml of buffer A in a 15ml plastic conical tube (Falcon, catalog number 2097). Centrifuge at 6,000 x g for 20 minutes at 4°C.

3. Remove the supernatant and place on column. Allow plasma solution to elute through column without pressure. During elution, the columns are to be kept at 4°C.

4. Discard eluant and add 3ml of solution B.

5. Discard eluant and again add 3ml of solution B.

6. Discard eluant and add 3ml of solution A. Collect the eluant in a 15ml plastic conical tube. This tube contains the extracted peptides.

7. Evaporate the eluant to dryness using a centrifuge lyophilizer (Savant Speed Vac, catalog number SVC100).

8. Dissolve the lyophilized sample in 300ul of RIA buffer (Advanced Chemtech, catalog number JRK0001). This quantity is sufficient for 3 assays.

9. Store the reconstituted specimens at -15°C until assay.

B. Extraction of Peptides from Brain Tissue

Brain tissue is extracted using the following procedure [167]:

1. Approximately 1g of brain tissue is removed from autopsy subjects, rinsed in saline and boiled for 10 minutes in 5ml of water before cooling on ice. Tissue is removed from the periaqueductal gray region of the midbrain.

2. Following the addition of 0.5ml of 10N acetic acid (Fisher, catalog number A38-212), containing 0.2N hydrochloric acid (Fischer, catalog A144^C-212) and 1% beta mercaptoethanol (Sigma, catalog number M6250), homogenize the tissue with a tissue grinder (Wheaton, 357544).

3. Centrifuge the homogenized tissue at 20,000 x g for 20 minutes at 4°C.

4. Evaporate the supernatant using a centrifuge lyophilizer.

5. Dissolve the lyophilized sample in 300ul RIA buffer. This quantity is good for 3 assays.

6. Store the reconstituted specimens at -15°C until assay.

C. Radioimmunoassay (RIA)

Assays for substance P, bradykinin, and met-enkephalin are performed using commercially prepared radioimmunoassay kits (Advanced Chemtech. catalog numbers JRR4269, JRR1437, and JRR2279). The procedure is as follows [131]:

1. Allow frozen lyophilized specimens to thaw completely on ice.
2. Prepare dilutions of known peptide for standard curve in 1.5ml plastic microcentrifuge tubes. Standards of 100pg/100ul, 50pg/100ul, 25pg/100ul, 12.5pg/100ul, 6.25pg/100ul, 3.13pg/100ul, 1.56pg/100ul, and 0.16pg/100ul are prepared.
3. Set up duplicate RIA reactions that will determine total counts and non-specific binding by pipetting 200ul RIA buffer into reaction tubes (all RIA reactions are done in 1.5ml plastic microcentrifuge tubes).

4. Set up duplicate RIA reaction that will determine total binding by pipetting 100ul RIA buffer into a reaction tube.
5. Pipette 100ul of each standard into respective reaction tubes in duplicate.
6. Pipette 100ul of samples to be assayed into respective reaction tubes in duplicate.
7. Pipette 100ul of antiserum containing polyclonal antibodies of the peptide to be assayed into respective reaction tubes and vortex. Incubate tubes for 4-6 hours at room temperature.
8. Determine the amount of ^{125}I labeled tracer needed to complete the assay. This is done as follows:
 - A. Remove 5ul of the labeled tracer and place in a poly-Q vial (Beckman, catalog number 566740) containing 3 ml of liquid scintillation cocktail (Beckman, catalog number 586605) and counted for one minute using a liquid scintillation counter (Beckman, model number LS 6000SE). Before use the liquid scintillation counter is calibrated using a C^{14} standard (Beckman, catalog number 44439).

B. From the number of counts per minute (CPM), determine the volume of tracer needed to produce between 5,000 and 10,000 CPM (manufacturer recommendation; above 10,000 CPM stray radiation becomes problematic).

For instance, if a 5ul sample produces 2,500 CPM, then by simple multiplication (using a factor of 3), 15ul would produce 7,500 CPM.

C. Dilute the calculated amount of tracer with RIA buffer to 100ul. This is the quantity that needs to be added to all controls and test samples. Count the solution for one minute to make sure the calculation is correct.

9. Prepare enough diluted tracer to add 100ul to all reaction tubes. Add 100ul of the diluted tracer to all reaction tubes and incubate for 16-24 hours at 2-8°C.

10. Add 100ul of goat anti-rabbit IgG serum and 100ul of normal rabbit serum to each reaction tube.

11. Centrifuge all reaction tubes at 1,700 x g for 20 minutes at 4°C.

12. Remove the supernatant from all reaction tubes. Place the tube containing the precipitate into a poly-Q vial containing 3ml liquid scintillation cocktail and count for one minute in the liquid scintillation counter. Mean CPM values from each sample

are used for calculation. Samples with discrepant duplicate CPM results will not be used in the study.

D. Preparation of Standard Curve and Data Calculation

1. Calculate the maximum binding (B_o) by subtracting the number of CPM of the non-specific binding tube from the number of counts from the total binding tube.
2. Subtract the number of CPM for the non-specific binding tube from the number of CPM from all standards and test samples to obtain the corrected count.
3. Calculate the $\%B/B_o$ for each data point in the standard curve and in test samples by dividing the corrected count for each sample by B_o .
4. Prepare a standard curve by plotting the log value of each standard concentration on the x-axis against the corresponding $\%B/B_o$ value on the y-axis.
5. Plot the best fit straight line by using the linear least squares method [6] via a computer program.
6. Calculate x (log concentration) for each sample by using the equation $-y+b=mx$

(where $y = \%B/B_0$ for each sample, $b = y$ intercept of the standard curve, and $m =$ slope of the curve).

7. Take the antilog for each value of x . Multiply this value by 3 (samples are reconstituted with 300ul of RIA buffer, 100ul are used for each assay) to obtain the correct concentration per ml of serum or gram of brain tissue.

E. Statistical Evaluation of Data

Values for mean and standard deviations for pooled data are determined. Q testing is not conducted for outlying results because to do so would introduce bias into the data. Alternatively, median results will be given for pooled data to indicate the presence of outlying results in mean data. The problematic nature of introducing bias is typically offset by the risk of retaining results that are spurious and have an inordinate effect on the mean and standard deviation of the data. This would certainly be the case. for example, when averaging a series of readings determining the amount of titanium in a paint chip. However, the analogy cannot be applied in this study. Since no two deaths or two decedents are exactly the same, the possibility of controlling for the seemingly endless array of parameters is remote. Thus, it would be difficult to pinpoint "spurious" data. One possible consequence of this study is the production of ranges of peptide values expected for deaths with similar etiology and

time intervals between incident and death. If this could be determined experimentally then perhaps Q testing would be appropriate.

Testing of experimental means for significant difference will be performed by t testing at the 95% confidence interval [144]. If the observed difference between 2 means ($\bar{x}_1 - \bar{x}_2$) is greater than the computed value (equation 1), then the two means are significantly different.

$$\text{equation 1: } ts \pm \sqrt{\frac{N_1 + N_2}{N_1 N_2}}$$

where.

t = value of t at 95% confidence interval for specified degrees of freedom (degrees of freedom are defined as $N_1 + N_2 - 2$)

s = pooled standard deviation for both sets of pooled data

N_1 = number of samples in 1st group of pooled data

N_2 = number of samples in 2nd group of pooled data

XIV. Hypotheses

Several hypotheses will be offered and listed here:

1. Nociception transmission and modulation are associated with all death. Peptide levels in subjects who die instantaneously from violent traumatic causes will be higher and statistically different than peptide levels in living healthy subjects.

2. Peptide levels in serum will differ in acutely and chronically painful death. The longer the illness or the duration between traumatic incident and death (weeks to months), the higher the level of met-enkephalin and the lower the level of substance P and bradykinin. Conversely, in deaths where illness and the duration between traumatic incident and death is short (hours to days), the higher the level of substance P and bradykinin and the lower the met-enkephalin. Diagram V (Appendix A) gives a theoretical presentation of peptide levels in serum for various durations of pain. Since bradykinin and substance P play integral roles in the initial process of nociception, it is expected that the levels of these peptides will be higher than levels of met-enkephalin in cases of acutely painful death. Conversely, as acute pain turns into chronic pain, it is expected that met-enkephalin levels will become higher than levels of both substance P and bradykinin. Met-enkephalin is released in nerve tissue to inhibit the release of substance P and to modulate its effects. Similarly, the level of bradykinin should also be smaller in chronically painful deaths because its role in nociception is limited to the acute activation of peripheral nerve endings as a result of painful stimuli. Peptide levels in subjects who die because of painful chronic conditions will be similar to and not statistically different from those subjects who die violently and where the interval between incident and death is large. Levels for each peptide will be statistically different in deaths associated with acute and chronic pain.

3. Since bradykinin is nociceptive peripherally and antinociceptive centrally, levels of this peptide will be different in brain tissue and serum in both pre-mortem acute and chronic pain. In cases of chronically painful death, bradykinin levels will be higher in brain tissue than in serum. The converse should be true of acutely painful death. Unlike bradykinin levels, substance P and met-enkephalin levels in brain tissue should always reflect those in serum. Diagrams VI (Appendix A) gives a theoretical presentation of peptide levels for various durations of pain in brain tissue.

4. Medical conditions which effect peptide levels but which are not the cause of death may cause peptide levels in both the serum and brain tissue to be more reflective of the medical condition.

5. Changes in substance P and met-enkephalin peptide levels due to nociceptive activation and modulation will be observed in brain tissue before they are observed in serum. Substance P serum levels will usually not be reflective of acutely painful death where the duration between incident and death is very small (e.g. minutes). Expected substance P levels will, however, be observed in brain tissue.

6. Statistical differences due to psychological factors will not be seen in peptide levels between subjects who die traumatically from suicides, accidents, and homicides

where the duration between incident and death is similar.

7. Peptide levels and thus nociceptive and antinociceptive response will differ in adult violent death according to mode (i.e. gunshot, stabbing) because the degree of tissue damage typical of each mode will vary.

8. Peptide levels in violent infant and child deaths will mirror similar adult deaths.

9. Peptide levels in SIDS deaths will be statistically different than infant deaths caused by child abuse syndrome and other traumatic means.

10. Peptide levels in various non-violent deaths will differ due to the nature (acute and chronic) and extent of pain in each particular non-violent death.

XV. Results

Peptide levels are determined in pg/ml in serum and pg/g in midbrain tissue. Case summaries complete with peptide levels and causes of death with significant clinical manifestations are summarized in Appendix B.

XVI. Discussion of Results

A. Peptide Serum Levels in Living Adult Subjects with Varying Levels of Pain

The levels of peptides in serum of living subjects differ according to whether the subjects exhibit no pain, acute pain, or chronic pain. Mean and median values for substance P, bradykinin, and met-enkephalin serum levels in living subjects exhibiting varying levels of pain are tabulated in Tables 1a, 1b, and 1c. Peptide levels in the three states reflect expected values.

The subject with acute pain (77 year old man with acute pancreatitis) has a substance P serum level that is higher than the mean and median values for adults with either no pain (healthy adults having no known physical ailments) or chronic pain (subjects with a history of long term painful conditions such as arthritis, back injury, scoliosis, and spinal cord trauma). The substance P serum level in the acute pain subject is 2.7 times the mean value in the healthy adult subjects and 4.8 times the mean value in chronic pain subjects. Median values are similarly reflected. It is not surprising that the individual with acute pain has the highest level of substance P, given it's role in nociceptive transmission. Similarly, it is expected that the chronic pain group would show the lowest levels of substance P. As painful conditions become chronic, pain modulation processes become dominant which is best

Table 1a -Substance P Serum Levels In Living Adult Subjects With Varying Levels Of Pain

Level Of Pain	N	Mean	Median	Standard Deviation
Healthy Adults (No Pain)	10	24.59	24.90	9.98
Chronic Pain	4	13.87	12.75	9.81
Acute Pain	1	66.80	66.80	NA

Table 1b - Bradykinin Serum Levels In Living Adult Subjects With Varying Levels Of Pain

Level Of Pain	N	Mean	Median	Standard Deviation
Healthy Adults (No Pain)	10	19.35	21.05	7.35
Chronic Pain	4	36.82	38.00	13.01
Acute Pain	1	31.80	31.80	NA

Table 1c - Met-Enkephalin Serum Levels In Living Adult Subjects With Varying Levels Of Pain

Level Of Pain	N	Mean	Median	Standard Deviation
Healthy Adults (No Pain)	10	13.10	4.55	25.17
Chronic Pain	4	116.18	126.40	56.27
Acute Pain	1	1.20	1.20	NA

Mean and median levels are in pg/ml serum.

demonstrated by the inhibition of substance P release by met-enkephalin. Therefore, the high levels of met-enkephalin shown in the chronic pain group as compared with the other two groups is not surprising. Mean values of met-enkephalin in the chronic pain group are 8.9 times higher than the healthy group and 96.8 times higher than the acute pain group. Median values are similarly reflected. There is no apparent explanation for why met-enkephalin serum levels are higher in the no pain subjects as compared with the acute pain subject and may reflect the presence of a disease state in the acute pain subject that causes diminished met-enkephalin levels. Perhaps if more subjects were available, both the mean and median values would approach the values for healthy subjects.

Bradykinin serum levels in both the acute pain subject and the chronic pain group are higher than the healthy subjects. The value for the acute pain subject is 1.6 times higher than the mean value for the healthy subjects, while the mean value for the chronic pain group is 1.9 times greater. Higher bradykinin serum values in the acute pain subject is reflective of its synergistic role with substance P peripherally in nociception and tissue regeneration, while higher bradykinin levels in the chronic pain group is reflective of its antinociceptive role in the central nervous system.

B. Peptide Levels in Violent Deaths Having Varying Incident-Death Time Intervals

1. Serum study

The levels of substance P, bradykinin, and met-enkephalin changes as the incident-death time interval increases for all combined violent deaths. As stated in the foregoing, violent deaths are defined as any mode of death causing accidental, suicidal, or homicidal death. As the incident-death time interval increases, the mechanisms of acute pain diminish while the mechanisms of chronic pain become dominant as evident by changing peptide levels. Mean and median substance P, bradykinin, and met-enkephalin peptide serum levels in all violent deaths having varying incident-death time intervals are tabulated, respectively, in Tables 2a, 2b, and 2c. With the exception of no time interval between incident and death, incident-death time interval bins were arbitrarily defined by sample size. Efforts were made to keep sample size approximately the same in each bin. An assumption is made that all samples in a given bin experience the same mechanism of pain.

Substance P levels increase as the time interval between incident and death increases. Comparisons of mean substance P levels between time interval bins using the t-test for significance gives some insight into the changing mechanisms of pain (Table 2d). No significant difference was observed between 0 time and 2-15 minutes. However, a significant difference was observed between 0 time and 20-50 minutes as substance P levels rose by a factor of 1.6 (from 45.09 pg/ml to 70.86 pg/ml). Such

a difference may indicate that the activation of nociceptive processes are noticeable in serum at this time. In short, the mechanisms of acute pain are recognizable in serum 20-50 minutes after the violent incident. The concentration of substance P continues to increase until the last bin where it diminishes considerably. The concentration of substance P decreases by a factor of 2.5 (134.44 pg/ml to 54.59 pg/ml) from 8 days-3 weeks to 1-8 months. A significant difference using t-testing was observed between the 2 bins. This difference indicates that the processes of nociceptive modulation are observable in serum in the last bin. Thus, chronic pain mechanisms are dominant.

Table 2a - Substance P Serum Levels In All Violent Deaths

Incident-Death Time Interval	N	Mean	Median	Standard Deviation
0	83	45.09	30.70	37.96
2-15 minutes	17	32.43	18.30	25.07
20-50 minutes	13	70.86	62.50	27.53
1-4 hours	14	78.88	72.50	49.55
4.5-21 hours	14	90.54	88.25	56.50
1-3 days	12	108.22	102.10	64.73
3.5-7 days	9	114.12	108.90	61.15
8 days-3 weeks	11	134.44	114.30	72.90
1-8 months	9	54.59	70.10	44.16

Mean and median levels are in pg/ml serum.

Table 2b - Bradykinin Serum Levels In All Violent Deaths

Incident-Death Time Interval	N	Mean	Median	Standard Deviation
0	83	48.79	34.10	44.67
2-15 minutes	17	40.14	28.70	35.46
20-50 minutes	13	71.51	71.90	41.79
1-4 hours	14	85.64	57.00	68.93
4.5-21 hours	14	101.42	98.20	61.94
1-3 days	12	96.35	98.50	65.25
3.5-7 days	9	119.48	66.60	94.80
8 days-3 weeks	11	114.29	113.10	76.70
1-8 months	9	54.20	52.10	41.99

Table 2c -Met-Enkephalin Serum Levels In All Violent Deaths

Incident-Death Time Interval	N	Mean	Median	Standard Deviation
0	83	24.44	18.50	24.21
2-15 minutes	17	13.52	7.70	15.08
20-50 minutes	13	33.38	36.30	15.34
1-4 hours	14	27.20	19.55	25.41
4.5-21 hours	14	26.64	25.35	24.33
1-3 days	12	31.16	11.55	37.67
3.5-7 days	9	34.53	27.40	30.52
8 days-3 weeks	11	79.58	56.60	90.18
1-8 months	9	134.63	98.40	105.99

Mean and median levels are in pg/ml serum.

Bradykinin serum concentration mimics substance P levels. The concentration of bradykinin increases up to and through the 8 days-3 week bin. The first significant difference based on t testing (Table 2e) exists between the 0 time bin and the 1-4 hour time bin although increases similar to substance P are found at the 20-50 minute time interval. Since most violent death is associated with tissue damage, the increase in bradykinin may also signal the start of tissue regeneration. Also, similar to substance P, bradykinin diminishes significantly from the 8 days-3 week bin to the 1-8 month bin by a factor of 2.1 (from 114.29 pg/ml to 54.20 pg/ml). Like substance P, this difference indicates that the processes of chronic pain and nociceptive modulation are observable in serum in the last bin. Furthermore, such decrease may indicate that the initial steps of tissue repair and regeneration are slowing.

Met-enkephalin serum levels do not increase to any appreciable degree until the 8 days-3 week time interval. However, the increase is not significant (Table 2f) when compared with the 1-4 hour time interval (the interval where substance P and bradykinin levels are indicative of acute pain) indicating that the processes of nociceptive modulation and chronic pain are not yet dominant. Conversely, pain modulation and chronic pain do become dominant in the 1-8 month time interval since a significant difference exists between this bin and the 1-4 hour time interval.

Table 2d - t Testing Comparisons Of Mean Substance P Serum Levels At Various Incident-Death Time Intervals In All Violent Deaths

Incident-Death Time Interval 1	Incident-Death Time Interval 2	$x_1 - x_2$	Degrees Of Freedom	Critical t Value At 95% Confidence Level	Calculated t Value	Type Of Difference
0	2-15 minutes	12.66	98	1.96	18.94	Not Significant
0	20-50 minutes	25.77	94	1.96	22.01	Significant
0	1-4 hours	33.79	95	1.96	23.39	Significant
1-4 hours	8 days - 3 weeks	55.56	23	1.96	52.00	Significant
1-4 hours	1-8 months	24.29	21	1.96	40.22	Not Significant
8 days - 3 weeks	1-8 months	79.85	18	1.96	64.01	Significant

Table 2c - t Testing Comparisons Of Mean Bradykinin Serum Levels At Various Incident-Death Time Intervals In All Violent Deaths

Incident-Death Time Interval 1	Incident-Death Time Interval 2	$x_1 - x_2$	Degrees Of Freedom	Critical t Value At 95% Confidence Level	Calculated t Value	Type Of Difference
0	2-15 minutes	8.65	98	1.96	22.54	Not Significant
0	20-50 minutes	22.72	94	1.96	25.90	Not Significant
0	1-4 hours	36.85	95	1.96	28.41	Significant
1-4 hours	8 days - 3 weeks	28.65	23	1.96	57.14	Not Significant
1-4 hours	1-8 months	31.44	21	1.96	50.91	Not Significant
8 days - 3 weeks	1-8 months	60.09	18	1.96	60.90	Not Significant

Table 2f - t Testing Comparisons Of Mean Met-Enkephalin Serum Levels At Various Incident-Death Time Intervals In All Violent Deaths

Incident-Death Time Interval 1	Incident-Death Time Interval 2	$x_1 - x_2$	Degrees Of Freedom	Critical t Value At 95% Confidence Level	Calculated t Value	Type Of Difference
0	2-15 minutes	10.92	98	1.96	12.19	Not Significant
0	20-50 minutes	8.94	94	1.96	13.60	Not Significant
0	1-4 hours	2.76	95	1.96	13.73	Not Significant
1-4 hours	8 days - 3 weeks	52.38	23	1.96	52.63	Not Significant
1-4 hours	1-8 months	80.58	21	1.96	71.75	Significant
8 days - 3 weeks	1-8 months	55.05	18	1.96	87.21	Not Significant

2. Serum/Brain study

The changing substance P concentration from subjects with varying incident-death time intervals in all violent deaths follows the same pattern whether substance P is measured in serum or midbrain (Table 2g). However, increases to substance P levels as a result of a violent episode appears to occur first in the midbrain. The initial increases of substance P seen in the brain tissue is the direct result of the activation of nociceptive pathways in both the peripheral and central nervous systems. Increases in serum substance P levels would only be seen through either spillage into the general circulation from nervous tissue or from inflammatory processes at the site of tissue injury creating an inevitable lag time in serum concentration.

At each time interval where acute pain mechanisms are thought to be dominant, the level of substance P is higher in midbrain tissue. Tests for significance between serum and midbrain levels for each time interval (Table 2h) show that for the first two intervals (0 and 15-40 minutes), a significant difference exists. This is consistent with acute pain processes in midbrain tissue during these time intervals. In contrast, the serum study shows that acute pain processes are not significantly noticeable in serum until 20-50 minutes after a violent incident.

Substance P levels diminish in both serum and midbrain tissue in the longest two time intervals (10-32 days and 3 months, 3 weeks) indicating that the mechanisms of chronic pain are beginning to become dominant. Although it is difficult to draw

any conclusion from one test sample, the larger concentration of substance P in serum (18.30 pg/ml as opposed to 3.0 pg/g in midbrain tissue) in the sample having a 3 month, 3 week incident-death time interval may be indicative of the initiation of nociceptive modulation in nervous tissue.

Like substance P, met-enkephalin midbrain levels are typically higher than in serum for various incident-death time intervals (Table 2i) . Although the data suggest that substance P release is inhibited at only the longer incident-time intervals and thus at the largest met-enkephalin concentrations, t testing comparisons of serum and midbrain levels (Table 2j) indicates that nociceptive modulating processes may be activated soon after violent incident. Significant differences in levels are observed at both the 0 and 1½ -4½ hour incident-death time interval.

Since bradykinin helps initiate nociception by helping to stimulate peripheral nerve endings and plays an antinociceptive role in the central nervous system, it is not surprising that bradykinin levels in both serum and midbrain tissue follow different patterns as the incident-death time interval increases (Table 2k).

Like met-enkephalin levels, bradykinin midbrain levels do not increase to any large degree until the larger incident-death time intervals are reached (10-32 days and 3 months, 3 weeks) suggesting its antinociceptive role.

Conversely, serum bradykinin levels begin to increase in the early incident-death time intervals (for instance, levels increase from 67.09 pg/ml at 0 time to

Table 2g - Substance P Serum And Midbrain Levels In All Violent Deaths

Incident-Death Time Interval	N	Midbrain			Serum		
		Mean	Median	Standard Deviation	Mean	Median	Standard Deviation
0	28	105.25	88.15	73.27	49.70	42.55	31.89
15-40 minutes	10	136.65	128.80	46.11	62.31	59.45	25.41
1½-4½ hours	4	152.68	157.05	88.71	72.45	67.40	35.53
5½-10 hours	3	200.30	186.70	24.60	104.63	135.90	68.94
1 day-5 days, 18 hours	4	208.08	209.50	140.74	148.75	148.30	80.92
10-32 days	2	100.65	100.65	13.36	87.90	87.90	30.55
3 months, 3 weeks	1	3.0	3.0	NA	18.30	18.30	NA

NA = Not Applicable

Mean and median levels are in pg/ml serum or pg/g brain tissue.

Table 2h - t Testing Comparisons Of Mean Substance P Serum And Midbrain Levels At Various Incident-Death Time Intervals In All Violent Deaths

Incident-Death Time Interval	$x_1 - x_2$	Degrees Of Freedom	Critical t Value At 95% Confidence Level	Calculated t Value	Type Of Difference
0	55.55	54	1.96	32.80	Significant
15-40 minutes	74.34	18	1.96	46.11	Significant
1½-4½ hours	80.23	6	2.45	131.40	Not Significant
5½-10 hours	95.67	4	2.78	158.71	Not Significant
1 day-5 days, 18 hours	59.33	6	2.45	192.14	Not Significant
10-32 days	15.30	2	4.30	88.62	Not Significant

Table 2i - Met-Enkephalin Serum And Midbrain Levels In All Violent Deaths

Incident-Death Time Interval	N	Midbrain			Serum		
		Mean	Median	Standard Deviation	Mean	Median	Standard Deviation
0	28	49.50	51.95	29.13	28.39	22.95	18.37
15-40 minutes	10	36.85	28.45	17.11	35.75	38.25	14.69
1½-4½ hours	4	47.68	49.00	5.13	19.23	19.55	5.41
5½-10 hours	3	48.97	48.30	4.34	20.87	25.50	9.54
1 day-5 days, 18 hours	4	70.45	33.25	78.01	22.13	20.80	14.51
10-32 days	2	118.80	118.80	49.50	122.50	122.50	96.87
3 months, 3 weeks	1	236.60	236.60	NA	171.40	171.40	NA

NA = Not Applicable

Mean and median levels are in pg/ml serum or pg/g brain tissue.

Table 2j - t Testing Comparisons Of Mean Met-Enkephalin Serum And Midbrain Levels At Various Incident-Death Time Intervals In All Violent Deaths

Incident-Death Time Interval	$\bar{x}_1 - \bar{x}_2$	Degrees Of Freedom	Critical t Value At 95% Confidence Level	Calculated t Value	Type Of Difference
0	21.11	54	1.96	13.81	Significant
15-40 minutes	1.10	18	1.96	13.61	Not Significant
1½-4½ hours	28.45	6	2.45	27.67	Significant
5½-10 hours	28.10	4	2.78	38.04	Not Significant
1 day-5 days, 18 hours	48.32	6	2.45	100.50	Not Significant
10-32 days	3.70	2	4.30	270.21	Not Significant

Table 2k - Bradykinin Serum And Midbrain Levels In All Violent Deaths

Incident-Death Time Interval	N	Midbrain			Serum		
		Mean	Median	Standard Deviation	Mean	Median	Standard Deviation
0	28	56.37	57.55	29.91	67.09	48.65	56.43
15-40 minutes	10	40.28	41.25	13.63	78.05	72.60	43.20
1½-4½ hours	4	37.00	37.35	10.24	110.53	106.50	83.34
5½-10 hours	3	34.63	36.90	4.37	101.00	99.60	56.91
1 day-5 days, 18 hours	4	78.03	49.95	88.61	113.00	119.25	43.59
10-32 days	2	145.85	145.85	58.76	63.60	63.60	11.03
3 months, 3 weeks	1	202.10	202.10	NA	122.20	122.20	NA

NA = Not Applicable

Mean and median levels are in pg/ml serum or pg/g brain tissue.

Table 21- t Testing Comparisons Of Mean Bradykinin Serum And Midbrain Levels At Various Incident-Death Time Intervals In All Violent Deaths

Incident-Death Time Interval	$x_1 - x_2$	Degrees Of Freedom	Critical t Value At 95% Confidence Level	Calculated t Value	Type Of Difference
0	10.72	54	1.96	23.61	Not Significant
15-40 minutes	37.77	18	1.96	32.17	Significant
1½-4½ hours	73.53	6	2.45	117.06	Not Significant
5½-10 hours	66.37	4	2.78	116.28	Not Significant
1 day-5 days, 18 hours	34.97	6	2.45	116.57	Not Significant
10-32 days	82.25	2	4.30	252.45	Not Significant

110.53 pg/ml at 1½ -4½ hours). This increase is consistent with both its peripheral role in nociception and inflammation. Although t-testing comparisons of bradykinin serum and midbrain levels (Table 2l) show that a significant difference exists at only one time interval (15-40 minutes), the larger bradykinin serum levels cannot be disputed in time intervals up to 10 hours after violent incident. The non-significant result in several of the early time intervals may be due more to a lack of sample size [149] than actual group similarity.

Furthermore, small sample size is the probable reason that significant differences in bradykinin levels are not observed in the two largest incident-death time intervals. In these intervals where chronic pain and antinociceptive processes are dominant, midbrain bradykinin levels are approximately 2 fold greater than in serum.

C. Comparison of Peptide Levels at Various Incident-Death Time Intervals in all Violent Deaths with Peptide Levels of Alive Adult Subjects with Varying Levels of Pain: Determination of Time Interval for Onset of Acute and Chronic Pain After Violent Episode in Post-Mortem Serum

The comparison of substance P, bradykinin, and met-enkephalin serum levels at various incident-death time intervals in all violent deaths with similar peptide levels in living adult subjects with varying levels of pain (Tables 3a, 3b, and 3c) gives an estimate of how long after the critical incident acute and chronic pain markers are observable in serum.

Acute pain markers are observable shortly after a violent incident. Using t-

testing. substance P serum levels in violent deaths become significantly different than levels in healthy adults at the 20-50 minute time interval. At no time interval between incident and death are bradykinin serum levels in violent death significantly different than normal adult levels. A significant difference is also observed at the 20-50 minute time interval. The rise in bradykinin levels before substance P increase may be due to the better accessibility of bradykinin to the general circulation. Since bradykinin is produced primarily in the liver [135], it is easily released into the general circulation from the hepatic portal system. In contrast, substance P release from the peripheral and central nervous system into the general circulation occurs through much thinner capillaries. When compared with bradykinin and substance P levels from the adult subject with acute pain, no significant difference is observed at the 20-50 minute incident-death time interval indicating that acute pain and nociceptive processes are observable.

Substance P serum level comparisons also show when chronic pain and antinociceptive processes are observable. When comparing substance P levels from subjects having a history of chronic pain with violent deaths having an incident-death time interval of 8 days to 3 weeks, a significant difference is observed. This significant difference is indicative that chronic pain processes are not yet dominant at this time interval. However, no such difference is observed at the 1-8 month time interval. During this time interval, it is obvious that chronic pain processes are

observable in serum. Significant differences are also not observed for met-enkephalin and bradykinin levels in violent deaths having incident-death time intervals of 8 days to 3 weeks and 1-8 months when compared with chronic pain alive subjects. Caution should always be used when comparisons are made with chronic pain living subjects because chronic pain is often variable and transient among subjects.

In summary, peptide serum levels indicative of acute pain are observable in minutes after a violent incident, while peptide serum levels suggestive of chronic pain are observable at a minimum of one month after a violent incident.

D. Comparison of Serum Peptide Levels in Various Types of Instantaneous Death with Serum Peptide Levels in Healthy Adults: Determination of Death-Induced Nociception

Conventional logic dictates that individuals who die instantaneously do not experience conscious pain. However, data comparing serum peptide levels in various types of violent instantaneous death with serum peptide levels in healthy adults suggests that nociceptive activation and perhaps modulation does occur. Mean and median levels of substance P, bradykinin, and met-enkephalin are higher in each respective violent instantaneous death (burns/electrocution, blunt trauma, gunshot, stabbing, hanging/strangulation/asphyxia, drug overdose, drowning, and smoke inhalation) than in alive healthy adults (Tables 4a, 4b, and 4c). The increased

Table 3a - t Testing Comparisons Of Mean Substance P Serum Levels At Various Incident-Death Time Intervals In All Violent Deaths With Those Of Living Adult Subjects With Varying Levels Of Pain

Incident-Death Time Interval In All Violent Deaths	Level Of Pain In Living Adult Subjects	$x_1 - x_2$	Degrees Of Freedom	Critical t Value At 95% Confidence Level	Calculated t Value	Type Of Difference
0	Healthy (No Pain)	20.50	91	1.96	23.97	Not Significant
20-50 minutes	Healthy (No Pain)	46.27	21	1.96	26.12	Significant
20-50 minutes	Acute Pain	40.60	12	2.18	59.88	Not Significant
8 days - 3 weeks	Chronic Pain	120.57	13	2.16	104.46	Significant
1-8 months	Chronic Pain	40.72	11	2.20	54.61	Not Significant

Table 3b - t Testing Comparisons Of Mean Bradykinin Serum Levels At Various Incident-Death Time Intervals In All Violent Deaths With Those Of Living Adult Subjects With Varying Levels Of Pain

Incident-Death Time Interval In All Violent Deaths	Level Of Pain In Living Adult Subjects	$x_1 - x_2$	Degrees Of Freedom	Critical t Value At 95% Confidence Level	Calculated t Value	Type Of Difference
0	Healthy (No Pain)	29.44	91	1.96	28.35	Significant
20-50 minutes	Healthy (No Pain)	52.16	21	1.96	33.73	Significant
20-50 minutes	Acute Pain	39.71	12	2.18	93.95	Not Significant
8 days - 3 weeks	Chronic Pain	77.47	13	2.16	93.49	Not Significant
1-8 months	Chronic Pain	17.38	11	2.20	47.45	Not Significant

Table 3c - t Testing Comparisons Of Mean Met-Enkephalin Serum Levels At Various Incident-Death Time Intervals In All Violent Deaths With Those Of Living Adult Subjects With Varying Levels Of Pain

Incident-Death Time Interval In All Violent Deaths	Level Of Pain In Living Adult Subjects	$x_1 - x_2$	Degrees Of Freedom	Critical t Value At 95% Confidence Level	Calculated t Value	Type Of Difference
0	Healthy (No Pain)	11.34	91	1.96	16.09	Not Significant
20-50 minutes	Healthy (No Pain)	20.28	21	1.96	18.31	Not Significant
20-50 minutes	Acute Pain	32.18	12	2.18	38.62	Not Significant
8 days - 3 weeks	Chronic Pain	36.64	13	2.16	103.76	Not Significant
1-8 months	Chronic Pain	18.45	11	2.20	120.89	Not Significant

concentration of each peptide is variable for each type of death and may depend on the extent of tissue invasion and injury. For example, deaths by drug overdose and drowning (deaths in which the extent of tissue injury may be minimal) exhibit the lowest levels of substance P and bradykinin. Conversely, deaths caused by burns or electrocution and stabbing (deaths in which tissue injury can be extensive) show the highest levels of substance P and bradykinin.

One factor which may affect data interpretation is the reliance on case information. Whether or not an individual dies instantaneously is often difficult, if not impossible, to ascertain. Each subject placed in the instantaneous category was either found dead or witnessed to be killed instantaneously. It is certainly possible that a certain percentage of subjects who were found dead may have survived a short time between incident and death. Subjects who do survive unreported for a short time would be expected to have higher peptide levels (at least substance P and bradykinin) than those who truly die instantaneously. A case in point is peptide levels for death by gunshot. Peptide levels for death by gunshot are closer to those of drowning and drug overdose deaths than those of burns and stabbing, although the level of tissue injury should be similar to the latter. The apparent discrepancy may be due to unreported survival time between incident and death. Gunshot victims may be more apt to die instantaneously than burn or stabbing victims. In a similar manner, smoke inhalation deaths, which may be expected to be less painful than other types

Table 4a - Substance P Serum Levels In Various Types Of Violent Instantaneous Death

Type Of Death	N	Mean	Median	Standard Deviation
Burns/Electrocution	7	66.03	55.30	51.79
Blunt Trauma	21	54.15	28.30	47.14
Gunshot	19	34.16	27.80	24.08
Stabbing	6	87.20	72.95	66.80
Hanging/Strangulation/Asphyxia	17	40.94	32.40	30.17
Drug Overdose	26	27.99	22.10	18.35
Drowning	5	28.96	26.50	12.82
Smoke Inhalation	7	56.31	60.20	18.01

Table 4b - Bradykinin Serum Levels In Various Types Of Violent Instantaneous Death

Type Of Death	N	Mean	Median	Standard Deviation
Burns/Electrocution	7	89.10	66.70	81.75
Blunt Trauma	21	51.41	28.00	49.25
Gunshot	19	37.31	34.10	32.40
Stabbing	6	62.38	60.00	38.06
Hanging/Strangulation/Asphyxia	17	44.59	33.10	37.29
Drug Overdose	26	24.41	18.30	21.58
Drowning	5	35.90	29.00	30.46
Smoke Inhalation	7	63.87	59.50	20.75

Mean and median levels are in pg/ml serum.

Table 4c - Met-Enkephalin Serum Levels In Various Types Of Violent Instantaneous Death

Type Of Death	N	Mean	Median	Standard Deviation
Burns/Electrocution	7	48.94	40.40	45.98
Blunt Trauma	21	27.61	13.80	26.76
Gunshot	19	17.02	15.50	9.15
Stabbing	6	23.47	18.55	21.34
Hanging/Strangulation/Asphyxia	17	27.18	18.70	21.97
Drug Overdose	26	25.02	16.45	24.72
Drowning	5	26.78	30.90	18.89
Smoke Inhalation	7	48.34	49.70	21.19

Mean and median levels are in pg/ml serum.

of violent death, give unexpected and comparatively high peptide levels. In this case, smoke inhalation may cause high serum peptide levels due to extensive lung and other respiratory tissue damage or case information may not reflect the extent of pre-mortem burn injury often found with smoke inhalation deaths. Alternatively, carbon monoxide toxicity in smoke inhalation deaths may stimulate peptide release from blood-borne tissues irrespective of nociceptive activity [126].

Comparisons of peptide levels in instantaneous deaths with healthy adult levels using t-testing show a predicable pattern (Tables 4d, 4e, and 4f). Bradykinin levels appear to show the most significant difference. Significant differences in

Table 4d - t Testing Comparisons Of Mean Substance P Serum Levels In Instantaneous Death With Healthy Adult Levels

Type Of Death	$\bar{x} - \bar{x}_2$	Degrees Of Freedom	Critical t Value At 95% Confidence Level	Calculated t Value	Type Of Difference
Electrocution/Burns	41.44	15	1.96	37.46	Significant
Blunt Trauma	29.56	29	1.96	31.12	Not Significant
Gunshot	9.60	27	1.96	14.97	Not Significant
Stabbing	62.60	14	2.14	55.55	Significant
Strangulation/ Hanging/Asphyxia	16.30	25	1.96	20.05	Not Significant
Drug Overdose	3.40	34	1.96	11.95	Not Significant
Drowning	4.40	13	2.16	12.72	Not Significant
Smoke Inhalation	31.70	15	1.96	20.19	Significant

$\bar{x}_2$ = mean substance P value in healthy adults

Table 4e - t Testing Comparisons Of Mean Bradykinin Serum Levels In Instantaneous Death With Healthy Adult Levels

Type Of Death	$\bar{x} - \bar{x}_2$	Degrees Of Freedom	Critical t Value At 95% Confidence Level	Calculated t Value	Type Of Difference
Electrocution/Burns	69.75	15	1.96	59.45	Significant
Blunt Trauma	32.09	29	1.96	32.52	Not Significant
Gunshot	17.96	27	1.96	21.22	Not Significant
Stabbing	43.03	25	2.14	34.57	Significant
Strangulation/ Hanging Asphyxia	25.24	25	1.96	25.05	Significant
Drug Overdose	5.06	34	1.96	13.67	Not Significant
Drowning	16.55	13	2.16	22.60	Not Significant
Smoke Inhalation	44.49	15	1.96	25.59	Significant

$\bar{x}_2$ = mean bradykinin value in healthy adults

Table 4f - t Testing Comparisons Of Mean Met-Enkephalin Serum Levels In Instantaneous Death With Healthy Adult Levels

Type Of Death	$\bar{x} - \bar{x}_2$	Degrees Of Freedom	Critical t Value At 95% Confidence Level	Calculated t Value	Type Of Difference
Electrocution/Burns	35.84	15	1.96	37.16	Not Significant
Blunt Trauma	14.51	29	1.96	20.14	Not Significant
Gunshot	3.92	27	1.96	12.37	Not Significant
Stabbing	10.37	14	2.14	26.14	Not Significant
Strangulation Hanging/Asphyxia	14.08	25	1.96	18.56	Not Significant
Drug Overdose	11.92	34	1.96	18.28	Not Significant
Drowning	13.68	13	2.16	27.84	Not Significant
Smoke Inhalation	35.24	15	1.96	28.07	Significant

$\bar{x}_2$ = mean met-enkephalin value in healthy adults

bradykinin levels were observed in four types of violent death: electrocution/burns, stabbing, strangulation/hanging/asphyxia, and smoke inhalation. Since it is likely that bradykinin levels in serum are the first to be elevated due to violent assault (for reasons previously mentioned), it should not be surprising that bradykinin levels show the most significant difference.

Significant elevation in substance P levels are observed in three types of deaths: electrocution/burns, stabbings, and smoke inhalation. Increases in substance

P levels may suggest the presence of a short survival time between incident and death.

No significant increases in met-enkephalin levels were observed in any of the violent deaths. This is not unexpected since nociceptive and acute pain processes should be dominant in deaths with little or no incident-death time interval.

Although peptide levels for some types of violent death with little or no incident-death time interval are not significantly different from levels in healthy adults, the empirical data show that the levels of each peptide increase for each type of violent death. Substance P increases range from 1.2 (drug overdose) to 3.5 (stabblings) fold, bradykinin increases range from 1.3 (drug overdose) to 4.6 (burns/electrocution) fold, and met-enkephalin increases range from 1.3 (gunshot) to 3.7 (burns/electrocution) fold. These increases, although not conclusive or significant in some types of violent instantaneous death, suggests that death does activate nociceptive transmission and modulating pathways.

E. Comparison of Serum Peptide Levels with Midbrain Peptide Levels in Various Types of Violent Instantaneous Death - An Indication of the Relationship Between Trauma and Nociceptive Induction

The examination of serum and midbrain peptide mean and median levels in various types of violent instantaneous death (Tables 5a, 5b, and 5c) indicates that the degree of nociceptive induction may be dependant on the amount of associated

trauma. Substance P midbrain levels are highest for deaths by thermal burns (189.70 pg/g) and blunt trauma (212.06 pg/g). Both deaths are often typified by extensive trauma encompassing multiple effected areas. Blunt trauma deaths, for example, usually involve injury to the whole body such as in the usual case of a pedestrian involved in a motor vehicle accident. Similarly, second and third degree burns over a majority of the body surface area is characteristic of fire deaths caused by burns.

Substance P midbrain levels are smaller for the other types of violent death. Deaths by gunshot, hanging/strangulation/asphyxia, drowning, and smoke inhalation give similar values ranging from 83.13 pg/g (gunshot) to 101.95 (smoke inhalation). In contrast to blunt trauma and burn deaths, trauma in these deaths may be localized to a generalized location. For instance, death by gunshot may occur by a single wound to a vital area (e.g. head, chest). The lowest substance P midbrain levels (39.23 pg/g) are found in drug overdose deaths. This low value may be indicative of a lack of tissue injury associated with death by drug overdose.

The magnitude of difference between midbrain and serum substance P values may also be indicative of the extent of tissue trauma. Given that the incident-death interval is either non-existent or unmeasurable in instantaneous violent death, it is likely that increases in substance P levels would be found in the midbrain without seeing appreciable increases in the serum. For instance, deaths due to gunshot, hanging/strangulation/asphyxia, drug overdose, and smoke inhalation all have similar

Table 5a - Substance P Serum And Midbrain Levels In Various Types Of Violent Instantaneous Death

Type Of Death	N	Midbrain			Serum		
		Mean	Median	Standard Deviation	Mean	Median	Standard Deviation
Thermal Burns	1	189.70	189.70	NA	106.10	106.10	NA
Blunt Trauma	5	212.06	225.40	74.56	92.98	99.70	29.45
Gunshot	7	83.13	63.20	44.58	36.24	31.90	17.18
Hanging/Strangulation/Asphyxia	4	85.38	80.00	34.27	38.80	36.15	20.70
Drug Overdose	6	39.23	40.45	28.09	27.32	26.65	12.31
Drowning	2	83.50	83.50	10.47	35.30	35.30	12.45
Smoke Inhalation	2	101.95	101.95	55.08	42.15	42.15	18.60

NA = Not Applicable

Mean and median levels are in pg/ml serum or pg/g brain tissue.

Table 5b - Bradykinin Serum And Midbrain Levels In Various Types Of Violent Instantaneous Death

Type Of Death	N	Midbrain			Serum		
		Mean	Median	Standard Deviation	Mean	Median	Standard Deviation
Thermal Burns	1	50.20	50.20	NA	267.10	267.10	NA
Blunt Trauma	5	92.28	74.90	46.20	91.16	89.90	52.36
Gunshot	7	48.04	49.80	13.19	59.99	43.90	40.89
Hanging/Strangulation/Asphyxia	4	38.85	43.05	18.77	44.95	47.60	26.07
Drug Overdose	6	42.25	44.95	25.57	32.18	29.30	11.40
Drowning	2	62.15	62.15	5.02	49.80	49.80	52.47
Smoke Inhalation	2	59.35	59.35	15.91	58.80	58.80	11.17

NA = Not Applicable

Mean and median levels are in pg/ml serum or pg/g brain tissue.

Table 5c - Met-Enkephalin Serum And Midbrain Levels In Various Types Of Violent Instantaneous Death

Type Of Death	N	Midbrain			Serum		
		Mean	Median	Standard Deviation	Mean	Median	Standard Deviation
Thermal Burns	1	37.10	37.10	NA	12.60	12.60	NA
Blunt Trauma	5	73.68	82.00	40.10	35.88	44.80	23.49
Gunshot	7	39.59	26.30	27.56	16.76	16.20	5.22
Hanging/Strangulation/Asphyxia	4	37.53	44.95	21.85	42.90	42.80	22.65
Drug Overdose	6	55.17	62.95	26.84	30.33	38.25	20.39
Drowning	2	40.20	40.20	36.77	19.80	19.80	15.70
Smoke Inhalation	2	58.15	58.15	10.39	36.75	36.75	12.37

NA = Not Applicable

Mean and median levels are in pg/ml serum or pg/g brain tissue.

substance P levels in serum. Mean serum levels range from 27.32 pg/ml (drug overdose) to 42.15 pg/ml (smoke inhalation). Corresponding values in the midbrain show the extent that tissue trauma has on the initiation of nociception. Deaths by gunshot, hanging/strangulation/asphyxia, drowning, and smoke inhalation have similar mean values, ranging from 83.13 pg/g (gunshot) to 101.95 pg/g (smoke inhalation). The range is slightly greater (63.20 pg/g to 101.95 pg/g) when examining median values. In each case, mean levels of substance P in the midbrain are approximately 2.3-2.4 times greater than in the serum. Conversely, the increase in substance P levels in midbrain for drug overdose deaths is not nearly as high. The mean value of substance P concentration in the midbrain is 39.23 pg/g, a magnitude of only 1.4 over the level in serum. The lower value may be indicative of the minimal amount of tissue damage in drug overdose deaths.

Similar to levels in serum, substance P levels in deaths due to thermal burns and blunt trauma show the highest mean values (189.70 pg/g and 212.06 pg/g, respectively). Like the values in serum, these inordinate high values reflect the extent of tissue damage associated with burn and blunt trauma deaths.

The difference between mean substance P serum and midbrain levels in several types of deaths were found not to be statistically different (Table 5d). With the exception of values obtained for drug overdose deaths, these results may be more reflective of the small sample size in each type of death than to actual similarities in

serum and midbrain values.

Bradykinin serum and midbrain levels appear to be very similar in gunshot, hanging/strangulation/asphyxia, drug overdose, drowning, and smoke inhalation deaths. Unlike substance P levels, no apparent spike in bradykinin levels was observed in the midbrain data. Since bradykinin has no nociceptive function in the midbrain, this result is not surprising.

Contrasting bradykinin levels were found in the thermal burn and blunt trauma deaths. Although the level of bradykinin in the midbrain is similar to the other deaths, the serum level in the thermal burn death is approximately 4-5 fold higher (267.10 pg/g). This very high level is undoubtedly due to the large influx of bradykinin from internal organs to the multiple sites of tissue injury and inflammation typical of burn deaths via the general circulation. The serum level of bradykinin is more than twice the level of substance P demonstrating the speed to which bradykinin gets to the site of injury. Like substance P increases, the high level of bradykinin is reflective of the extent of tissue damage.

Although lower than the serum value in the burn death, bradykinin levels in blunt trauma deaths are higher than the other types of death, again demonstrating a larger extent of tissue damage. Although the mean value in midbrain tissue is similar to the mean value in serum (due to outlying data), the median value gives the more expected result of a higher bradykinin level in serum. What is perplexing is that

the serum level of bradykinin is almost identical to the level of substance P. The only plausible explanation is the presence of unknown clinical conditions which may cause substance P levels to rise or that previously unknown incident-death time intervals of the order that will allow substance P serum levels to rise may actually exist in some cases.

Like substance P levels, the difference between mean bradykinin serum and midbrain levels in several types of deaths were found not to be statistically different (Table 5e). Given the closeness of the values, these results are not unexpected.

In all types of deaths, respective serum and midbrain met-enkephalin levels are relatively similar. With one exception (hanging/strangulation/asphyxia), midbrain levels are approximately twice as large as serum levels. These levels probably represent normal endogenous values since midbrain levels are comparatively high for drug overdose deaths. The only hint of the activation of nociceptive modulation is in midbrain levels obtained for blunt trauma deaths. The modestly high mean value of 73.68 pg/g may represent a response to high levels of substance P (212.06 pg/g) in the midbrain and consequently the initiation of nociceptive modulation.

The difference between mean met-enkephalin serum and midbrain levels in several types of deaths are not statistically different (Table 5f). Since values obtained probably approximate endogenous levels, the lack of significance is expected.

Table 5d - t Testing Comparisons Of Mean Substance P Serum And Midbrain Levels In Various Types Of Violent Instantaneous Death

Type Of Death	$x_1 - x_2$	Degrees Of Freedom	Critical t Value At 95% Confidence Level	Calculated t Value	Type Of Difference
Blunt Trauma	119.08	8	2.31	120.43	Not Significant
Gunshot	46.89	12	2.18	47.26	Not Significant
Hanging/Strangulation/Asphyxia	46.58	6	2.45	62.63	Not Significant
Drug Overdose	11.91	10	2.23	27.80	Not Significant

Table 5e - t Testing Comparisons Of Mean Bradykinin Serum And Midbrain Levels In Various Types Of Violent Instantaneous Death

Type Of Death	$\bar{x}_1 - \bar{x}_2$	Degrees Of Freedom	Critical t Value At 95% Confidence Level	Calculated t Value	Type Of Difference
Blunt Trauma	1.12	8	2.31	68.02	Not Significant
Gunshot	11.95	12	2.18	34.77	Not Significant
Hanging/Strangulation/Asphyxia	6.10	6	2.45	36.87	Not Significant
Drug Overdose	10.04	10	2.23	25.22	Not Significant

Table 5f - t Testing Comparisons Of Mean Met-Enkephalin Serum And Midbrain Levels In Various Types Of Violent Instantaneous Death

Type Of Death	$\bar{x}_1 - \bar{x}_2$	Degrees Of Freedom	Critical t Value At 95% Confidence Level	Calculated t Value	Type Of Difference
Blunt Trauma	37.80	8	2.31	53.82	Not Significant
Gunshot	22.83	12	2.18	26.15	Not Significant
Hanging/Strangulation/Asphyxia	5.37	6	2.45	36.03	Not Significant
Drug Overdose	24.84	10	2.23	33.69	Not Significant

F. Comparison of Peptide Levels in Accidental, Homicidal, and Suicidal Violent Death - An Assessment of the Effect of Manner of Violent Death and Psychological Factor on Substance P, Bradykinin, and Met-Enkephalin Levels

1. Serum study

If an assumption is made that victims of accidental violent death, homicides, and suicides experience different psychological stresses, then the effect these stresses have on peptide levels may be determined by measuring peptide levels in the respective manners of violent death. For instance, it may be assumed that psychological factors such as depression, fear, and panic are present in different degrees in accidental deaths, homicides, and suicides. For instance, depression may be more of a psychological factor in suicides than in homicides or accidental deaths. In contrast, fear and panic may play larger roles in accidental deaths and homicides. Any effect these or other psychological factors play on peptide levels would be observed if peptide levels were substantially different in the three manners of death. By examining the data, it can be concluded that this is not the case to any significant degree.

Peptide level comparisons were made between accidental violent death, homicides, and suicides for different incident-death time intervals. Incident-death time intervals were selected arbitrarily with emphasis placed on total sample number (combined sample number for the three manners of death) for each interval.

Substance P mean and median serum levels appear to follow the same pattern

with increasing incident-death time intervals (Table 6a-c). In all three manners of death, substance P levels increase until they drop off dramatically in the 1-8 month incident-death time interval. Comparisons using t-testing show that no significant differences exist between substance P serum levels in accidental violent death and homicides for all incident-death time intervals (Table 6d). This is reflected in the data since it is apparent that only negligible differences exist at every time interval with the exception of 0. At 0 time interval (instantaneous death), levels are slightly higher in homicides (63.78 pg/ml) than in accidental violent death (41.55 pg/ml). This difference may be related to the impact drug overdose deaths have on lowering mean and median levels at the 0 incident-death time interval in accidental violent deaths.

Due to the lack of availability of suicide cases at certain incident-death time intervals, comparisons could not be made at every interval. Data for suicides was obtained at the 0, 2-20 minute, 25 minute - 4 hour, and 5 day - 3 week incident-death time intervals. Like the data obtained for accidental violent deaths and homicides, substance P levels increase as the incident-death time interval increases. The increase, however, is not as large as the other two manners of death as evident by the lower level at the 5 days - 3 week time interval. Additionally, the data suggests that the increase may occur later since, unlike accidental violent deaths and homicides, no increase is observed at the 25 minute - 4 hour time interval. Comparisons made

Table 6a - Substance P Serum Levels In All Accidental Violent Deaths

Incident-Death Time Interval	N	Mean	Median	Standard Deviation
0	48	41.55	31.20	29.79
2-20 minutes	5	33.46	22.10	29.15
25 minutes - 4 hours	5	77.72	74.20	39.32
4.5 hours - 21 hours	6	78.12	88.25	50.09
1-4 days	11	87.95	92.90	53.71
5 days - 3 weeks	7	143.57	114.30	56.91
1-8 months	4	53.80	44.55	51.43

Table 6b - Substance P Serum Levels In All Homicides

Incident-Death Time Interval	N	Mean	Median	Standard Deviation
0	19	63.78	56.10	50.69
2-20 minutes	4	41.05	35.05	28.53
25 minutes - 4 hours	16	82.40	71.50	42.67
4.5 hours - 21 hours	8	99.86	105.85	62.48
1-4 days	5	132.02	121.90	70.88
5 days - 3 weeks	5	148.60	144.30	74.23
1-8 months	5	55.22	70.10	43.77

Mean and median levels are in pg/ml.

Table 6c - Substance P Serum Levels In All Suicides

Incident-Death Time Interval	N	Mean	Median	Standard Deviation
0	38	34.69	25.05	29.68
2-20 minutes	10	34.74	18.05	26.38
25 minutes - 4 hours	3	32.27	24.80	15.97
5 days - 3 weeks	3	80.07	50.60	82.16

Mean and median levels are in pg/ml.

with t-testing (Table 6d) do show that significant differences in mean substance P levels do exist at the 0 time interval between suicides and homicides. Although the reason for the lower values may ultimately be case specific (i.e. cases where chronic pain is present), the explanation is unclear.

Bradykinin serum levels (Tables 6e-f) in accidental violent deaths and homicides mimic substance P serum levels, although increases in bradykinin levels appear to plateau at intermediate incident-time intervals before dropping sharply at the 1-8 month time interval. This plateau may be related to the antinociceptive qualities of central nervous system bradykinin. Since no increases in central nervous system bradykinin will occur as a result of acute pain, no causative rise in serum levels will occur. Conversely, substance P levels will increase in central nervous system tissue during nociception subsequently causing serum substance P levels to

Table 6d - t Testing Comparisons Of Serum Substance P Levels In Accidental (A), Homicidal (H), And Suicidal (S) Violent Deaths At Varying Incident-Death Time Intervals

Incident-Death Time Interval	Type Of Violent Deaths	$x_1 - x_2$	Degrees Of Freedom	Critical t Value At 95% Confidence Level	Calculated t Value	Type Of Difference
0	A/H	22.23	65	1.96	20.06	Significant
0	A/S	6.86	84	1.96	12.67	Not Significant
0	H/S	29.09	55	1.96	21.95	Significant
2-20 minutes	A/H	7.59	7	2.36	43.24	Not Significant
2-20 minutes	A/S	1.28	13	2.16	31.09	Not Significant
2-20 minutes	H/S	2.15	12	2.18	33.58	Not Significant
25 minutes-4 hours	A/H	4.68	19	1.96	41.16	Not Significant
25 minutes-4 hours	A/S	42.98	6	2.45	69.53	Not Significant
25 minutes-4 hours	H/S	50.13	17	1.96	53.73	Not Significant
4.5 hours-21 hours	A/H	21.74	12	2.18	66.52	Not Significant
1-4 days	A/H	44.07	14	2.14	70.28	Not Significant
5 days-3 weeks	A/H	5.03	10	2.23	80.24	Not Significant
5 days-3 weeks	A/S	63.50	8	2.31	95.34	Not Significant
5 days-3 weeks	H/S	68.53	6	2.45	142.40	Not Significant
1-8 months	A/H	1.42	7	2.36	69.91	Not Significant

Table 6e - Bradykinin Serum Levels In All Accidental Violent Deaths

Incident-Death Time Interval	N	Mean	Median	Standard Deviation
0	48	43.53	34.10	44.10
2-20 minutes	5	63.50	71.90	49.29
25 minutes - 4 hours	5	108.76	86.20	56.88
4.5 hours - 21 hours	6	103.17	94.65	91.52
1-4 days	11	101.12	92.80	33.744
5 days - 3 weeks	7	109.41	110.20	65.19
1-8 months	4	49.65	37.35	52.60

Table 6f - Bradykinin Serum Levels In All Homicides

Incident-Death Time Interval	N	Mean	Median	Standard Deviation
0	19	54.16	42.10	47.90
2-20 minutes	4	35.30	32.55	33.06
25 minutes - 4 hours	16	75.55	54.95	59.81
4.5 hours - 21 hours	8	100.11	98.20	33.74
1-4 days	5	74.82	66.60	57.60
5 days - 3 weeks	5	136.64	164.80	85.08
1-8 months	5	47.84	24.60	38.68

Mean and median levels are in pg/ml.

Table 6g - Bradykinin Serum Levels In All Suicides

Incident-Death Time Interval	N	Mean	Median	Standard Deviation
0	38	40.23	27.20	36.16
2-20 minutes	10	36.82	30.05	26.35
25 minutes - 4 hours	3	28.73	28.90	19.75
5 days - 3 weeks	3	101.83	56.30	125.93

Mean and median levels are in pg/ml.

later increase. Thus, serum substance P levels may continue to increase for a longer duration of time.

As in serum substance P levels, bradykinin levels in suicides increase at a slower rate with longer incident-death time intervals than in accidental violent deaths and homicides (Table 6g). This is demonstrated in the median values, which at the 5 day - 3 week interval, is approximately 2 fold lower than accidental violent deaths (56.30 pg/ml compared to 110.20 pg/ml) and 3 fold lower than homicides (164.80 pg/ml). Again the explanation, though unclear, may reside in case specifics. For instance, since comatose conditions prior to death are often not mentioned in case reports, the effect of such conditions on peptide levels is unclear. If comatose conditions are more or less prevalent in the suicide cases used in this study, it may explain lower serum levels. Test for significant differences in mean serum bradykinin levels were conducted using t-testing (Table 6h). Comparisons were

Table 6h - t Testing Comparisons Of Serum Bradykinin Levels In Accidental (A), Homicidal (H), And Suicidal (S) Violent Deaths At Varying Incident-Death Time Intervals

Incident-Death Time Interval	Type Of Violent Deaths	$x_1 - x_2$	Degrees Of Freedom	Critical t Value At 95% Confidence Level	Calculated t Value	Type Of Difference
0	A/H	10.63	65	1.96	23.96	Not Significant
0	A/S	3.30	84	1.96	17.27	Not Significant
0	H/S	13.93	55	1.96	22.34	Not Significant
2-20 minutes	A/H	28.20	7	2.36	68.01	Not Significant
2-20 minutes	A/S	26.68	13	2.16	42.82	Not Significant
2-20 minutes	H/S	1.52	12	2.18	34.93	Not Significant
25 minutes-4 hours	A/H	33.21	19	1.96	59.75	Not Significant
25 minutes-4 hours	A/S	80.03	6	2.45	108.48	Not Significant
25 minutes-4 hours	H/S	71.18	17	1.96	46.82	Not Significant
4.5 hours-21 hours	A/H	3.06	12	2.18	72.92	Not Significant
1-4 days	A/H	26.30	14	2.14	78.19	Not Significant
5 days-3 weeks	A/H	27.23	10	2.23	93.67	Not Significant
5 days-3 weeks	A/S	7.58	8	2.31	112.21	Not Significant
5 days-3 weeks	H/S	34.81	6	2.45	169.67	Not Significant
1-8 months	A/H	1.81	7	2.36	69.91	Not Significant

conducted between accidental violent death and homicides, accidental violent death and suicides, and homicides and suicides at each incident-death time interval. In no instance was a significant difference in serum bradykinin levels noted.

Serum met-enkephalin levels in accidental violent deaths and homicides do not differ to any appreciable degree (Tables 6i-j). Levels stay relatively low until the last incident-death time interval (1-8 months), where levels increase dramatically. This large increase represents the dominance of chronic pain processes at this point. Significant differences using t testing were observed at the 25 minute - 4 hour and 4.5 - 21 hour time intervals (Table 6l). These differences are not the result of any increase in met-enkephalin serum levels but rather due to the inordinate low levels of met-enkephalin found in the accidental violent death cases at these time intervals (13.96 pg/ml and 9.93 pg/ml, respectively). A significant difference was also observed at 0 time interval between suicides and accidental violent deaths despite the similarities of the actual mean values (20.74 pg/ml and 35.26 pg/ml, respectively). Again this difference does not appear to be related to any large increase in met-enkephalin serum levels.

Serum met-enkephalin levels in suicides show a large increase at an earlier interval (Table 6k). At the interval of 5 days - 3 weeks, the mean met-enkephalin value is 146.00 pg/ml and the median value is 100.30 pg/ml. This is substantially higher than corresponding values for homicides and accidental violent deaths

Table 6i - Met-Enkephalin Serum Levels In All Accidental Violent Deaths

Incident-Death Time Interval	N	Mean	Median	Standard Deviation
0	48	35.26	34.50	25.59
2-20 minutes	5	14.26	5.90	18.54
25 minutes - 4 hours	5	13.96	17.20	8.94
4.5 hours - 21 hours	6	9.93	5.95	9.97
1-4 days	11	32.07	11.40	38.99
5 days - 3 weeks	7	54.93	39.40	36.57
1-8 months	4	95.28	79.20	54.72

Table 6j -Met-Enkephalin Serum Levels In All Homicides

Incident-Death Time Interval	N	Mean	Median	Standard Deviation
0	19	21.49	18.50	16.81
2-20 minutes	4	12.28	9.75	8.29
25 minutes - 4 hours	16	35.74	32.10	21.79
4.5 hours - 21 hours	8	39.16	30.85	24.69
1-4 days	5	34.34	27.40	38.13
5 days - 3 weeks	5	40.32	53.10	23.31
1-8 months	5	116.12	216.00	132.05

Mean and median levels are in pg/ml.

Table 6k - Met-Enkephalin Serum Levels In All Suicides

Incident-Death Time Interval	N	Mean	Median	Standard Deviation
0	38	20.74	12.90	20.02
2-20 minutes	10	16.67	11.45	18.59
25 minutes - 4 hours	3	21.23	17.60	19.80
5 days - 3 weeks	3	146.00	100.30	165.36

Mean and median levels are in pg/ml.

(however, t testing comparisons found no significant difference because of high calculated t values due to small sample size). This may explain the apparent low substance P and bradykinin levels found in suicides at this incident-death time interval. Substance P serum levels may be depressed due to inhibition by met-enkephalin in the central nervous system. Similarly, the levels of serum bradykinin may be related more to antinociceptive stimulation in the central nervous system than to its release into the circulation as a response to tissue injury and therefore may not be as high.

The large standard deviation (165.36), however, makes it probable that the serum levels for met-enkephalin at the 5 day - 3 week incident- death time interval is reflective of the cases in the study and not to any difference in peptide release for suicides. Although the median level is still quite high (100.30 pg/ml), the serum met-

enkephalin concentration for all cases at this time interval range from 8.3 pg/ml to 329.40 pg/ml (clinical conditions that may alter peptide levels were not reported for any case). Only results from a larger sample group could clarify the question of whether peptide release in suicides is different than accidental violent deaths or homicides.

2. Serum/Brain study

Substance P, bradykinin, and met-enkephalin levels in the midbrain and corresponding serum samples follow the same basic pattern in accidental violent deaths, homicides, and suicides with increased incident-death time interval. Even though comparisons between manners of death are made with different incident-time intervals, many of which contain limited samples (in most cases, one), the overall pattern of peptide increase or decrease can still be discerned.

For reasons previously cited, midbrain and serum peptide levels in accidental violent deaths give expected values with increasing incident-death time interval (Tables 6m, 6n, and 6o). In the midbrain, high substance P levels characteristic of acute pain are found from the 0 time interval through the 2 hour incident-death time interval. Also characteristic of acute pain, bradykinin and met-enkephalin midbrain levels in these time intervals are comparatively lower and do not increase through 2 hours. In the serum, bradykinin levels begin to increase substantially during the early time intervals and peak at the 28 hour interval (134.30 pg/ml). Serum substance P

levels increase at a slower rate as evident at the 2 hour interval where levels increase from a mean value of 44.66 pg/ml at the 0 interval to only 59.0 pg/ml. In contrast, the bradykinin serum concentration at 2 hours is 116.20 pg/ml as compared to a mean value of 63.40 pg/ml at the 0 time interval. No increase in serum met-enkephalin levels is seen through the 2 hour interval.

At the 28 hour interval, midbrain peptide levels become characteristic of chronic pain and antinoceptive processes since met-enkephalin and bradykinin both show large increases from the previous interval. Met-enkephalin levels show even higher levels at the 3 month, 3 week interval, while bradykinin levels appear to plateau. Since met-enkephalin midbrain levels increase during the latter incident-death time intervals, it is not surprising that substance P levels correspondingly decrease. The decrease is most substantial at the 3 month, 3 week interval where substance P levels are at their lowest concentration (3 pg/g) and met-enkephalin levels are highest (236.60 pg/g).

The onset of chronic pain markers in the serum is characteristically much later. In fact, at the 28 hour interval where substance P levels in the midbrain are already beginning to decrease, serum substance P levels show their largest increase. It is not until the final interval (3 month, 3 week) that serum substance P concentration shows a level consistent with chronic pain (18.30 pg/ml). Serum met-enkephalin levels do not show any large increase until the 32 day interval and, as expected, remain high

Table 6I - t Testing Comparisons Of Serum Met-Enkephalin Levels In Accidental (A), Homicidal (H), And Suicidal (S) Violent Deaths At Varying Incident-Death Time Intervals

Incident-Death Time Interval	Type Of Violent Deaths	$\bar{x}_1 - \bar{x}_2$	Degrees Of Freedom	Critical t Value At 95% Confidence Level	Calculated t Value	Type Of Difference
0	A/H	13.77	65	1.96	14.45	Not Significant
0	A/S	14.52	84	1.96	11.35	Significant
0	H/S	0.75	55	1.96	10.39	Not Significant
2-20 minutes	A/H	1.52	7	2.36	34.93	Not Significant
2-20 minutes	A/S	2.41	13	2.16	21.22	Not Significant
2-20 minutes	H/S	4.39	12	2.18	20.76	Not Significant
25 minutes-4 hours	A/H	21.78	19	1.96	21.59	Significant
25 minutes-4 hours	A/S	7.27	6	2.45	23.36	Not Significant
25 minutes-4 hours	H/S	14.51	17	1.96	26.70	Not Significant
4.5 hours-21 hours	A/H	29.23	12	2.18	28.63	Significant
1-4 days	A/H	2.27	14	2.14	43.23	Not Significant
5 days-3 weeks	A/H	14.61	10	2.23	40.95	Not Significant
5 days-3 weeks	A/S	91.07	8	2.31	132.65	Not Significant
5 days-3 weeks	H/S	105.68	6	2.45	188.62	Not Significant
1-8 months	A/H	20.84	7	2.36	167.81	Not Significant

through 3 month, 3 week interval. Serum bradykinin levels peak at 28 hours (134.40 pg/ml) then fall to 55.80 pg/ml at the 32 day incident-death time interval. The high value obtained at the 3 month, 3 week interval may be due to migration of antinociceptive central nervous system bradykinin into the general circulation and not to any nociceptive or inflammatory process.

Data from homicides is much less complete than that of accidental violent deaths due to the inability to obtain samples from deaths with an incident-death time interval greater than 38½ hours. Little difference is observed between homicide and accidental violent death peptide levels up to this interval (Tables 6p, 6q, and 6r: Graph 6j). Midbrain levels in homicides at the 1day - 1 day, 14½ hour interval appear to have lower met-enkephalin and bradykinin levels and higher substance P levels than at the closest interval (28 hours) in accidental deaths. With small sample size at each interval and no homicide data past the 1day - 1 day, 14½ hour interval, any conclusions drawn must be judged accordingly. However, it may seem reasonable that the difference may be due to the type of cases in each manner of death. As noted earlier, tissue injury may be more significant in accidental violent deaths (e.g., multiple trauma in blunt impact deaths) than in homicides (e.g. single stab wound or gunshot). In situations where there is extensive tissue damage, antinociceptive processes may be accelerated causing midbrain levels of bradykinin and met-enkephalin levels to increase (and as a consequence substance P levels to

decrease) sooner. It is doubtful that the presence of psychological factors endemic to accidental violent death and homicides play a role in this difference in peptide levels. Serum levels between the two manners of death do not vary to any significant degree.

Like homicides, data from suicides are limited and contain only two incident-death time intervals, 0 and 5 day, 18 hour - 10 day (Tables 6s, 6t, and 6u). No difference in midbrain or serum peptides levels at the 0 incident-death time interval is observed between suicides and the other two manners of death. However, differences are observed in the 5 day, 18 hour - 10 day time interval. Unlike similar incident-death time intervals in accidental violent deaths, no increases in levels are observed in any of the peptides in both the midbrain and serum. The lack of increase is not easily explained and is similar to data obtained in the serum study. The data at this interval was obtained from a hanging and a blunt trauma suicide. Both deaths are associated with tissue injury (extensive in the blunt trauma death) and would be expected to show peptide increases at this time interval. The presence of clinical conditions which may affect peptide levels was not noted in the blunt trauma death. In the hanging death, however, it was documented that the victim was being treated medicinally for depression. Although the release of substance P, bradykinin, and met-enkephalin is believed not be autonomically influenced [1], the lack of increase in both midbrain and serum peptide levels at the 5 day, 18 hour - 10 day time interval

cannot discount the possibility that psychological factors (or medicinal treatments) in suicides affect peptide levels. More likely, however, is the presence of chronic pain in suicide victims prior to death. It seems reasonable that chronic pain may be a reason for an individual to commit suicide. A definitive determination, however, cannot be made without data from suicides with larger incident-death time intervals.

Comparisons of the three manners of death in the midbrain/serum study using t-testing were not conducted because of small sample size in many of time intervals.

Table 6m - Substance P Serum And Midbrain Levels In All Accidental Violent Deaths

Incident-Death Time Interval	N	Midbrain			Serum		
		Mean	Median	Standard Deviation	Mean	Median	Standard Deviation
0	13	101.04	63.00	92.54	44.66	38.70	32.60
20 minutes	1	157.20	157.20	NA	49.10	49.10	NA
2 hours	1	130.20	130.20	NA	59.0	59.0	NA
28 hours	1	72.0	72.0	NA	158.30	158.30	NA
32 days	1	91.20	91.20	NA	109.50	109.50	NA
3 months, 3 weeks	1	3.0	3.0	NA	18.30	18.30	NA

NA = Not Applicable

Mean and median levels in pg/ml serum or pg/g brain tissue.

Table 6n - Bradykinin Serum And Midbrain Levels In All Accidental Violent Deaths

Incident-Death Time Interval	N	Midbrain			Serum		
		Mean	Median	Standard Deviation	Mean	Median	Standard Deviation
0	13	50.71	59.10	20.46	63.40	50.70	64.01
20 minutes	1	40.40	40.40	NA	71.90	71.90	NA
2 hours	1	41.70	41.70	NA	116.20	116.20	NA
28 hours	1	206.80	206.80	NA	134.30	134.30	NA
32 days	1	187.40	187.40	NA	55.80	55.80	NA
3 months, 3 weeks	1	202.10	202.10	NA	122.20	122.20	NA

NA = Not Applicable

Mean and median levels in pg/ml serum or pg/g brain tissue.

Table 6o - Met-Enkephalin Serum And Midbrain Levels In All Accidental Violent Deaths

Incident-Death Time Interval	N	Midbrain			Serum		
		Mean	Median	Standard Deviation	Mean	Median	Standard Deviation
0	13	53.92	60.30	25.22	32.19	36.90	11.40
20 minutes	1	57.30	57.30	NA	47.40	47.40	NA
2 hours	1	52.30	52.30	NA	19.90	19.90	NA
28 hours	1	187.40	187.40	NA	38.60	38.60	NA
32 days	1	153.80	153.80	NA	191.0	191.0	NA
3 months, 3 weeks	1	236.60	236.60	NA	171.40	171.40	NA

NA = Not Applicable

Mean and median levels in pg/ml serum or pg/g brain tissue.

Table 6p - Substance P Serum And Midbrain Levels In All Homicides

Incident-Death Time Interval	N	Midbrain			Serum		
		Mean	Median	Standard Deviation	Mean	Median	Standard Deviation
0	6	106.38	103.85	43.76	53.28	44.0	36.02
15-40 minutes	9	134.37	127.70	48.30	63.78	61.20	26.50
1½-10 hours	5	179.50	186.70	81.47	85.02	75.80	57.46
1 day-1 day, 14½ hours	2	329.20	329.20	17.11	193.05	193.05	77.43

NA = Not Applicable

Mean and median levels in pg/ml serum or pg/g brain tissue.

Table 6q - Bradykinin Serum And Midbrain Levels In All Homicides

Incident-Death Time Interval	N	Midbrain			Serum		
		Mean	Median	Standard Deviation	Mean	Median	Standard Deviation
0	6	55.73	52.00	17.91	83.72	54.10	61.80
15-40 minutes	9	40.27	42.00	14.45	78.73	73.30	45.76
1½-10 hours	5	37.06	36.90	7.09	82.58	96.80	55.96
1 day-1 day, 14½ hours	2	49.95	49.95	10.96	130.70	130.70	37.48

NA = Not Applicable

Mean and median levels in pg/ml serum or pg/g brain tissue.

Table 6r - Met-Enkephalin Serum And Midbrain Levels In All Homicides

Incident-Death Time Interval	N	Midbrain			Serum		
		Mean	Median	Standard Deviation	Mean	Median	Standard Deviation
0	6	42.23	34.45	29.59	17.70	17.35	5.28
15-40 minutes	9	34.58	28.30	16.47	34.46	36.30	14.97
1½-10 hours	5	47.12	48.30	4.86	20.08	25.50	8.27
1 day-1 day, 14½ hours	2	30.50	30.50	3.68	20.80	20.80	3.68

Table 6s - Substance P Serum And Midbrain Levels In All Suicides

Incident-Death Time Interval	N	Midbrain			Serum		
		Mean	Median	Standard Deviation	Mean	Median	Standard Deviation
0	9	110.59	89.60	63.33	54.58	50.80	30.83
5 days, 18 hours-10 days	2	106.0	106.0	5.80	58.45	58.45	11.10

NA = Not Applicable

Mean and median levels in pg/ml serum or pg/g brain tissue.

Table 6t - Bradykinin Serum And Midbrain Levels In All Suicides

Incident-Death Time Interval	N	Midbrain			Serum		
		Mean	Median	Standard Deviation	Mean	Median	Standard Deviation
0	9	64.97	58.60	45.34	61.33	46.60	44.15
5 days, 18 hours-10 days	2	54.85	54.85	69.93	63.85	63.85	10.68

Table 6u - Met-Enkephalin Serum And Midbrain Levels In All Suicides

Incident-Death Time Interval	N	Midbrain			Serum		
		Mean	Median	Standard Deviation	Mean	Median	Standard Deviation
0	9	47.96	11.97	35.90	30.02	17.50	22.04
5 days, 18 hours-10 days	2	58.60	58.60	35.64	31.15	31.15	32.31

NA = Not Applicable

Mean and median levels in pg/ml serum or pg/g brain tissue.

G. Comparison of Peptide Levels in Various Modes (Blunt Trauma, Gunshot, Stabbing, and Thermal Burns) of Violent Death - Possibility of a Bimodal Nociceptive Response

1. Blunt Trauma Deaths - Serum study

Due to a large amount of cases spread out over a range of incident-death time intervals, serum levels of substance P, bradykinin, and met-enkephalin from all blunt trauma deaths are examined with incident-death time intervals of smaller duration than data previously presented. The examination of data over smaller time intervals allows for more precise determination of the time after incident when increased peptide levels are observed in serum.

The overall pattern of peptide levels with increasing incident-death time interval is the same as previously described. In the case of substance P and bradykinin, however, the smaller time intervals show two large peaks in serum concentration (Tables 7a and 7b) not previously noted. Peaks in bradykinin levels are observed at 5-7 hours and 5-8 days, while peaks in substance P levels occur at 18-28 hours and 9-13 days. With both peptides, levels begin to drop after the second peak indicating the onset of chronic pain.

With the onset of chronic pain, met-enkephalin levels begin to expectantly rise and are at their largest in the 2-8 month incident-death time interval. In addition to the typical rise in met-enkephalin serum levels found in incident-death time intervals measuring in months, a second small peak is also observed at 18-28 hours (Table 7c).

Table 7a - Substance P Serum Levels In Accidental, Homicidal, And Suicidal Blunt Trauma Deaths

Incident-Death Time Interval	N	Mean	Median	Standard Deviation
0	21	32.45	33.80	15.09
5-20 minutes	4	22.65	17.70	18.81
1-2 hours	5	53.97	54.80	26.17
3-4.5 hours	6	96.73	84.90	32.37
5-7 hours	3	83.60	82.40	68.61
18-28 hours	7	127.50	126.20	43.19
2-4 days	6	58.88	30.65	73.68
5-8 days	4	132.83	145.15	68.73
9-13 days	5	140.53	161.00	96.51
17-32 days	3	103.60	114.30	22.54
2-8 months	4	39.65	40.25	35.59

Mean and median levels in pg/ml serum.

Table 7b - Bradykinin Serum Levels In Accidental, Homicidal, And Suicidal Blunt Trauma Deaths

Incident-Death Time Interval	N	Mean	Median	Standard Deviation
0	21	51.41	28.00	49.24
5-20 minutes	4	48.40	48.85	41.47
1-2 hours	5	52.10	48.60	40.49
3-4.5 hours	6	103.53	84.20	75.45
5-7 hours	3	167.33	177.70	75.09
18-28 hours	7	79.98	85.75	57.72
2-4 days	6	80.88	41.30	87.47
5-8 days	4	143.75	149.00	115.63
9-13 days	5	58.16	44.60	42.07
17-32 days	3	125.00	110.20	81.32
2-8 months	4	51.25	48.60	47.71

Mean and median levels in pg/ml serum.

Table 7c - Met-Enkephalin Serum Levels In Accidental, Homicidal, And Suicidal Blunt Trauma Deaths

Incident-Death Time Interval	N	Mean	Median	Standard Deviation
0	21	27.61	13.80	26.76
5-20 minutes	4	16.48	6.55	20.63
1-2 hours	5	23.32	19.90	15.02
3-4.5 hours	6	17.23	12.50	18.77
5-7 hours	3	10.40	7.60	7.88
18-28 hours	7	45.45	42.55	47.65
2-4 days	6	9.13	8.90	4.01
5-8 days	4	25.68	20.65	20.59
9-13 days	5	72.38	61.20	40.85
17-32 days	3	52.27	60.00	25.40
2-8 months	4	120.83	74.85	131.17

Mean and median levels in pg/ml serum.

There are several possible explanations for the dual peak in peptide serum levels. First, the initial peak in substance P and bradykinin may be due to the early migration of tissue bound substance P and bradykinin to the site(s) of tissue injury to begin the inflammatory process and to stimulate peripheral nerve endings to initiate nociception. The initial peak of bradykinin occurring before substance P is entirely reasonable in light of the accessibility of bradykinin to the general circulation from the liver [135]. The small peak in met-enkephalin serum levels at 18-28 hours

corresponds with the initial peak in substance P and may be reflective of the dual function of both in leukocyte chemotaxis [162]. The second peak may represent the consequential buildup of substance P, bradykinin, and met-enkephalin in the serum due to large increases in central nervous system levels.

A second possible explanation for a second peak is the enzymatic production of peptides from larger translationally produced precursors as a response to tissue injury [103]. The two peaks in serum levels, therefore, may represent the respective rise in existing endogenous peptides from tissues and the post-injury enzymatic production of peptides.

A third possible explanation for a second peak is the increased translational production of peptides due to genetic activation as a response to tissue injury and nociception. Such increased production may be due to enhanced activation of primary genes or to the initial activation of second genes which may also respectively control the production of substance P, bradykinin, and met-enkephalin.

2. Gunshot, Stabbing, and Thermal Burn Deaths - Serum study

Due to a lack of availability of cases in certain incident-death time intervals found in the blunt trauma study, the existence of a bimodal peptide increase in serum due to tissue trauma cannot be demonstrated in gunshot, stabbing, and thermal burn deaths. Mean and median peptide levels presented for deaths due to gunshot (Tables 7d, 7e, and 7f), stabbing (Tables 7g, 7h, and 7i), and thermal burns (Tables 7j, 7k,

and 71), however, do show little deviation from each other and from the blunt trauma deaths (minus the bimodal response) with increasing incident-death time intervals. There are a few notable exceptions.

First, bradykinin serum levels in thermal burn deaths show extremely high values at the 17.5 day (244.20 pg/ml) and 4 month (122.20 pg/ml) intervals. Both values are much higher than levels for the other types of death at similar incident-death time intervals. Although the 4 month value is smaller than the 17.5 day value, as expected, the 122.20 pg/ml value is hardly consistent with the onset of chronic pain processes. Conversely, values at similar incident-death time intervals from the other types of deaths (e.g. 36.17 pg/ml at 4-8 months in gunshot deaths) reflect serum levels typical in chronic pain. In addition, the met-enkephalin serum level in the thermal burn death with the 17.5 day incident-death time interval (329.40 pg/ml) is much larger than levels from the other deaths at corresponding time intervals. Both peptide levels are reflective of the quick onset of central nervous system antinociceptive processes due to the extensive amount of tissue damage in thermal burn deaths. As previously mentioned, the victims of thermal burn death in this study typically have over 90% of total body surface burned.

Second, substance P and bradykinin serum levels at the 0 incident-death time interval in stabbing deaths are approximately 2.5 and 1.7 times higher respectively than in gunshot deaths at the same time interval. If an assumption is made that on

average a similar amount of tissue damage occurs in both types of death, similar values in substance P and bradykinin serum levels would be expected. The apparent discrepancy may be explained by the fact that many of the gunshot deaths in this study occurred in the brain. Since the brain is not strongly innervated with nociceptive neurons [18], brain injury is not expected to stimulate nociception quickly. Thus, substance P and bradykinin serum levels would be expected to be lower in incident-death time intervals of small duration. Furthermore, the relatively high substance P and bradykinin levels at the 0 time interval in stabbing deaths may be simply explained if the duration of the time interval is longer than reported. This may occur more often in stabbing than gunshot deaths since logic dictates that gunshot deaths are more likely to be instantaneous.

Table 7d - Substance P Serum Levels In Homicidal And Suicidal Gunshot Deaths

Incident-Death Time Interval	N	Mean	Median	Standard Deviation
0	19	34.16	27.80	24.08
2-15 minutes	10	30.85	18.05	23.50
25-90 minutes	4	63.63	55.00	31.98
4.5-9 hours	4	71.90	65.20	48.20
14-40 hours	3	182.87	162.50	57.52
4-14 days	3	118.07	121.90	28.35
4-8 months	3	65.20	78.90	50.66

Mean and median levels in pg/ml serum.

Table 7e - Bradykinin Serum Levels In Homicidal And Suicidal Gunshot Deaths

Incident-Death Time Interval	N	Mean	Median	Standard Deviation
0	19	37.31	34.10	32.40
2-15 minutes	10	32.16	26.75	27.23
25-90 minutes	4	38.30	33.40	27.97
4.5-9 hours	4	91.48	94.25	9.69
14-40 hours	3	120.93	104.20	31.44
4-14 days	3	90.80	66.60	73.07
4-8 months	3	36.17	24.50	28.64

Table 7f - Met-Enkephalin Serum Levels In Homicidal And Suicidal Gunshot Deaths

Incident-Death Time Interval	N	Mean	Median	Standard Deviation
0	19	17.02	15.50	9.15
2-15 minutes	10	11.18	10.25	6.14
25-90 minutes	4	35.05	35.70	18.43
4.5-9 hours	4	31.00	30.00	18.45
14-40 hours	3	43.90	29.90	41.03
4-14 days	3	45.60	27.40	47.59
4-8 months	3	165.67	216.00	117.67

Mean and median levels in pg/ml serum.

Table 7g - Substance P Serum Levels In Homicidal And Suicidal Stabbing Deaths

Incident-Death Time Interval	N	Mean	Median	Standard Deviation
0	6	87.20	72.95	66.80
4-20 minutes	4	50.78	57.35	30.07
30-40 minutes	7	78.64	72.20	25.57
50-60 minutes	3	68.57	70.80	43.49
3-10 hours	3	99.20	119.60	65.82
8-15 days	2	148.40	148.40	137.46

Table 7h - Bradykinin Serum Levels In Homicidal And Suicidal Stabbing Deaths

Incident-Death Time Interval	N	Mean	Median	Standard Deviation
0	6	62.38	60.00	38.06
4-20 minutes	4	46.95	53.45	27.30
30-40 minutes	7	78.53	55.10	52.75
50-60 minutes	3	64.20	54.80	27.63
3-10 hours	3	139.86	158.60	87.13
8-15 days	2	110.20	110.20	77.22

Mean and median levels in pg/ml serum.

Table 7i - Met-Enkephalin Serum Levels In Homicidal And Suicidal Stabbing Deaths

Incident-Death Time Interval	N	Mean	Median	Standard Deviation
0	6	23.47	18.55	21.34
4-20 minutes	4	26.00	17.05	28.31
30-40 minutes	7	34.09	36.30	12.59
50-60 minutes	3	47.23	30.80	47.34
3-10 hours	3	23.97	25.50	4.21
8-15 days	2	38.75	38.75	25.24

Table 7j - Substance P Serum Levels In Accidental And Suicidal Deaths Due To Thermal Burns

Incident-Death Time Interval	N	Mean	Median	Standard Deviation
0-15 minutes	8	54.86	57.65	29.26
1 day-1 week	3	128.90	127.00	36.99
17.5 days	1	172.90	172.90	NA
4 months	1	18.30	18.30	NA

Mean and median levels in pg/ml serum.

Table 7k - Bradykinin Serum Levels In Accidental And Suicidal Deaths Due To Thermal Burns

Incident-Death Time Interval	N	Mean	Median	Standard Deviation
0-15 minutes	8	94.07	73.20	76.69
1 day-1 week	3	155.67	170.10	57.04
17.5 days	1	244.20	244.20	NA
4 months	1	122.20	122.20	NA

Table 7l - Met-Enkephalin Serum Levels In Accidental And Suicidal Deaths Due To Thermal Burns

Incident-Death Time Interval	N	Mean	Median	Standard Deviation
0-15 minutes	8	43.50	34.20	45.27
1 day-1 week	3	44.50	57.50	29.44
17.5 days	1	329.40	329.40	NA
4 months	1	171.40	171.40	NA

Mean and median levels in pg/ml serum.

3. Blunt Trauma Deaths - Serum/Brain study

Due to limitations on the availability of blunt trauma deaths having varied incident-death time intervals, the number of cases examined and the number of time intervals in the serum/brain study is substantially less than the serum study. Conclusions drawn from the data, therefore, must be limited.

At first glance, peptide levels found in both the midbrain and serum in blunt

trauma deaths (Tables 7m, 7n, and 7o) show evidence of the bimodal response found in the serum study. However, examination of the case which may lead to such a conclusion shows that the apparent bimodal response in the serum/brain study is nothing more than an anomaly of the data. The case listed at the 28 hour incident-death time interval shows a quicker than usual antinociceptive response in the midbrain. This is evident by the comparatively high met-enkephalin and bradykinin levels (187.40 pg/g and 206.80 pg/g, respectively) and the comparatively low substance P level (72.0 pg/g). These values create a peak in the met-enkephalin and bradykinin data and a valley in the substance P data. Without the data from this case, only one peak in midbrain levels would be found for each peptide. The accelerated antinociceptive response in this case may be due to a greater amount of tissue damage and nociception. A tissue damage scale may be used to correlate the rate of nociception with the amount of tissue damage. Review of peptide levels in the corresponding serum sample show that the antinociceptive response is not yet evident. Serum levels for each peptide are reflective of an acute pain response. In particular, the substance P serum value (158.30 pg/ml) is high compared with the levels in adjacent time intervals. Evidently, the time interval between antinociception and death was so small that serum substance P levels could not reflect levels in the midbrain. As demonstrated at 0, 20 minutes, 2 hours, and 5 days - 18 hours and in studies previously examined, substance P serum levels typically lag behind midbrain

values at short incident-death time intervals. It is usually not until later incident-death time intervals, where parameters of chronic pain typically become apparent, that serum levels either reflect or exceed midbrain values.

The absence of any data in this study reflective of bimodal peptide release because of tissue injury or nociception does not prove that one does not exist in the midbrain. In fact, corresponding serum levels do not show the bimodal response evident in the previous serum study. The serum study had the benefit of cases with such varied incident-death time intervals that a more continual time frame was possible. The large gaps between time intervals (e.g. gap between 28 hours and 5 days - 18 hours) in the serum/brain study makes possible the chance of not finding the bimodal response when, in fact, it exists.

4. Gunshot, Stabbing, and Thermal Burn Deaths - Serum/Brain study

Mean and median peptide levels in the midbrain and corresponding serum samples show little deviation from one another and from blunt trauma deaths. Peptide levels in serum also correspond with levels found in the serum study. Despite limited number of incident-death time intervals in each type of death, the pattern of each peptide level with increasing time interval can still be discerned.

Like the serum study, levels of substance P in the midbrain of gunshot victims do not show the same level of immediate increase as blunt trauma deaths (Tables 7p, 7q, and 7r). At 0 time interval, mean substance P midbrain levels show a value of

Table 7m - Substance P Serum And Midbrain Levels In Blunt Trauma Deaths Classified As Accidents, Homicides, And Suicides

Incident-Death Time Interval	N	Midbrain			Serum		
		Mean	Median	Standard Deviation	Mean	Median	Standard Deviation
0	5	212.06	225.40	74.56	92.98	99.70	29.45
20 minutes	1	157.20	157.20	NA	49.10	49.10	NA
2 hours	1	130.20	130.20	NA	59.0	59.0	NA
28 hours	1	72.0	72.0	NA	158.30	158.30	NA
5 days - 18 hours	1	101.90	101.90	NA	50.60	50.60	NA
32 days	1	91.20	91.20	NA	109.50	109.50	NA

NA = Not Applicable

Mean and median levels in pg/ml serum or pg/g brain tissue.

Table 7n - Bradykinin Serum And Midbrain Levels In Blunt Trauma Deaths Classified As Accidents, Homicides, And Suicides

Incident-Death Time Interval	N	Midbrain			Serum		
		Mean	Median	Standard Deviation	Mean	Median	Standard Deviation
0	5	92.28	74.90	46.20	91.16	89.90	52.36
20 minutes	1	40.40	40.40	NA	71.90	71.90	NA
2 hours	1	41.70	41.70	NA	116.20	116.20	NA
28 hours	1	206.80	206.80	NA	134.30	134.30	NA
5 days - 18 hours	1	5.40	5.40	NA	56.30	56.30	NA
32 days	1	187.40	187.40	NA	55.80	55.80	NA

NA = Not Applicable

Mean and median levels in pg/ml serum or pg/g brain tissue.

Table 7o - Met-Enkephalin Serum And Midbrain Levels In Blunt Trauma Deaths Classified As Accidents, Homicides, And Suicides

Incident-Death Time Interval	N	Midbrain			Serum		
		Mean	Median	Standard Deviation	Mean	Median	Standard Deviation
0	5	73.68	82.00	40.10	35.88	44.80	23.49
20 minutes	1	57.30	57.30	NA	47.40	47.40	NA
2 hours	1	52.30	52.30	NA	19.90	19.90	NA
28 hours	1	187.40	187.40	NA	38.60	38.60	NA
5 days - 18 hours	1	33.40	33.40	NA	8.30	8.30	NA
32 days	1	153.80	153.80	NA	191.00	191.00	NA

NA = Not Applicable

Mean and median levels in pg/ml serum or pg/g brain tissue.

83.13 pg/g (the median value is even lower at 63.20 pg/g), while the mean level in blunt trauma deaths is 212.06 pg/g. Although the levels in both types of death begin to correspond at the next time interval (20 minutes for blunt trauma deaths and 15-40 minutes for gunshot deaths), the discrepancy at the 0 time interval may again indicate a diminished amount of nociceptive receptors in the brain (a common site of gunshot wounds causing death) and a larger amount of tissue trauma in blunt trauma deaths.

The thermal burn death at the 0 time interval show substance P midbrain and serum levels similar to those in blunt trauma deaths (Table 7s). Since only thermal burn death can rival blunt trauma death in the magnitude of tissue damage, this observation is not surprising.

In no better instance is the role of bradykinin in nociception and antinociception better demonstrated than in the comparison of the two thermal burn deaths in Table 7t. The case with no incident-death time interval shows a serum bradykinin level approximately five times higher than in the midbrain (267.10 pg/ml and 50.20 pg/g, respectively). The immediate rise in serum bradykinin levels is indicative of nociception and acute pain. Conversely, the case with a 3 month, 3 week incident-death time interval gives a midbrain bradykinin level of 202.10 pg/g and a serum level of 127.20 pg/ml. The large midbrain value is indicative of antinociception and chronic pain, while the still high bradykinin serum level represents the accumulation of bradykinin in the serum as a result of high levels in

the central nervous system.

Met-enkephalin midbrain and serum levels for both thermal burn deaths are given in Table 7u and reflect expected values.

Mean and median peptide levels in stabbing deaths are given for two incident-death time intervals (30-40 minutes and 3-10 hours) and are presented in Tables 7v, 7w, and 7x. For all three peptides, values reflect expected results.

Table 7p - Substance P Serum And Midbrain Levels In Homicidal And Suicidal Gunshot Deaths

Incident-Death Time Interval	N	Midbrain			Serum		
		Mean	Median	Standard Deviation	Mean	Median	Standard Deviation
0	7	83.13	63.20	44.58	36.24	31.90	17.18
15-40 minutes	4	142.53	136.25	39.82	59.40	55.50	37.95
1½-8 hours	3	160.70	185.50	107.27	82.37	75.80	50.57
24-38½ hours	2	329.20	329.20	17.11	193.05	193.05	77.43

Table 7q - Bradykinin Serum And Midbrain Levels In Homicidal And Suicidal Gunshot Deaths

Incident-Death Time Interval	N	Midbrain			Serum		
		Mean	Median	Standard Deviation	Mean	Median	Standard Deviation
0	7	48.04	49.80	0	59.99	43.90	40.89
15-40 minutes	4	38.55	41.25	9.56	58.40	60.75	24.72
1½-8 hours	3	39.60	37.40	7.93	69.83	96.80	49.15
24-38½ hours	2	49.95	49.95	10.96	130.70	130.70	37.48

Mean and median levels in pg/ml serum or pg/g brain tissue.

Table 7r - Met-Enkephalin Serum And Midbrain Levels In Homicidal And Suicidal Gunshot Deaths

Incident-Death Time Interval	N	Midbrain			Serum		
		Mean	Median	Standard Deviation	Mean	Median	Standard Deviation
0	7	39.57	26.30	27.56	16.76	16.20	5.22
15-40 minutes	4	33.33	27.60	17.51	33.15	30.10	18.85
1½-8 hours	3	45.67	48.30	4.56	15.90	12.30	8.40
24-38½ hours	2	30.50	30.50	3.68	20.80	20.80	12.87

Table 7s - Substance P Serum And Midbrain Levels In Accidental Deaths Due To Thermal Burns

Incident-Death Time Interval	N	Midbrain			Serum		
		Mean	Median	Standard Deviation	Mean	Median	Standard Deviation
0	1	189.70	189.70	NA	106.10	106.10	NA
3 months, 3 weeks	1	3.0	3.0	NA	18.3	18.3	NA

NA = Not Applicable

Mean and median levels in pg/ml serum or pg/g brain tissue.

Table 7t - Bradykinin Serum And Midbrain Levels In Accidental Deaths Due To Thermal Burns

Incident-Death Time Interval	N	Midbrain			Serum		
		Mean	Median	Standard Deviation	Mean	Median	Standard Deviation
0	1	50.20	50.20	NA	267.10	267.10	NA
3 months, 3 weeks	1	202.10	202.10	NA	127.20	127.20	NA

Table 7u - Met-Enkephalin Serum And Midbrain Levels In Accidental Deaths Due To Thermal Burns

Incident-Death Time Interval	N	Midbrain			Serum		
		Mean	Median	Standard Deviation	Mean	Median	Standard Deviation
0	1	37.10	37.10	NA	12.60	12.60	NA
3 months, 3 weeks	1	236.60	236.60	NA	171.40	171.40	NA

NA = Not Applicable

Mean and median levels in pg/ml serum or pg/g brain tissue.

Table 7v - Substance P Serum And Midbrain Levels In Homicidal Stabbing Deaths

Incident-Death Time Interval	N	Midbrain			Serum		
		Mean	Median	Standard Deviation	Mean	Median	Standard Deviation
30-40 minutes	5	127.84	127.70	57.94	67.28	62.50	17.03
3-10 hours	3	199.77	186.70	25.10	99.20	119.60	65.82

Table 7w - Bradykinin Serum And Midbrain Levels In Homicidal Stabbing Deaths

Incident-Death Time Interval	N	Midbrain			Serum		
		Mean	Median	Standard Deviation	Mean	Median	Standard Deviation
30-40 minutes	5	41.64	44.20	18.55	95.00	117.90	54.64
3-10 hours	3	30.47	29.60	6.05	139.80	158.60	87.13

NA = Not Applicable

Mean and median levels in pg/ml serum or pg/g brain tissue.

Table 7x - Met-Enkephalin Serum And Midbrain Levels In Homicidal Stabbing Deaths

Incident-Death Time Interval	N	Midbrain			Serum		
		Mean	Median	Standard Deviation	Mean	Median	Standard Deviation
30-40 minutes	5	35.58	28.60	17.60	35.50	36.30	13.36
3-10 hours	3	49.43	49.70	4.31	23.97	25.50	4.21

NA = Not Applicable

Mean and median levels in pg/ml serum or pg/g brain tissue.

H. Peptide Levels in all Infant/Child Deaths - Comparisons Between SIDS Deaths and Deaths Caused By Violent Trauma, Infection, and Other Clinical Etiologies

The determination of infant death caused by SIDS is often difficult for medical examiners since there are no clinical or physical manifestations associated with it. Often the determination of SIDS as the cause of death is made through a process of elimination with other possible causes. Very often, family history is taken into account. Since the determination of SIDS as a cause of death is made in such a fashion, the possibility of a misdiagnosis exists. For instance, since subtle forms of child abuse syndrome such as shaking baby syndrome may not produce any outward physical signs, it can easily be missed if the medical examiner does not suspect enough to look for it. In a case such as this, the diagnosis of SIDS is a distinct possibility. If such subtle forms of trauma cause the activation of nociception and SIDS does not, differences in serum and midbrain peptide levels may help distinguish the two. The following studies explore the possibility that peptide levels could distinguish SIDS from other types of infant-child deaths. SIDS deaths are compared against deaths caused by infections (e.g. pneumonia), other clinical etiologies (e.g. complications due to maturity, hypoglycemia, sickle cell anemia, congenital defects), and violent deaths. Violent deaths include child abuse homicides, blunt trauma accidents, and choking. Additionally, violent deaths are divided into four incident-death time intervals: 0, 10 minutes - 8 hours, 4-6 days, and 4 months, 15 days. Since the central and peripheral nervous system are still growing in children as well as

infants (which may cause peptide levels to differ from adults), all categories will include subjects up to 13 years of age.

1. Serum study

Mean and median substance P, bradykinin, and met-enkephalin serum levels for each category of death are presented respectively in Tables 8a, 8b, and 8c.

Table 8a - Substance P Serum Levels In All Infant-Child Deaths

Type Of Death	N	Mean	Median	Standard Deviation
SIDS	12	22.56	18.25	17.11
Infectious	10	24.13	23.75	8.98
Other Clinical	17	22.41	20.70	12.27
Violent. Incident-Death Time Interval = 0	8	22.37	22.30	9.36
Violent. Incident-Death Time Interval = 10 minutes-8 hours	5	70.64	75.90	35.16
Violent. Incident-Death Time Interval = 4-6 days	3	159.23	187.20	61.98
Violent. Incident-Death Time Interval = 4 months. 15 days	1	13.8	13.8	NA

Mean and median levels in pg/ml serum.

Table 8b - Bradykinin Serum Levels In All Infant-Child Deaths

Type Of Death	N	Mean	Median	Standard Deviation
SIDS	12	31.09	23.90	24.84
Infectious	10	35.34	34.40	29.53
Other Clinical	17	19.49	19.30	13.97
Violent. Incident-Death Time Interval = 0	8	26.21	26.35	20.19
Violent. Incident-Death Time Interval = 10 minutes-8 hours	5	48.52	41.80	16.49
Violent. Incident-Death Time Interval = 4-6 days	3	272.77	275.60	86.08
Violent. Incident-Death Time Interval = 4 months. 15 days	1	375.60	375.6	NA

Table 8c - Met-Enkephalin Serum Levels In All Infant-Child Deaths

Type Of Death	N	Mean	Median	Standard Deviation
SIDS	12	18.75	16.40	13.17
Infectious	10	14.82	12.80	8.86
Other Clinical	17	19.95	13.90	17.62
Violent. Incident-Death Time Interval = 0	8	23.15	18.25	19.63
Violent. Incident-Death Time Interval = 10 minutes-8 hours	5	41.16	14.60	46.81
Violent. Incident-Death Time Interval = 4-6 days	3	42.87	54.30	20.41
Violent. Incident-Death Time Interval = 4 months. 15 days	1	166.20	166.20	NA

Mean and median levels in pg/ml serum.

Substance P, bradykinin, and met-enkephalin serum levels in SIDS deaths, and deaths due to infection and other clinical reasons are all comparable. The concentration of each peptide in these types of death is not suggestive of either acute or chronic pain. Additionally, no deviation in serum peptide levels is seen in violent death with no incident-death time interval.

Changes in peptide levels, however, are observed in violent death with a measurable incident-death interval. At the 10 minute-8 hour interval, peptide levels begin to elevate suggestive of the initiation of nociception and the inflammatory process. At the 4-6 day interval, the parameters of acute pain are fully evident as the serum levels of substance P and bradykinin are at their highest. The mean level of substance P at this interval (159.23 pg/ml) is more than twice the value at the 10 minute-8 hour interval. Furthermore, the mean substance P level is more than six fold the value of each category of clinical death and violent deaths with no incident-death time interval. Similarly, the mean level of bradykinin (272.77 pg/ml) at this interval is more than five times the value at the 10 minute-8 hour interval. The mean value of bradykinin at this interval is also more than seven times the levels in each category of clinical death and violent deaths with no incident-death time interval. Met-enkephalin serum levels at the 4-6 day interval remain unchanged from the value observed at the 10 minute - 8 hour time interval.

In the case with the 4 month, 15 day incident-death time interval, substance

P and bradykinin levels reflective of chronic pain are observed. Met-enkephalin levels (166.20 pg/ml) rise to approximately four times the value at the 4-6 day interval. Conversely, substance P levels fall to 13.8 pg/ml. The high serum level of bradykinin (375.60 pg/ml) in this case may be due to expected high levels of antinociceptive bradykinin in the central nervous system entering the general circulation. Given the cause of death in the current case (6 month old immersed in boiling water causing second and third degree burns over 67% of the body), it is not surprising that serum bradykinin levels are high since antinociceptive processes in the central nervous system would be expected to be accelerated. Additionally, the extensive amount of tissue injury in this kind of death may prolong the inflammatory response keeping bradykinin serum levels elevated.

Peptide levels in SIDS death were compared with the other types of death using t testing to see if differences in peptide levels between deaths are statistically significant (Table 8d, 8e, and 8f). Substance P serum levels in SIDS deaths only show a significant difference when compared with violent deaths with incident-death time intervals of 10 minutes-8 hours and 4-6 days. This significant difference in substance P levels indicates that parameters of acute pain and nociception become apparent in the serum of violent death victims having incident-death time intervals in this range and are not apparent in the serum of SIDS victims. Similarly, the lack of significant difference in substance P levels between SIDS deaths and infectious

Table 8d - t Testing Comparisons Of Mean Substance P Serum Levels In SIDS Deaths With Other Infant-Child Deaths

Type Of Death	$\bar{x}_1 - \bar{x}_2$	Degrees Of Freedom	Critical t Value At 95% Confidence Level	Calculated t Value	Type Of Difference
Infectious	1.57	20	1.96	11.52	Not Significant
Other Clinical	0.15	27	1.96	10.48	Not Significant
Violent, Incident-Death Interval = 0	0.19	18	1.96	12.70	Not Significant
Violent, Incident-Death Interval = 10 minutes-8 hours	48.08	15	1.96	33.32	Significant
Violent, Incident-Death Interval = 4-6 days	136.67	13	2.16	87.98	Significant
Violent, Incident-Death Interval = 4 months, 15 days	8.76	11	2.20	37.92	Not Significant

Table 8c - t Testing Comparisons Of Mean Bradykinin Serum Levels In SIDS Deaths With Other Infant-Child Deaths

Type Of Death	$x_1 - x_2$	Degrees Of Freedom	Critical t Value At 95% Confidence Level	Calculated t Value	Type Of Difference
Infectious	4.25	20	1.96	22.23	Not Significant
Other Clinical	11.60	27	1.96	14.55	Not Significant
Violent, Incident-Death Interval = 0	4.88	18	1.96	20.27	Not Significant
Violent, Incident-Death Interval = 10 minutes-8 hours	17.43	15	1.96	24.67	Not Significant
Violent, Incident-Death Interval = 4-6 days	241.68	13	2.16	149.88	Significant
Violent, Incident-Death Interval = 4 months, 15 days	344.51	11	2.20	225.48	Significant

Table 8f - t Testing Comparisons Of Mean Met-Enkephalin Serum Levels In SIDS Deaths With Other Infant-Child Deaths

Type Of Death	$x_1 - x_2$	Degrees Of Freedom	Critical t Value At 95% Confidence Level	Calculated t Value	Type Of Difference
Infectious	3.93	20	1.96	9.51	Not Significant
Other Clinical	1.20	27	1.96	11.59	Not Significant
Violent, Incident-Death Interval = 0	4.4	18	1.96	14.06	Not Significant
Violent, Incident-Death Interval = 10 minutes-8 hours	22.41	15	1.96	29.10	Not Significant
Violent, Incident-Death Interval = 4-6 days	24.12	13	2.16	23.97	Significant
Violent, Incident-Death Interval = 4 months, 15 days	147.45	11	2.20	97.98	Significant

and other clinical deaths also indicates that acute pain parameters are not present in the serum of victims of these types of death. A significant difference also does not exist between SIDS death and the violent death having an incident-death time interval of 4 months, 15 days. Although the levels between the two types of death are comparable (SIDS deaths have a mean substance P serum value of 22.56 pg/ml, while the substance P levels in the violent death is 13.8 pg/ml), the mechanism of obtaining the value is different. While the 22.56 pg/ml substance P value in SIDS deaths appears not be reflective of either nociception or antinociception, the low 13.8 pg/ml substance P value in the violent death is apparently due to antinociception.

Comparisons of met-enkephalin serum levels among the various types of death support this. The large discrepancy in met-enkephalin serum levels between SIDS deaths (mean level of 18.75 pg/ml) and the violent death with a 4 month, 15 day incident-death time interval (166.20 pg/ml) is due to antinociceptive processes in the violent death causing serum met-enkephalin levels to rise. Although the met-enkephalin level is much lower at the 4-6 day time interval (42.87 pg/ml), a significant difference still exists when compared to SIDS deaths indicating that chronic pain parameters begin to become evident in the serum of violent death victims having incident-death time intervals in this range.

Significant differences do not exist in met-enkephalin serum levels between SIDS deaths and infectious deaths, other clinical deaths, and violent deaths with no

incident-death time interval. Since mean and median serum levels are all very low and comparable, chronic pain parameters are not evident.

A significant difference also does not exist in met-enkephalin serum levels between SIDS deaths and violent deaths having an incident-death time interval of 10 minutes-8 hours. In this case, the mean met-enkephalin serum level in the violent death is slightly higher than that of SIDS deaths (41.16 pg/ml compared to 18.75 pg/ml) and not much different than violent deaths with a 4-6 day time interval (42.87 pg/ml). Despite the lack of significant difference, it is apparent that the rise in the level of serum met-enkephalin indicates that chronic pain and antinociceptive processes are becoming evident.

Similar to substance P, bradykinin serum levels in SIDS deaths are not significantly different from levels in infectious deaths, other clinical deaths, and violent death with no incident-death time interval (Table 8e). Since mean and median levels are all low and comparable, acute pain and nociception activation are not evident in the serum from these deaths.

A significant difference in bradykinin serum levels is also not observed between SIDS deaths and violent deaths having an incident-death time interval of 10 minutes-8 hours. This is in contrast to substance P levels where a significant difference was observed. Although no statistical difference is observed when compared to SIDS deaths, the mean bradykinin serum level in violent deaths with an

incident-death time interval of 10 minutes-8 hours follows the trend of increasing with longer incident-death time intervals. Mean levels almost double from violent deaths with no incident-death time interval (48.52 pg/ml compared to 26.21 pg/ml).

Significant differences do exist in bradykinin serum levels between SIDS deaths and violent deaths with incident-death time intervals of 4-6 days and 4 months, 15 days. The former appears to be due to nociceptive and acute pain processes, while the latter appears to be due to high levels of antinociceptive bradykinin in the central nervous system entering the general circulation.

2. Serum/Brain study

Due to the inherent limitation in the availability of cases, only four types of infant/child deaths are presented in the serum/brain study: SIDS, other clinical deaths, and violent deaths with incident-death time intervals of 30-40 minutes and 4 months, 15 days, respectively. The latter violent death category encompasses only one case (the same case that made up this interval in the serum study). Since the total number of cases in this study is not enough for reliable interpretation of t-testing results [9], statistical comparison of SIDS cases with the other three categories of death were not conducted.

Substance P, bradykinin, and met-enkephalin midbrain and serum levels do not provide any evidence of activation of either nociceptive or antinociceptive processes in SIDS deaths (Tables 8g, 8h, and 8i). In both the midbrain and serum,

Table 8g - Substance P Serum And Midbrain Levels In All Infant-Child Deaths

Type Of Death	N	Midbrain			Serum		
		Mean	Median	Standard Deviation	Mean	Median	Standard Deviation
SIDS	3	31.47	28.10	17.99	29.77	33.11	18.03
Clinical	2	54.45	54.45	8.13	17.80	17.80	4.38
Violent, Incident-Death Time Interval = 30-40 minutes	3	182.37	172.30	135.28	82.83	36.10	90.55
Violent, Incident-Death Time Interval = 4 months, 15 days	1	23.40	23.40	NA	13.80	13.80	NA

NA = Not Applicable

Mean and median levels in pg/ml serum or pg/g brain tissue.

Table 8h - Bradykinin Serum And Midbrain Levels In All Infant-Child Deaths

Type Of Death	N	Midbrain			Serum		
		Mean	Median	Standard Deviation	Mean	Median	Standard Deviation
SIDS	3	35.0	33.40	8.61	42.20	34.90	32.67
Clinical	2	48.50	48.50	38.33	38.45	38.45	12.66
Violent, Incident-Death Time Interval = 30-40 minutes	3	67.23	66.10	2.41	152.73	58.50	177.43
Violent, Incident-Death Time Interval = 4 months, 15 days	1	295.30	295.30	NA	375.6	375.6	NA

NA = Not Applicable

Mean and median levels in pg/ml serum or pg/g brain tissue.

Table 8i - Met-Enkephalin Serum And Midbrain Levels In All Infant-Child Deaths

Type Of Death	N	Midbrain			Serum		
		Mean	Median	Standard Deviation	Mean	Median	Standard Deviation
SIDS	3	30.80	22.30	26.59	13.20	11.40	3.38
Clinical	2	30.50	30.50	13.15	25.20	25.20	4.81
Violent, Incident-Death Interval = 30-40 minutes	3	64.83	64.86	10.95	30.27	20.90	20.98
Violent, Incident-Death Interval = 4½ months	1	183.10	183.10	NA	166.20	166.20	NA

NA = Not Applicable

Mean and median levels in pg/ml serum or pg/g brain tissue.

peptide levels are all comparably low. In comparison, other clinical deaths do show slightly higher substance P (54.45 pg/g compared to 31.47 pg/g) and bradykinin (48.50 pg/g compared to 35.0 pg/g) midbrain levels, albeit not large enough to conclude that nociceptive or antinociceptive activation has occurred.

Conversely, it is evident by the values reported that nociceptive activation has occurred in the violent deaths with an incident-death time interval of 30-40 minutes. Consistent with nociception, substance P midbrain levels and bradykinin serum levels are inordinately high (182.37 pg/g and 152.73, respectively). Furthermore, substance P serum levels (82.83 pg/ml) are high compared with those of both SIDS and other clinical deaths.

When compared with SIDS and other clinical deaths, bradykinin and met-enkephalin midbrain levels hint that antinociception activation is in the early stages. With both peptides, the levels show slight increases. The level of met-enkephalin in the midbrain (64.83 pg/g) is more than twice the levels of both SIDS and other clinical deaths. Similarly, bradykinin levels in the midbrain (64.83 pg/g) are 1.9 and 1.4 fold higher than in SIDS and other clinical deaths, respectively.

Peptide levels in the midbrain of the violent death case with an incident-death time interval of 4 months, 15 days are reflective of antinociception and chronic pain. Bradykinin and met-enkephalin levels are high, as expected, (295.30 pg/g and 183.10 pg/g, respectively), while substance P levels are low (23.40 pg/g). Similar levels of

met-enkephalin and substance P in the serum also typify antinociception and chronic pain. Although the numerical value of serum bradykinin is more consistent with nociception, the high value (375.60 pg/ml) may, in part, be due to the migration of central nervous system antinociceptive bradykinin into the general circulation. Additionally, inflammatory processes still at work in the serum may contribute to the high bradykinin level. This is certainly possible considering that the cause of death in this case is thermal burns with extensive tissue damage.

I. Comparison of Mean Peptide Serum Levels at Similar Incident-Death Time Intervals in all Adult and Infant/Child Violent Deaths

Mean substance P, bradykinin, and met-enkephalin serum levels at similar incident-death time intervals in all adult and infant/child violent deaths were compared to determine if significant differences in peptide levels exist between adult and infant/child violent death. Comparisons in four ranges of incident-death time intervals were made using t testing (Tables 9a, 9b, and 9c). The four ranges include time intervals of zero, minutes-hours, days, and months. By performing these comparisons, an assessment of the effect of age on peptide levels in violent death can be made.

In each of the four incident-death time ranges, substance P and met-enkephalin serum levels show no significant difference between adult and infant/child violent deaths. Similarly, bradykinin serum levels show no significant difference in 3 of the

4 time ranges. Only in the time range measuring in months is a significant difference observed. In this comparison, nine adult deaths were compared against one infant/child death. It is difficult to draw any conclusion from a comparison which includes only one case in one of the compared groups. Statistical analysis may be unduly affected if the one case includes potential anomalies particular to the case. Indeed, both nociception and antinociception appear to be accelerated in the case in question due to an atypical amount of tissue damage (the case having an incident-death time interval of 4 months, 15 days was discussed in Section G in both the serum and serum/brain study). Despite this discrepancy, no evidence exists that serum peptide levels differ in adult and infant/child violent deaths.

Table 9a - t Testing Comparisons Of Mean Substance P Serum Levels At Similar Incident-Death Time Intervals In All Adult And Infant-Child Violent Deaths

Incident-Death Time Interval (Adult)	Incident-Death Time Interval (Infant/Child)	$x_1 - x_2$	Degrees Of Freedom	Critical t Value At 95% Confidence Level	Calculated t Value	Type Of Difference
0	0	22.72	89	1.96	26.77	Not Significant
1-4 hours	10 minutes-8 hours	8.24	17	1.96	46.37	Not Significant
3.5-7 days	4-6 days	45.11	11	2.20	90.82	Not Significant
1-8 months	4 months, 15 days	40.79	8	2.31	106.14	Not Significant

Table 9b - t Testing Comparisons Of Mean Bradykinin Serum Levels At Similar Incident-Death Time Intervals In All Adult And Infant-Child Violent Deaths

Incident-Death Time Interval (Adult)	Incident-Death Time Interval (Infant/Child)	$x_1 - x_2$	Degrees Of Freedom	Critical t Value At 95% Confidence Level	Calculated t Value	Type Of Difference
0	0	22.58	89	1.96	31.56	Not Significant
1-4 hours	10 minutes-8 hours	37.12	17	1.96	62.73	Not Significant
3.5-7 days	4-6 days	153.29	11	2.20	165.22	Not Significant
1-8 months	4 months, 15 days	321.40	8	2.31	265.60	Significant

Table 9c - t Testing Comparisons Of Mean Met-Enkephalin Serum Levels At Similar Incident-Death Time Intervals In All Adult And Infant-Child Violent Deaths

Incident-Death Time Interval (Adult)	Incident-Death Time Interval (Infant/Child)	$\bar{x}_1 - \bar{x}_2$	Degrees Of Freedom	Critical t Value At 95% Confidence Level	Calculated t Value	Type Of Difference
0	0	1.29	89	1.96	17.25	Not Significant
1-4 hours	10 minutes-8 hours	13.96	17	1.96	32.18	Not Significant
3.5-7 days	4-6 days	8.34	11	2.20	40.63	Not Significant
1-8 months	4 months, 15 days	31.57	8	2.31	244.54	Not Significant

J. Peptide Levels in Clinical Non-Violent Deaths

Levels of substance P, bradykinin, and met-enkephalin were determined in the serum and brain tissue of victims who die from clinical and non-violent means. Depending on the nature of the illness, markers of acute or chronic pain may be present. It may be more likely that post-mortem peptide levels typical of chronic pain in long-term illnesses are typically accompanied by pain during their duration (i.e. AIDS, some malignancies). However, many long-term illnesses are latent and may not be characterized by pain until the disease becomes symptomatic. Pain often develops just prior to death (i.e. cardiovascular diseases, diabetes). In these instances, peptide levels may be more reflective of acute pain. Furthermore, some etiologies of clinical or non-violent death may develop acutely (i.e. acute pancreatitis, acute renal failure). Post-mortem peptide levels are similarly likely to be consistent with nociception and acute pain [18]. The types of cases examined were listed above.

1. Serum study

Serum peptide levels are unremarkable for many of the clinical deaths examined (Tables 10a-c). Very little deviation (in both mean and median levels) from normal (serum values obtained in alive healthy subjects) was observed in the levels of all three peptides in deaths caused by non-diabetes pancreatic, hepatic, renal, central nervous system (this category contains only diseases of the brain; no deaths caused by spinal cord disease were available during the study), non-AIDS infectious

disease, cardiovascular, respiratory, emboli, and immunological/hematological etiologies. Mean peptide values ranged from 26.50-56.63 pg substance P/ml, 21.60-72.99 pg bradykinin/ml, and 25.45-54.37 pg met-enkephalin/ml in this types of death. For all three peptides, the low range value came from the immunological/hematological disease category. Deaths caused by sickle cell anemia, autoimmune disease, and non-cancer blood cell disease are found in this group. One surprising result is the normal peptide serum levels found in deaths with a non-diabetes pancreatic etiology. This group is made up primarily of individuals who have died from either acute or chronic pancreatitis. Although acute pancreatitis is by far the more painful, it is commonly known that both conditions typically cause various degrees of pain [18]. The cases making up this group in the study may simply not demonstrate the level of pain expected in these kinds of deaths.

Evidence of acute pain is demonstrated by relatively high serum substance P and bradykinin levels in deaths caused by gastrointestinal disease, non-cerebral hemorrhage, diabetes, and embolism (Tables 10a-c). Though the levels of substance P and bradykinin are only modestly increased from normal ranges, they nonetheless are suggestive of the activation or early stages of nociception. In particular, all four types of death have elevated bradykinin serum levels ranging from 70.31-96.94 pg/ml (mean values) and with met-enkephalin levels not suggestive of antinociception.

Table 10a - Substance P Serum Levels In Clinical Non-Violent Deaths

Etiology	N	Mean	Median	Standard Deviation
Gastrointestinal	16	80.41	78.90	57.27
Non-Diabetes Pancreatic	7	42.90	35.20	28.46
Hepatic	12	28.74	20.30	25.28
Renal	10	40.47	26.15	33.75
CNS (brain)	18	42.09	28.35	32.27
Non-Cerebral Hemorrhage	6	75.45	67.80	57.25
Non-AIDS Infectious Disease	15	56.63	40.90	43.85
Cardiovascular	50	35.54	28.45	31.53
Respiratory	18	35.42	29.05	17.89
Malignancy	28	32.26	11.90	56.81
Diabetes Mellitus	10	44.65	42.20	27.87
AIDS	18	23.98	9.75	30.45
Embolism	16	58.84	65.50	41.51
Immunological/Hematological	4	26.50	17.30	27.56

Mean and median levels in pg/ml serum.

Table 10b - Bradykinin Serum Levels In Clinical Non-Violent Deaths

Etiology	N	Mean	Median	Standard Deviation
Gastrointestinal	16	96.94	82.25	59.83
Non-Diabetes Pancreatic	7	55.29	55.00	27.52
Hepatic	12	35.58	29.10	29.12
Renal	10	72.99	66.90	55.06
CNS (brain)	18	31.09	24.45	27.34
Non-Cerebral Hemorrhage	6	78.13	62.25	71.98
Non-AIDS Infectious Disease	15	65.04	37.70	74.37
Cardiovascular	50	34.13	19.45	34.17
Respiratory	18	58.57	53.70	33.73
Malignancy	28	133.63	124.05	102.27
Diabetes Mellitus	10	80.71	51.60	78.85
AIDS	18	102.10	82.15	60.19
Embolism	16	70.31	62.05	61.39
Immunological/Hematological	4	21.60	23.55	16.05

Mean and median levels in pg/ml serum.

Table 10c - Met-Enkephalin Serum Levels In Clinical Non-Violent Deaths

Etiology	N	Mean	Median	Standard Deviation
Gastrointestinal	16	57.16	59.25	29.23
Non-Diabetes Pancreatic	7	46.66	32.60	38.94
Hepatic	12	28.07	17.85	33.53
Renal	10	54.37	58.55	33.65
CNS (brain)	18	28.43	20.10	25.28
Non-Cerebral Hemorrhage	6	54.12	53.55	43.15
Non-AIDS Infectious Disease	15	40.29	14.60	63.98
Cardiovascular	50	26.17	12.35	32.69
Respiratory	18	34.98	25.45	32.44
Malignancy	28	137.95	143.50	66.09
Diabetes Mellitus	10	63.85	28.65	69.12
AIDS	18	120.61	135.00	48.51
Embolism	16	35.89	28.60	32.32
Immunological/Hematological	4	25.45	10.70	33.08

Mean and median levels in pg/ml serum.

Consistent with the early stages of nociception, only two of the four types show discernible increases in serum substance P (gastrointestinal disease and non-cerebral hemorrhage shows respective values of 80.41 pg/ml and 75.45 pg/ml). For both substance P and bradykinin, the high range value is observed in deaths caused by gastrointestinal disease. This is not surprising because ailments of the gastrointestinal tract (i.e. ulcers, diverticulitis, gastritis) are known to be painful.

Evidence of chronic pain and antinociception is demonstrated by high serum levels of met-enkephalin and bradykinin and low serum levels of substance P in deaths caused by AIDS and malignancy (Tables 10a-c). These results are similar to those obtained in violent deaths with a long duration (usually months) between incident and death. In particular, mean serum met-enkephalin levels in AIDS (120.61 pg/ml) and malignancy (137.95 pg/ml) deaths are suggestive of chronic pain. These values are approximately double the disease with the next highest level (diabetes mellitus, 63.85 pg/ml). Additionally, the high level of serum bradykinin in both types of death (mean values of 102.10 pg/ml and 133.63 pg/ml in AIDS and malignancy deaths, respectively) reflect the probable high level of antinociceptive bradykinin in the central nervous system. Furthermore, the low mean levels of substance P in both AIDS (23.98 pg/ml) and malignancy (32.26 pg/ml) deaths is consistent with the inhibition of substance P release by high levels of met-enkephalin. Given the often long duration of these typically painful diseases [18], these results are not surprising.

2. Insight Into Chronic and Acute Pain - t Testing of Mean Serum Levels in Non-Violent Deaths with all Instantaneous Violent Deaths and all Violent Deaths with a 1-8 Month Incident-Death Time Interval

Statistical tests for significance using t testing were conducted on mean peptide serum levels in various non-violent deaths with all instantaneous violent deaths and all violent deaths with a 1-8 month incident-death time interval (Tables 10d-e). Tests for significance were performed to try to gain greater insight into the state of nociception and antinociception in various types of non-violent or clinical death. Comparisons were made with instantaneous violent death in order to better determine if serum peptide levels are reflective of nociception and acute pain. In a previous discussion (pp. 64-70) of the violent death study, it was determined that nociceptive activity could not be detected in serum in instantaneous violent death. Peptide levels indicative of acute pain began to appear in the 20-50 minute incident-death time interval. If the substance P and/or the bradykinin serum level in a non-violent death is higher and to be significantly different than mean values found in instantaneous violent death, then the activation of nociception apparent in the serum of the non-violent death is indicated. Conversely, no significant difference is to be seen in met-enkephalin serum levels. Although, it has been observed in cases of extreme pain (such as in burning deaths), nociceptive and antinociceptive activity could be detected simultaneously. In these cases, a significant difference in met-enkephalin serum level may result.

Table 10d - t Testing Comparisons Of Mean Substance P Serum Levels In Various Non-Violent Deaths With All Instantaneous Violent Deaths And All Violent Deaths With A 1-8 Month Incident-Death Time Interval

Etiology Of Non-Violent Death	Incident-Death Time Interval In All Violent Deaths	$\bar{x}_1 - \bar{x}_2$	Degrees Of Freedom	Critical t Value At 95% Confidence Level	Calculated t Value	Type Of Difference
Gastrointestinal	0/1-8 months	35.32/44.16	97/14	1.96/2.16	23.19/37.28	Significant/Significant
Non-Diabetes Pancreatic	0/1-8 months	2.19/11.69	88/14	1.96/2.14	28.68/40.36	Not Significant/Not Significant
Hepatic	0/1-8 months	16.35/25.85	93/19	1.96/1.96	22.34/32.20	Not Significant/Not Significant
Renal	0/1-8 months	4.62/14.12	91/17	1.96/1.96	24.52/34.74	Not Significant/Not Significant
CNS (brain)	0/1-8 months	3.00/12.50	99/25	1.96/1.96	18.79/29.04	Not Significant/Not Significant
Non-Cerebral Hemorrhage	0/1-8 months	30.36/20.86	87/13	1.96/2.16	33.01/55.74	Not Significant/Not Significant
Non-AIDS Infectious Disease	0/1-8 months	11.54/2.04	96/22	1.96/1.96	21.39/35.54	Not Significant/Not Significant
Cardiovascular	0/1-8 months	9.55/19.05	131/57	1.96/1.96	12.58/24.14	Not Significant/Not Significant
Respiratory	0/1-8 months	9.67/19.17	99/25	1.96/1.96	18.01/23.92	Not Significant/Not Significant
Malignancy	0/1-8 months	12.83/22.33	109/35	1.96/1.96	18.66/40.78	Not Significant/Not Significant
Diabetes Mellitus	0/1-8 months	0.44/9.94	91/17	1.96/1.96	24.20/32.24	Not Significant/Not Significant
AIDS	0/1-8 months	21.11/30.61	99/25	1.96/1.96	24.10/30.78	Not Significant/Not Significant
Emboli	0/1-8 months	13.75/4.25	97/23	1.96/1.96	20.69/33.98	Not Significant/Not Significant
Immunological/Hematological	0/1-8 months	18.59/28.09	85/11	1.96/2.20	37.75/54.06	Not Significant/Not Significant

Table 10e - t Testing Comparisons Of Mean Bradykinin Serum Levels In Various Non-Violent Deaths With All Instantaneous Violent Deaths And All Violent Deaths With A 1-8 Month Incident-Death Time Interval

Etiology Of Non-Violent Death	Incident-Death Time Interval In All Violent Deaths	x_1-x_2	Degrees Of Freedom	Critical t Value At 95% Confidence Level	Calculated t Value	Type Of Difference
Gastrointestinal	0/1-8 months	48.15/42.74	97/14	1.96/2.16	26.94/39.28	Significant/Significant
Non-Diabetes Pancreatic	0/1-8 months	6.50/1.09	88/14	1.96/2.14	33.56/38.04	Not Significant/Not Significant
Hepatic	0/1-8 months	13.21/18.62	93/19	1.96/1.96	26.10/30.69	Not Significant/Not Significant
Renal	0/1-8 months	24.20/18.79	91/17	1.96/1.96	30.29/44.05	Not Significant/Not Significant
CNS (brain)	0/1-8 months	17.70/23.11	99/25	1.96/1.96	21.68/27.19	Not Significant/Not Significant
Non-Cerebral Hemorrhage	0/1-8 months	29.34/23.93	87/13	1.96/2.16	38.94/62.41	Not Significant/Not Significant
Non-AIDS Infectious Disease	0/1-8 months	16.25/10.84	96/22	1.96/1.96	27.61/52.32	Not Significant/Not Significant
Cardiovascular	0/1-8 months	14.66/20.07	131/57	1.96/1.96	14.77/25.42	Not Significant/Not Significant
Respiratory	0/1-8 months	9.78/4.37	99/25	1.96/1.96	21.88/28.75	Not Significant/Not Significant
Malignancy	0/1-8 months	84.84/79.43	109/35	1.96/1.96	31.55/72.93	Significant/Significant
Diabetes Mellitus	0/1-8 months	31.92/26.51	91/17	1.96/1.96	32.71/57.51	Not Significant/Not Significant
AIDS	0/1-8 months	53.31/47.90	99/25	1.96/1.96	26.35/46.94	Significant/Significant
Emboli	0/1-8 months	21.52/16.11	97/23	1.96/1.96	25.72/44.78	Not Significant/Not Significant
Immunological/Hematological	0/1-8 months	27.19/32.60	85/11	1.96/2.20	44.24/50.95	Not Significant/Not Significant

Table 10f - t Testing Comparisons Of Mean Met-Enkephalin Serum Levels In Various Non-Violent Deaths With All Instantaneous Violent Deaths And All Violent Deaths With A 1-8 Month Incident-Death Time Interval

Etiology Of Non-Violent Death	Incident-Death Time Interval In All Violent Deaths	$\bar{x}_1 - \bar{x}_2$	Degrees Of Freedom	Critical t Value At 95% Confidence Level	Calculated t Value	Type Of Difference
Gastrointestinal	0/1-8 months	32.72/77.47	97/14	1.96/2.16	14.66/52.72	Significant/Significant
Non-Diabetes Pancreatic	0/1-8 months	22.22/87.97	88/14	1.96/2.14	19.99/85.19	Significant/Significant
Hepatic	0/1-8 months	3.63/106.56	93/19	1.96/1.96	15.35/77.46	Not Significant/Significant
Renal	0/1-8 months	29.93/80.26	91/17	1.96/1.96	17.46/76.70	Significant/Significant
CNS (brain)	0/1-8 months	3.99/106.2	99/25	1.96/1.96	12.38/64.40	Not Significant/Significant
Non-Cerebral Hemorrhage	0/1-8 months	29.68/80.51	87/13	1.96/2.16	21.93/76.50	Significant/Significant
Non-AIDS Infectious Disease	0/1-8 months	15.85/94.34	96/22	1.96/1.96	18.66/76.53	Not Significant/Significant
Cardiovascular	0/1-8 months	1.73/108.46	131/57	1.96/1.96	9.68/44.85	Not Significant/Significant
Respiratory	0/1-8 months	10.54/99.65	99/25	1.96/1.96	13.19/64.20	Not Significant/Significant
Malignancy	0/1-8 months	113.51/3.32	109/35	1.96/1.96	26.67/57.07	Significant/Not Significant
Diabetes Mellitus	0/1-8 months	39.41/70.78	91/17	1.96/1.96	22.01/84.00	Significant/Not Significant
AIDS	0/1-8 months	96.17/14.02	99/25	1.96/1.96	23.86/56.81	Significant/Not Significant
Emboli	0/1-8 months	11.45/98.74	97/23	1.96/1.96	13.77/67.03	Not Significant/Significant
Immunological/Hematological	0/1-8 months	1.01/109.18	85/11	1.96/2.20	24.51/95.56	Not Significant/Significant

Comparisons were made with violent deaths with a 1-8 month incident-death time interval to ascertain if mean serum peptide levels are reflective of chronic pain. In the violent death study, it was clear by serum peptide levels that antinociception and chronic pain mechanisms were dominant in this incident-death time interval. It is expected that no significant difference in met-enkephalin levels will be observed if serum levels in the non-violent death are reflective of chronic pain. Conversely, the higher met-enkephalin levels are expected to be significantly different than the low met-enkephalin serum levels found in instantaneous violent death. A significant difference in met-enkephalin levels between a particular non-violent death and all violent deaths with an incident-death time interval of 1-8 months will indicate that the mechanisms of chronic pain and antinociception are not found in the serum of victims in the particular non-violent death.

Depending on the level of antinociception, serum bradykinin may or may not show a significant difference. If antinociceptive activity is optimal in a particular non-violent death resulting in significantly higher antinociceptive bradykinin present in the central nervous system, a significant difference may be observed in serum bradykinin levels (a mean value of 54.20 pg bradykinin/ml was observed in all violent deaths).

Results from t testing show more evidence of chronic than of acute pain in non-violent clinical deaths. Death due to gastrointestinal etiology is the sole example

of non-violent death showing evidence of acute pain. Significant differences are observed in substance P and bradykinin serum levels when compared with all instantaneous violent deaths. Serum bradykinin levels are also significantly different when compared with all violent deaths with a 1-8 month incident-death time interval. Since a significant difference was observed in met-enkephalin levels between gastrointestinal based clinical death and all violent deaths with the 1-8 month time interval, this significant difference in bradykinin levels is probably the result of nociception.

Evidence of antinociception is evident in several types of non-violent death to varying degrees. Deaths due to AIDS and malignancy show the largest activity of antinociception and chronic pain. For both types of death, mean met-enkephalin serum levels are not significantly different than all violent deaths with a 1-8 month incident-death time interval, but are significantly different than levels in violent instantaneous death. In addition, bradykinin serum levels in AIDS and malignancy deaths are significantly different when compared with both types of violent death. Given the high level of serum met-enkephalin and low levels of serum substance P in the two non-violent deaths, the high level of serum bradykinin is the obvious result of high antinociceptive activity.

Several types of non-violent, clinical death (diabetes mellitus, gastrointestinal, non-diabetes pancreatic, renal, and non-cerebral hemorrhage) show intermediate

antinociceptive activity. This group of non-violent deaths all have mean serum met-enkephalin values between the low value (24.44 pg/ml) found in instantaneous violent deaths and the high value (134.63 pg/ml) found in all violent deaths with a 1-8 month incident-death time interval. As a result, four of the five non-violent deaths show significant differences in serum met-enkephalin levels with both violent deaths. Having the largest serum met-enkephalin level (63.85 pg/ml) of this group, diabetes mellitus did not show a significant difference when compared with all violent deaths having a 1-8 month incident-death time interval.

By showing no significant difference in met-enkephalin levels with those of all instantaneous violent deaths but a significant difference when compared with all violent deaths with an incident-death time interval of 1-8 months, the remaining non-violent deaths (hepatic, central nervous system, non-AIDS infectious disease, cardiovascular, respiratory, embolism, and immunological/hematological etiologies) show no evidence of antinociceptive activity in the serum.

3. Serum/Brain study

With the exception of deaths with a gastrointestinal etiology, substance P serum levels show no evidence of nociception in non-violent deaths. Midbrain substance P levels, however, do show evidence of nociception in many of the other non-violent deaths. In several instances, the level of substance P in the midbrain approximately doubles that of levels in the serum (Table 10g). In addition to death

with a gastrointestinal etiology (where the midbrain substance P is appreciably higher than in serum), non-violent deaths caused by embolism, non-cerebral hemorrhage, and cardiovascular, respiratory, renal, and non-AIDS infectious disease show evidence of nociception in the midbrain due to substance P levels substantially higher than in serum. Two of these deaths, cardiovascular and respiratory, have midbrain and serum levels that are significantly different statistically (comparisons of midbrain and serum substance P levels for each non-violent death using t-testing are summarized in Table 10h). Marginally high substance P serum levels which are only slightly indicative of nociception are found only in gastrointestinal and non-cerebral hemorrhage deaths in this study.

Perhaps the best explanation of why these deaths have substance P levels in the midbrain indicative of nociception is that these deaths may characteristically have only a short duration of painful symptoms prior to death [18]. For example, deaths due to cardiovascular disease or embolism may be sudden and occur without prior painful symptoms. Similarly, hemorrhages and non-AIDS infectious diseases causing death are usually not chronic conditions and may be of short duration. The episodic event causing death, however, may initiate nociception and cause substance P levels in the brain to rise. Furthermore, substance P levels in serum may be unremarkable due to a lack of tissue injury or the necessary time lag between episode and death allowing for equilibration of substance P in the serum and nociceptive tissues. Long

term painful chronic conditions such as AIDS, non-diabetes pancreatic disease, and malignancy showed non-nociceptive levels of substance P in the midbrain as expected..

Alternatively, chronic clinical conditions may become more painful prior to death. Such a condition may cause peptide levels characteristic of both acute and chronic pain. Examination of peptide levels in renal etiologic deaths may be a case in point. Typical of early nociceptive activity, mean substance P levels in the midbrain are moderately high (97.23 pg/g) but are much lower in the serum (48.50 pg/ml). Mean bradykinin levels in the serum (126.0 pg/ml), however, are indicative of nociceptive activation. As shown in studies previously discussed (pp.81-91), it is not uncommon to find both high levels of serum bradykinin and unremarkable levels of serum substance P in the early stages of nociception.

The presence of antinociceptive activity in renal deaths is best demonstrated by high mean met-enkephalin levels in the midbrain (129.67 pg/g). Midbrain levels in renal deaths is the third highest in the study behind only AIDS and cancer deaths. Somewhat contrary to this finding, however, are the mean levels of serum met-enkephalin (63.23 pg/ml) and midbrain bradykinin (73.97 pg/g). Although slightly higher than normal (with the exception of bradykinin levels in burn deaths, these values exceed those found in each type of violent instantaneous death), these levels are only slightly indicative of antinociception or a chronically painful state. Perhaps

Table 10g - Substance P Serum And Midbrain Levels In Clinical Non-Violent Deaths

Etiology	N	Midbrain			Serum		
		Mean	Median	Standard Deviation	Mean	Median	Standard Deviation
Gastrointestinal	6	163.35	168.35	106.29	92.18	88.05	49.63
Non-Diabetes Pancreatic	3	52.43	29.20	51.72	51.83	35.20	43.41
Hepatic	7	66.19	46.50	66.47	45.67	33.70	32.79
Renal	3	97.23	94.10	91.84	48.50	37.80	34.42
CNS (brain)	3	87.60	93.60	19.12	60.13	47.50	37.29
Non-Cerebral Hemorrhage	2	172.20	172.20	37.48	89.80	89.80	16.69
Non-AIDS Infectious Disease	4	109.15	113.45	48.59	56.43	55.0	14.62
Cardiovascular	14	94.47	92.80	43.95	46.44	42.50	23.85
Respiratory	9	97.71	107.40	32.15	40.46	31.40	17.55
Malignancy	12	50.11	22.25	84.90	37.59	12.60	76.84
Diabetes	3	43.87	44.90	34.76	41.07	40.80	21.10
AIDS	7	18.03	16.60	11.78	16.19	13.40	9.72
Embolism	5	142.50	165.40	53.39	81.56	68.20	42.09

Mean and median levels in pg/ml serum or pg/g brain tissue.

Table 10h - t Testing Comparisons Of Mean Substance P Serum And Midbrain Levels In Various Types Of Clinical Non-Violent Death

Etiology Of Death	$\bar{x}_1 - \bar{x}_2$	Degrees Of Freedom	Critical t Value At 95% Confidence Level	Calculated t Value	Type Of Difference
Gastrointestinal	71.17	10	2.23	112.51	Not Significant
Non-Diabetes Pancreatic	0.60	4	2.78	96.95	Not Significant
Hepatic	20.52	12	2.18	60.51	Not Significant
Renal	48.73	4	2.78	153.28	Not Significant
CNS (brain)	27.47	4	2.78	69.18	Not Significant
Non-Cerebral Hemorrhage	82.90	2	4.30	228.50	Not Significant
Non-AIDS Infectious Disease	52.72	6	2.45	75.46	Not Significant
Cardiovascular Disease	48.03	26	1.96	31.45	Significant
Respiratory	57.25	16	1.96	35.77	Significant
Malignancy	12.52	22	1.96	63.57	Not Significant
Diabetes	2.80	4	2.78	58.47	Not Significant
AIDS	1.84	12	2.18	12.14	Not Significant
Embolism	60.94	8	2.31	81.16	Not Significant

these values reflect a lower level of pain experienced over a long duration.

The data examined previously from burn deaths may be analogous. It may be recalled that antinociceptive activity was detected at an earlier incident-death time interval than other types of violent death. It was postulated that antinociception was accelerated in these cases as a consequence of elevated nociceptive activity due to the extensive nature of tissue damage. If this is true, then it is reasonable to believe that intense pain prior to death may cause both the levels of nociceptive and antinociceptive peptides to rise.

Like renal etiologic deaths, met-enkephalin serum and midbrain levels of the other non-violent deaths (Table 10i) indicate the state of antinociception prior to death. The highest levels of met-enkephalin in both the serum and midbrain are found in deaths caused by AIDS (116.40 pg/ml and 143.31 pg/g) and malignancy (148.45 pg/ml and 192.62 pg/g). These levels indicate that AIDS and malignancy have the highest level of chronic pain just prior to death in the study.

Several other non-violent deaths have met-enkephalin serum and midbrain levels that are elevated to a lesser extent. Chronic pain is associated with diabetic deaths and deaths with a gastrointestinal, non-diabetes pancreatic, and hepatic etiology. Peptide levels indicative of chronic pain, however, are not as pronounced as those in AIDS and malignancy. In other words, the disease leading up to death may be painful but not as painful as AIDS or cancer. Mean met-enkephalin levels

in the midbrain range from 67.97 pg/g (diabetes) to 91.80 pg/g (non-diabetes pancreatic) and 53.40 pg/ml (hepatic) to 77.93 (diabetes) in the serum.

Elevated met-enkephalin levels in the midbrain in other deaths may have occurred as a result of acute antinociception brought on by severe nociceptive stimuli just prior to death. In each of these deaths (non-cerebral hemorrhage, embolism, central nervous system, cardiovascular, and respiratory), met-enkephalin levels in the serum are not suggestive of antinociception (mean serum levels range from 18.73 pg/ml to 37.30 pg/ml) despite the slight elevation in the midbrain (mean midbrain levels range from 43.66 pg/g to 60.10 pg/g). Such slight elevation in the midbrain only suggest that a chronically painful state did not exist prior to death.

Low levels of met-enkephalin in both the serum (mean value-22.08 pg/ml) and midbrain (mean value-24.18 pg/g) were found in deaths caused by non-AIDS infectious disease.

Comparisons of met-enkephalin serum and midbrain levels in each type non-violent death were performed using t-testing (Table 10j). Significant differences were found in only malignancy and cardiovascular deaths. The significant difference found in cardiovascular deaths lends some credence to the acute antinociception theory where elevated levels of met-enkephalin are found in the midbrain but not in the serum. The significant difference found in malignancy deaths shows the extent of chronic pain in this disease prior to death. Even though met-enkephalin levels are

Table 10i - Met-Enkephalin Serum And Midbrain Levels In Clinical Non-Violent Deaths

Etiology	N	Midbrain			Serum		
		Mean	Median	Standard Deviation	Mean	Median	Standard Deviation
Gastrointestinal	6	90.75	65.50	56.34	60.25	63.15	11.61
Non-Diabetes Pancreatic	3	91.80	132.40	71.62	63.0	82.70	50.24
Hepatic	7	72.80	40.80	52.92	53.40	32.60	40.18
Renal	3	129.67	134.60	121.28	63.23	72.20	43.74
CNS (brain)	3	58.03	35.50	60.82	18.73	20.90	8.75
Non-Cerebral Hemorrhage	2	60.10	60.10	16.26	20.60	20.60	10.32
Non-AIDS Infectious Disease	4	22.08	24.35	10.91	24.18	17.85	14.90
Cardiovascular	14	49.89	45.65	25.70	26.84	24.25	18.68
Respiratory	9	49.96	39.40	24.29	37.30	28.50	24.37
Malignancy	12	192.62	185.65	45.96	148.45	154.90	32.86
Diabetes	3	67.97	51.10	63.99	77.93	56.30	83.67
AIDS	7	143.31	130.20	25.71	116.40	119.30	33.85
Embolism	5	43.66	41.70	19.11	32.34	30.40	17.96

Mean and median levels in pg/ml serum or pg/g brain tissue.

very high in the serum indicating chronic pain, the even higher midbrain levels producing the significant difference suggest that met-enkephalin is still being released at a high rate in the central nervous system.

Levels of antinociceptive bradykinin in the midbrain are generally consistent with the state of antinociception determined by serum and midbrain levels of met-enkephalin for each non-violent death. Similar to the results obtained for midbrain met-enkephalin levels, AIDS and malignancy deaths show the highest mean level of antinociceptive bradykinin (180.70 pg/g and 188.89 pg/g, respectively) further demonstrating the extent of chronic pain in these diseases prior to death (Table 10k)

Other deaths show elevated bradykinin levels in the midbrain to a lesser extent. Many of these deaths (hepatic, renal, gastrointestinal, and diabetes) have similar levels of met-enkephalin, demonstrating a level of chronic pain prior to death lower than that of AIDS and malignancy. Mean midbrain bradykinin levels range from 65.65 pg/g (gastrointestinal) to 91.40 pg/g (hepatic).

Unlike these deaths, bradykinin midbrain levels found in non-diabetes pancreatic deaths are not consistent with midbrain met-enkephalin levels. Mean bradykinin levels in this type of death are lower than expected (56.20 pg/g) and are something of an enigma. Acute nociceptive stimuli can be ruled out as a reason for the higher met-enkephalin levels because substance P midbrain and serum levels and bradykinin serum levels do not reflect nociception. Although the standard deviation

Table 10j - t Testing Comparisons Of Mean Met-Enkephalin Serum And Midbrain Levels In Various Types Of Clinical Non-Violent Death

Etiology Of Death	$\bar{x}_1 - \bar{x}_2$	Degrees Of Freedom	Critical t Value At 95% Confidence Level	Calculated t Value	Type Of Difference
Gastrointestinal	30.50	10	2.23	53.98	Not Significant
Non-Diabetes Pancreatic	28.80	4	2.78	130.61	Not Significant
Hepatic	19.40	12	2.18	53.89	Not Significant
Renal	66.44	4	2.78	202.68	Not Significant
CNS (brain)	39.30	4	2.78	100.83	Not Significant
Non-Cerebral Hemorrhage	39.50	2	4.30	109.09	Not Significant
Non-AIDS Infectious Disease	2.10	6	2.45	21.03	Not Significant
Cardiovascular	23.05	26	1.96	18.51	Significant
Respiratory	12.66	16	1.96	22.63	Not Significant
Malignancy	44.17	22	1.96	36.10	Significant
Diabetes	9.96	4	2.78	151.72	Not Significant
AIDS	26.91	12	2.18	37.38	Not Significant
Embolism	11.32	8	2.31	27.0	Not Significant

of the three cases in this group is low (13.49), a larger sample size may be needed to determine if this result is disparate.

Other deaths (central nervous system, non-cerebral hemorrhage, cardiovascular, respiratory, and embolism) which have slightly elevated met-enkephalin levels as a probable consequence of acute antinociception also have supportive bradykinin midbrain levels. Mean levels range from 43.48 pg/g (embolism and respiratory) to 63.30 pg/g (central nervous system). In each of these cases, midbrain substance P levels are high reflecting nociception.

Similar to met-enkephalin values, deaths from non-AIDS infectious disease have bradykinin midbrain levels that are not suggestive of antinociceptive (mean value-31.13 pg/g). These low values are in contrast with those of the deaths just mentioned since, like the others, midbrain substance P levels (109.15 pg/g) reflect nociception. In addition, the high levels of bradykinin (102.50 pg/ml) and the low levels of substance P (56.43pg/ml) in the serum further demonstrates the presence of nociceptive stimuli just prior to death.

Bradykinin serum levels for all non-violent deaths are also presented in Table 10k. Unlike substance P serum data, the data from bradykinin serum levels in many of the non-violent death suggests the initiation of nociception prior to death. In addition to deaths caused by non-AIDS infectious disease, deaths caused by non-cerebral hemorrhage, embolism, and gastrointestinal, renal, and respiratory disease

have bradykinin serum levels reflective of nociception. Levels range from 81.41 pg/ml (respiratory) to 109.65 pg/ml (non-cerebral hemorrhage). With the exception of gastrointestinal disease, these deaths all have low levels of serum substance P indicating that nociception was in the early stages prior to death.

The level of nociceptive bradykinin in respiratory deaths is particularly noteworthy. Respiratory deaths are the only non-violent death that has a serum bradykinin level that is significantly different statistically from the bradykinin level in the midbrain (Table 10l). The significant difference suggests that any pain experienced prior to respiratory death is due solely to nociception. The difference in the level of nociceptive bradykinin compared to antinociceptive bradykinin appears also to be large in deaths caused by non-cerebral hemorrhage and non-AIDS infectious disease also indicating nociceptive activity. Statistical significance between nociceptive and antinociceptive bradykinin levels is not seen because of large standard deviations present in serum bradykinin levels in both deaths.

Bradykinin serum levels are also very high in malignancy (178.50 pg/ml) and AIDS deaths (96.26 pg/ml). Since the level of bradykinin is very high in the midbrain of these deaths, the high serum value may simply be due to antinociceptive bradykinin entering the general circulation from the central nervous system. Any influence that nociception has on bradykinin serum levels in these deaths therefore is not known.

Table 10k - Bradykinin Serum And Midbrain Levels In Clinical Non-Violent Deaths

Etiology	N	Midbrain			Serum		
		Mean	Median	Standard Deviation	Mean	Median	Standard Deviation
Gastrointestinal	6	65.65	52.40	31.74	99.38	88.45	34.79
Non-Diabetes Pancreatic	3	56.20	59.70	13.49	68.50	69.80	34.97
Hepatic	7	91.40	76.10	59.00	57.44	55.0	24.98
Renal	3	73.97	59.70	66.56	126.0	145.80	67.80
CNS (brain)	3	63.30	45.20	56.66	31.30	37.0	26.42
Non-Cerebral Hemorrhage	2	48.80	48.80	6.65	109.65	109.65	62.86
Non-AIDS Infectious Disease	4	31.13	29.95	16.39	102.50	71.85	86.65
Cardiovascular Disease	14	57.01	55.55	20.80	53.06	44.95	29.01
Respiratory	9	43.48	36.40	23.43	81.41	90.90	20.71
Malignancy	12	188.89	169.90	71.36	178.50	143.30	124.73
Diabetes	3	66.90	40.60	75.37	70.37	63.90	67.33
AIDS	7	180.70	187.80	75.22	96.26	70.80	55.39
Emboli	5	43.48	39.40	26.54	96.80	61.70	82.34

Mean and median levels in pg/ml serum or pg/g brain tissue.

Table 101 - t Testing Comparisons Of Mean Bradykinin Serum And Midbrain Levels In Various Types Of Clinical Non-Violent Death

Etiology Of Death	$x_1 - x_2$	Degrees Of Freedom	Critical t Value At 95% Confidence Level	Calculated t Value	Type Of Difference
Gastrointestinal	33.73	10	2.23	46.75	Not Significant
Non-Diabetes Pancreatic	12.30	4	2.78	55.93	Not Significant
Hepatic	33.96	12	2.18	54.72	Not Significant
Renal	52.03	4	2.78	150.97	Not Significant
CNS (brain)	32.0	4	2.78	98.17	Not Significant
Non-Cerebral Hemorrhage	60.85	2	4.30	217.84	Not Significant
Non-AIDS Infectious Disease	71.37	6	2.45	119.88	Not Significant
Cardiovascular Disease	3.95	26	1.96	18.41	Not Significant
Respiratory	37.93	16	1.96	26.79	Significant
Malignancy	10.39	22	1.96	79.63	Not Significant
Diabetes	3.47	4	2.78	145.16	Not Significant
AIDS	84.44	12	2.18	89.86	Not Significant
Emboli	53.32	8	2.31	93.74	Not Significant

Bradykinin serum levels in non-diabetes pancreatic (68.50 pg/ml), hepatic (68.50 pg/ml), central nervous system (31.30 pg/ml), cardiovascular (53.06 pg/ml), and diabetes (70.37 pg/ml) deaths are not suggestive of nociception.

K. Nociceptive/Antinociceptive Peptide Ratios in Non-Violent Deaths - Providing Objective Criteria for the Assessment of Pain in Medicolegal Investigation

Assessing pain is a subjective exercise [62]. When providing medical care for patients suffering with pain, physicians usually determine the course of treatment by the assessment patients have of their pain. In lieu of objective criteria, patients will often be asked to assess their pain on a scale from 1 to 10. Although the same level of pain may be present in two individuals with similar affliction, subjective interpretation on the part of the two individuals may render the reporting of pain different. Subjective interpretation of pain will undoubtedly be influenced by emotional and psychological factors unrelated to nociception.

Like physicians, jurors may use similar subjective criteria for assessing the pain of crime victims. Their own personal experiences and perceptions of pain may play a role in their subjectivity. In order for jurors to assess pain in an objective fashion, they must be given frames of reference for differing levels of pain. Simple presentation of the numerical values expressing the levels of nociceptive and antinociceptive peptides by themselves cannot provide good frames of reference. It would be difficult for trained scientists let alone the average juror to assess the

amount of pain a crime victim suffered prior to death by the examination of peptide levels. "Real-life" examples of manifestations of differing levels of pain need to be presented. For instance, it would be far more meaningful for a juror to know that the victim prior to death had pain similar to the pain encountered in cancer death than knowing that the serum level of met-enkephalin is high while that of substance P is low. Although differing opinions about the level of pain in cancer death probably exist based on perception or experience, the comparison to cancer pain at least presents something relatively tangible. Unfortunately, most people have experience dealing with cancer. Subjectivity can never be entirely eliminated from pain assessment or from any other aspect of juror deliberation. Determination of nociceptive/antinociceptive peptide ratios for each example of clinical non-violent death creates an objective scale which can be used to gauge the level of pain in violent deaths. Since the level of pain in violent death varies according to the incident-death time interval, the pain scale is temporally dependent. This temporal pain scale will provide a frame of reference for pain of varying degrees. The comparison of a calculated peptide ratio with matching values on the scale will objectively compare the level of pain with that typically found in a particular clinical non-violent death.

Calculation of the peptide ratio can be determined using the equation 2:

$$\text{equation 2: } \frac{[SPs] + [SPmb] + [Bs]}{[Bmb] + [MEs] + [MEmb]}$$

where:

[SPs] = mean concentration of substance P in serum,

[SPmb] = mean concentration of substance P in midbrain,

[Bs] = mean concentration of bradykinin in serum,

[Bmb] = mean concentration of bradykinin in midbrain.

[MEs] = mean concentration of met-enkephalin in serum.

[MEmb] = mean concentration of met-enkephalin in midbrain.

Table 11 below lists noniceptive/antinociceptive peptide ratios for each clinical non-violent death examined in the study.

It is apparent from examining the ratios that the lower the value, the greater the contribution of chronic pain and antinociception to the total pain experienced by victims prior to death. Not surprisingly, deaths caused by AIDS and malignancy have the lowest ratios in the study (0.30 and 0.50, respectively) since peptide levels from both deaths demonstrated the largest level of chronic pain. Other non-violent deaths which show a majority contribution of chronic pain to the total amount of pain but were deemed less chronically painful include diabetes (0.73), hepatic (0.78), and non-diabetes pancreatic (0.78). Victims of violent death with a incident-death time interval of long duration (e.g. weeks to months), would be expected to similarly give ratios that are relatively low.

Conversely, the higher the ratio the greater the contribution of acute pain to

**Table 11 - Nociceptive/Antinociceptive Peptide Ratios In Clinical Non-Violent Deaths
(Temporal Pain Scale)**

Etiology Of Death	Nociceptive/Antinociceptive Peptide Ratio
AIDS	0.30
Malignancy	0.50
Diabetes	0.73
Hepatic	0.78
Non-Diabetes Pancreatic	0.82
Renal	1.02
CNS (brain)	1.28
Cardiovascular	1.45
Gastrointestinal	1.64
Respiratory	1.68
Embolism	2.68
Non-Cerebral Hemorrhage	2.87
Non-AIDS Infectious Disease	3.46

the total amount of pain experienced prior to death. The deaths with the highest ratios include non-AIDS infectious disease (3.46), non-cerebral hemorrhage (2.87) and embolism (2.68). In each of these three deaths, the presence of low levels of antinociceptive peptides helped produce the high peptide ratios. The apparent absence of chronic pain from these three deaths is consistent with the often non-chronic nature of these illnesses.

The high ratios, however, does not necessarily mean that these deaths have

pre-mortem levels of pain greater than those deaths with ratios closer to one. It simply means that the contribution of acute pain to the total amount of pain is greater. Some deaths which show higher levels of both nociceptive and antinociceptive peptides are by definition considered more painful. However, the presence of high levels of both types of peptides may produce a ratio closer to 1.0. Examination of the peptide ratios from renal (1.02) and gastrointestinal deaths (1.64) are cases in point. The high levels of both nociceptive and antinociceptive peptides suggests that either the mechanisms of both acute and chronic pain are present (as seems to be the case in gastrointestinal deaths) or that acute antinociception has occurred as a result of severe nociceptive stimuli prior to death (which may be the case in renal deaths). Violent deaths with a short incident-death time interval would be expected to produce a ratio greater than one.

When using the pain ratio scale for evaluating the level of pain in a violent death in comparison with a non-violent death as equivalent, one criterion must be met. Levels of at least one peptide showing evidence of nociception or antinociception must be present. It does not make sense to quantify pain when no pain exists. Furthermore, endogenous levels of each peptide may produce ratios similar to cases where the contributions of both acute and chronic pain to total pain are approximately the same. In both cases, the similar quantitative values of each peptide may lead to a ratio hovering around 1.0.

By comparing peptide levels in violent death with those found typically in particular non-violent deaths, a pain “equivalent” may be found. By being able to qualify peptide levels, lay jurors and other members of the criminal or civil trial process may assess the level of pre-mortem pain experienced by a victim of a violent death in an objective fashion.

XVII. Review of Hypotheses

1. Although peptide levels for some types of instantaneous violent death are not significantly different statistically from levels in healthy adults, the data show empirically that the levels of each peptide are higher for each type of violent instantaneous death. Substance P increases range from 1.2 (drug overdose) to 3.5 (stabblings) fold, bradykinin increases range from 1.3 (drug overdose) to 4.6 (burns/electrocution) fold, and met-enkephalin increases range from 1.3 (gunshot) to 3.7 (burns/electrocution) fold. These increases, although not conclusive or significant in some types of violent instantaneous death, suggest that death does activate nociceptive transmission and modulating pathways. Since, however, statistical significance does not exist for any peptide in four of the eight violent deaths, only part of the hypothesis can be accepted.

2. Peptide levels in serum do differ in acutely and chronically painful death. Parameters indicative of acute pain are apparent in the serum of violent death victims when the incident-death interval is very short. In instantaneous death, the level of serum bradykinin is significantly higher statistically than in healthy adult serum. Similarly, elevated substance P levels begin to show statistical significance in violent deaths having an incident-death time interval of 20-50 minutes. Conversely, met-enkephalin serum levels do not rise significantly until the mechanisms of nociceptive modulation or antinociception become dominant. These mechanisms become dominant in violent death when the incident-death time interval measures in months. During these long incident-death time intervals in violent death, substance P and bradykinin levels fall off appreciably. Substance P and met-enkephalin serum levels are analogous in chronically painful diseases such as malignancy or AIDS. No significant difference was found in serum levels between these two diseases (as well as the other diseases and causes of death in the non-violent death study) and violent deaths having an incident-death time interval of 1-8 months.

Significant differences are seen, however, in bradykinin serum levels. Bradykinin serum levels are higher than expected in three of the non-violent deaths studied (AIDS, malignancy, and gastrointestinal). These levels are significantly different statistically when compared with violent deaths having a 1-8 month incident-death time interval. Due to results in the serum/brain study, the larger than

expected serum values are probably reflective of much larger levels of central nervous system antinociceptive bradykinin in these non-violent deaths. It was not anticipated that antinociceptive levels of bradykinin would affect serum bradykinin levels. It may be concluded, therefore, that the high level of serum bradykinin in AIDS, malignancy, and gastrointestinal etiologic deaths is indicative of the extent of chronic pain associated with these deaths.

The results from the serum studies may be case specific. The serum bradykinin level in the one violent death case in the serum/brain study having an incident-death time interval measured in months is similar to those of the three non-violent deaths.

Despite bradykinin results that differed from expected, the stated hypothesis that serum peptide levels differ in both pre-mortem acute and chronic pain is proven.

3. Midbrain and serum bradykinin levels are different in pre-mortem acute and chronic pain. This is best demonstrated in the comparison of the two thermal burn deaths with contrasting incident-death time intervals in Table 7t. The case with no incident-death time interval shows a serum bradykinin level approximately five magnitudes higher than in the midbrain. The immediate rise in serum bradykinin levels is indicative of the role peripheral bradykinin plays in nociception and acute pain. Conversely, the case with a 3 month, 3 week incident-death time interval gives

a midbrain bradykinin level nearly double the serum level. The large midbrain value is indicative of the role that central nervous system bradykinin plays in nociceptive modulation and chronic pain. Countless other examples of the contrasting levels of midbrain and serum bradykinin in pre-mortem acute and chronic pain are presented in the study.

Conversely, regardless of the state of pre-mortem pain, the levels of substance P and met-enkephalin in the midbrain and serum always reflect the role of the former in nociception and the role of the latter in antinociception. Although levels of both peptides may be larger in the midbrain during the very early stages of nociceptive transmission and modulation, the levels of both peptides in the midbrain are normally reflected in the serum. The hypothesis is accepted.

4. Medical conditions which affect peptide levels but which are not the cause of death can cause peptide levels in both the serum and brain tissue to be more reflective of the medical condition. Several examples are found in the case summaries (Appendix B). For instance, the victim in case #60 shows serum peptide levels indicative of chronic pain caused by the presence of osteoarthritis, although the primary manner of death is cardiovascular disease. Cardiovascular disease is also the primary manner of death in case #462, despite peptide levels reflecting the stomach malignancy present in the victim. Similarly, victims in cases #139, #358, #429, and #455 all show serum and

peptide levels reflective of chronic AIDS pain, although each victim died from a drug overdose. The hypothesis is accepted.

5. Changes in substance P and met-enkephalin levels due to nociceptive activation and modulation are usually observed in the midbrain before they are observed in serum. This is apparent by reviewing the data from the serum/brain study in “all violent deaths” (Tables 2g and 2I). This conclusion is further reinforced when the “all violent death” data is separated into accidental violent deaths (Tables 6m and 6o), homicides (Tables 6p and 6r), and suicides (Tables 6s and 6u). In each case, changes in substance P and met-enkephalin levels in response to nociception and antinociception occurs first in the midbrain.

In addition, substance P serum levels are normally not reflective of acutely painful death where the duration between incident and death is very small. Substance P levels indicative of the presence of nociceptive stimuli are, however, observed in the midbrain. This is best observed when examining substance P levels in violent instantaneous deaths (Table 5a). In most of the violent instantaneous death examined (blunt trauma, gunshot, hanging, drowning, and smoke inhalation), nociceptive response based on substance P levels is seen only in the midbrain. In the case of death by thermal burns, however, both the midbrain and serum showed high substance P levels. This is probably due to the extensive nature of tissue damage in

this type of death. Conversely, deaths by drug overdose (where the potential for nociceptive stimuli is not as great), demonstrated low levels of substance P in both the midbrain and serum. Notwithstanding the thermal burn data, the hypothesis is accepted.

6. Although the possibility cannot be discounted, psychological factors such as fear or depression probably do not play a role in causing peptide levels to statistically differ in subjects who die traumatically from suicides, accidents, and homicides where the duration between incident and death is similar. The presence of drug overdose deaths is probably the reason substance P and bradykinin serum levels are lower in instantaneous accidental violent death than in instantaneous homicides. At measurable time intervals where nociceptive mechanisms usually dominate, the lower substance P and bradykinin midbrain and serum levels in accidental violent deaths are possibly due to the acceleration of antinociception as a consequence of typically larger tissue damage in accidental violent deaths.

In suicides, the lower substance P and bradykinin levels and the higher met-enkephalin levels in both the midbrain and serum at incident-death time intervals where nociceptive processes are expected to dominate may result from the presence of chronic pain prior to death. The study contains case examples where chronic pain may have been present prior to suicidal death (i.e. Appendix B, case #47). The

hypothesis is accepted.

7. The mode of violent death (i.e. gunshot, blunt trauma) does affect peptide levels at certain incident-death time intervals. The variable that appears to affect the nociceptive response is the level of tissue damage typically associated with a particular mode of death. Those modes of death which tend to be characterized by extensive tissue trauma (i.e. blunt trauma, thermal burns), show increases in nociceptive serum and midbrain peptides levels indicative of acute pain at earlier incident-death time intervals. Similarly, these deaths also show an accelerated pre-mortem antinociceptive response characterized by increases in antinociceptive peptides at earlier incident-death time intervals than modes of death (i.e. gunshot) which may have less extensive tissue damage.

The area of tissue damage may also affect nociceptive response. In instantaneous gunshot and stabbing deaths in the serum study, levels of nociceptive serum peptides are smaller in the gunshot deaths. This may be explained by the fact that many of the gunshot deaths in the study occurred in the brain. Since the brain is not strongly innervated with nociceptive neurons, brain injury is not expected to stimulate nociception quickly. Thus, substance P and bradykinin serum levels would be expected to be lower in gunshot deaths where the incident-death time interval is of small duration. The hypothesis is accepted.

8. Peptide levels in violent infant and child deaths do not differ from similar adult deaths regardless of the incident-death time interval. With one exception, no significant difference is observed in serum peptide levels between adult and infant/child violent deaths in all incident-death time intervals tested. A significant difference is only observed in serum bradykinin levels in the incident-death time interval measuring in months. In this time interval, nine adult deaths were compared against one infant/child death. It is difficult to draw any conclusion from a comparison which includes only one case in one of the compared groups. Statistical analysis may be unduly affected if the one case includes potential anomalies particular to the case. Indeed, both nociception and antinociception appear to be accelerated in the case in question due to an extensive amount of tissue damage. Despite this discrepancy, no evidence exists that serum peptide levels differ in adult and infant/child violent deaths. Although data is limited, midbrain peptide levels in child/infant violent deaths appear to also mirror values in adult violent death. The hypothesis is accepted.

9. Serum peptide levels in SIDS deaths do differ from those in violent infant/child deaths. Substance P, bradykinin, and met-enkephalin serum levels in SIDS deaths do not reflect either nociceptive or antinociceptive activation and are each statistically different at certain incident-death time intervals. The most significant finding is that

a statistical difference in substance P serum levels does exist between SIDS deaths and violent deaths with a 10 minute to 8 hour incident-death time interval. If the assumption can be made that occult violent death has a measurable incident-death time interval, then this finding may ultimately prove useful in the exclusion of SIDS as a cause of death in cases where pathological examination yields little to suggest death by violent means.

Although tests for significance were not conducted using the midbrain data due to limited sample size, peptide midbrain levels in SIDS deaths reflect those in the serum and appear to differ substantially from levels in violent infant/child deaths at both incident-death time intervals tested. Unfortunately, instantaneous violent death cases were not available during the serum/midbrain study. Midbrain peptide data in instantaneous violent death would be useful in helping to differentiate between SIDS death and death by occult violence. In both the serum and serum/midbrain study, peptide levels in SIDS deaths do not differ from those in infectious and other clinical infant/child deaths. In the serum study, tests for significance between SIDS and these other non-violent deaths confirm that no significant differences exist in peptide levels. The hypothesis is accepted.

10. Peptide levels in respective non-violent deaths differ due to the nature (acute and chronic) and extent of pain in each particular non-violent death. Ratios of nociceptive

to antinociceptive serum and midbrain peptides in various non-violent deaths demonstrate the extent of pre-mortem acute and chronic pain in each type of death. For instance, the ratio is heavily skewed toward antinociception in the most chronically painful deaths such as cancer and AIDS. Conversely, deaths caused by typically acute illnesses such as non-AIDS infectious disease and non-cerebral hemorrhage show peptide levels highly indicative of nociception. Intermediate to these extremes in the study are several other non-violent clinical deaths which demonstrate peptide levels indicative of varying degrees of both acute and chronic pain. The ratio of nociceptive to antinociceptive serum and midbrain peptides for each non-violent death may be used as a scale for comparing the amount of pain experienced in a violent death. The hypothesis is accepted.

XVIII. The Problem of Defining Negative Controls

In determining the existence of pain in violent death victims, it may be necessary to statistically compare peptide levels with a properly defined negative control. The negative control should be peptide levels from deceased individuals who do not show any peptide level consistent with the activation of nociception. Negative control peptide levels should be mean values taken from either one type of death or from several types of death with a commonality (for instance, all

instantaneous violent deaths regardless of mode). Unfortunately, results from the current study show that defining a proper negative control will almost always be difficult.

It would seem likely that the best chance of finding peptide levels reflective of a negative control would come from instantaneous violent deaths from decedents with no clinical medical conditions that can effect peptide levels. However, as stated in section D in the discussion of results, it appears that elevated peptide levels are associated with death. These elevated levels may be due to the initiation of nociception, peptide response to tissue injury, or to the cellular release of endogenous peptides into surrounding tissue as a result of cell lysis. Bradykinin serum levels in instantaneous violent death regardless of mode, for instance, are significantly different from levels in normal healthy adults. Since bradykinin is responsible for the initial activation of peripheral afferents, it is plausible that elevated peptides levels in instantaneous violent death are due to the initiation of nociception. In addition, substance P serum levels are significantly different in violent death victims with an incident-death time interval of 20-50 minutes. This later increase in serum substance P is also consistent with the role this peptide plays in nociceptive transmission from the peripheral nervous system to the spinal cord. Furthermore, the study consistently demonstrated that substance P midbrain levels in instantaneous violent death reflect nociceptive activation.

The only example of a violent instantaneous death that shows mean and median peptide levels not indicative of nociception in the brain/serum study is drug overdose deaths. Several factors, however, limit the idea about using drug overdose deaths as a negative control. First, mean peptide levels were determined from only 6 drug overdose deaths (Tables 5a-c). It is difficult to draw any conclusion about expected peptide levels in instantaneous drug overdose deaths from such a small number of cases. Potential variation in peptide levels in instantaneous drug overdose deaths is demonstrated by elevated bradykinin serum levels in one case from the serum study (case #378, Appendix B). Additionally, substance P and bradykinin serum levels were elevated in one drug overdose case from the serum study where hospital resuscitation lasted 10 minutes (case #321, Appendix B). Furthermore, it is hard to imagine that all drug overdose deaths are the same given the variety of drugs that ingested in a large enough dosage can kill. Toxicities involved in drug overdose deaths will differ according to the type or class of the drug ingested. It seems logical then that some drug overdose deaths may be painful while others are not. As with other types of deaths presented in the study, the large standard deviation associated with peptide levels is indicative that pain can vary in drug overdose deaths.

Another factor limiting the use of drug overdose deaths as a negative control is the difficulty in determining if death was instantaneous or whether time past between drug ingestion and death. Since these deaths are either suicides or accidents,

they may not be reported to the police or emergency medical services in a timely fashion. Without substantiated claims from witnesses, the incident-death interval cannot be known. Additionally, the time interval between incident and death may be dose related. As clearly demonstrated in the study, the incident-death time interval influences peptide levels.

It is reasonable to believe that suicidal gunshot wounds to the head may be the violent death with the best chance of being truly instantaneous and having peptide levels that give no hint of nociceptive activation. Additionally, nociceptive activation is less likely to be observed with injuries to the brain due to a lack of nociceptive neurons. However, an examination of peptide levels in one case in the study (case #400, Appendix B) where the cause of death was a suicidal gunshot wound to the head with no incident-death time interval shows that these deaths may also not be suitable as negative controls. By using equation 2, the nociceptive/antinociceptive peptide ratio in this case is calculated to be 1.7 emphasizing the activation of nociception and existence of acute pain. The measurement of nociception and acute pain in this case renders these cases inadequate as negative controls. Like other types of violent death, the same factors which cause variable peptide levels in deaths of similar modes undoubtedly occur in suicidal gunshot wounds to the head as well. The peptide ratio of 1.7 also lends support to the theory that death causes nociception.

The use of clinical deaths as potential negative controls is also severely limited. First, all types of clinical deaths examined exhibited peptide values indicative of some level of nociception or antinociception. Secondly, many of the illnesses leading to death can either be acute or chronic. Peptide levels in deaths caused by acute renal failure would not be expected to be the same as deaths caused by chronic renal disease. Thus, the potential variation of peptide levels in clinical deaths with a particular etiology renders any of these deaths useless as a negative control.

In the case of infant-child deaths, SIDS deaths offer some possibility as a negative control. In every SIDS death examined in the study, peptide levels in both the midbrain and serum showed no evidence of nociception. However, since the study advocates the use of peptide level determination in helping to identify SIDS death, these cases cannot be used as negative controls in the examination of infant-child deaths.

The use of peptide levels for the determination of the amount of pain a violent death victim experiences just prior to death is not invalidated because of a lack of a negative control. Nociceptive/antinociceptive peptide ratios greater or less than 1 clearly demonstrate the amount of nociceptive activity and the relative contribution of both acute and chronic pain. Ambiguity occurs, however, when the nociceptive/antinociceptive peptide ratio is around one. Two scenarios can account

for this ratio. Either nociception has not occurred (and thus no pain exists) or that the contribution of acute and chronic pain is approximately the same. In cases where peptide levels are very high, the latter scenario is easily deduced. Similarly, in cases where peptide levels are very low (those which approximate healthy living subjects), the former scenario can be inferred. However, in situations where intermediate peptide levels exist, no conclusion can be drawn about the state of nociception.

One possible remedy is to incorporate a measurement of tissue injury into the calculation of the nociceptive/antinociceptive peptide ratio. Since peptides involved in the activation and transmission of nociception are also involved in repair of tissue injury, it seems reasonable to do this. Such a measurement of tissue injury should consist of a numeric scale where the greater the amount of tissue injury the higher the value. Ambiguity caused by a peptide ratio which approximates 1 can be lessened if the measurement of tissue injury is either multiplied or added to the peptide ratio. Assuming the rate of nociception is related to the amount of tissue injury, a peptide ratio of 1 will only stay 1 when little or no tissue injury occurs (little or no tissue injury will be assigned a value of 1.0 if multiplied or 0.0 if added). A calculated value of 1.0 will therefore be more indicative of no pain.

The major problem with this approach is that it prevents the use of a clinical death pain scale as a measure of comparison, since the tissue injury modification cannot be applied in clinical deaths. The only way it would be possible to do this is

to standardize an amount of tissue injury with a corresponding amount of tissue necrosis. Since tissue necrosis occurs due to disease and not to injury this does not appear feasible. Furthermore, any comparison between tissue injury and tissue necrosis may be fruitless since clinical diseases are generally more localized than injuries due to violent trauma. Additionally, the incorporation of a tissue injury factor may cause peptide ratios tilted toward acute pain (less than 1.0) to be greater than 1.0 and thus be erroneously indicative of chronic pain.

A better approach to dealing with the ambiguity caused by a nociceptive/antinociceptive peptide ratio is to statistically compare peptide levels of a violent death in question with those levels previously determined to be indicative of both acute and chronic pain in violent death (Section XVI, Part D, Tables 3a-c). Perhaps the easiest way to do this is to apply the Q test [149] which can determine whether a particular peptide level statistically compares with mean levels found in acute and chronic premortem pain. Alternatively, t testing can be used to show if mean peptide levels in acute and chronic premortem pain are significantly different from levels found in the case in question. If peptide levels compare with levels experimentally found to be consistent with either acute or chronic pain, then evidence of nociception exists thus allowing a calculated peptide ratio near 1.0 to be compared on the pain scale. If, however, peptide levels in a particular violent death in question do not compare with levels found in either acute or chronic premortem pain, then no

evidence of nociception exists. If no evidence exists that nociception has occurred, then a calculated ratio near 1.0 cannot be compared on the pain scale.

XIX. Significance of Study and Future Considerations

It is difficult to imagine that forensic biology can have a greater impact on the criminal justice system than it currently has. With recent advances in DNA typing technologies, biological individualization would appear to be at its zenith. The likelihood of a DNA match occurring at random is typically of the order of billions and even trillions. Current debate revolves around the level of rareness that must be met before an individual sample can be deemed unique. In the foreseeable future, laboratory reports will often state that a biological sample is unique to a particular individual. The advent of DNA databases (e.g. CODIS) has allowed forensic biology to help find the perpetrator of crimes, particularly sexual assaults, when little or no investigative leads exist. Since the rate of recidivism is high, DNA profiles determined from biological evidence can be used to link a perpetrator to several crimes. The ability to provide such investigative leads has impacted greatly on police investigations. Similarly, the demand for DNA evidence in criminal trials by prosecutors, judges, and jurors is unmatched by any other type of forensic evidence, save fingerprints. Such unprecedented reliance on forensic DNA evidence by

investigators and prosecutors will continue with or without future improvements in the technology (i.e. the use of nested PCR in typing DNA polymorphisms from extremely low levels of DNA).

With the paramount importance that DNA technologies have on the investigative and trial phases of a criminal inquiry, the current study attempts to evaluate the potential for the utilization of forensic science in the sentencing phase of the process. The preceding study tries to offer objective criteria in the determination of pain experienced by victims of trauma and violence. An objective assessment of pain may offer unbiased guidance in the allocation of putative damages awarded to plaintiffs or the determination of aggravating factors that can influence the sentencing of individuals found culpable of a violent act.

The study leaves little doubt that the determination of pre-mortem acute and chronic pain can be made by the examination of peptide levels in both the serum and the midbrain. By determining ratios of nociceptive to antinociceptive peptides in various non-violent deaths, a pain scale can be created that can be used as a reference for the amount of pre-mortem pain suffered by a victim of violence. By being able to equate the ratio of nociceptive to antinociceptive peptide levels in individuals who were violently killed with a particular type of non-violent clinical death, a qualitative assessment can be made of the level of pain experienced by the victim. A clearer picture of the level of pain can be presented to jurors, judges, and attorneys if peptide

levels found in a victim of a violent death can, for example, be equated with death due to renal disease, embolism, or cancer.

Caution, however, must be advised in using this approach in the determination of premortem pain. It cannot be concluded that the sensation of pain is any more severe when it is acute or chronic at the moment of death. Therefore, the acute pain experienced just prior to death by gastrointestinal disease may or may not be as severe as chronic pain associated with cancer death. Additionally, pain associated with chronically painful conditions may get worse just prior to death. For example, peptide levels consistent with both acute and chronic pain are observed in deaths by renal disease. It will be difficult to objectively determine differences in the sensation of severe acute and chronic pain at the moment of death. What can be known is the severity of either chronic or acute painful death when compared with other cases having similar states of pain. Based on peptide levels, for instance, it is clearly demonstrated that the chronic pain associated with AIDS death is more severe than that associated with diabetic death.

The duration of pain can also be determined. Obviously, violent deaths with high met-enkephalin and low substance P levels are indicative of pain of long duration prior to death. Conversely, violent deaths with premortem pain of short duration would be expected to have high substance P and low met-enkephalin levels.

In most cases, a conclusion that no pain existed prior to death can also be

made. If serum and brain peptide levels are all unremarkable, then it can usually be concluded that no pain was experienced prior to death.

The possible existence of a bimodal nociceptive response (which was postulated when examining serum peptide levels in blunt trauma violent deaths) makes the determination of no pain less than absolute. If such a response occurs, then the conclusion that no pain exists based on low peptide levels cannot automatically be made in violent deaths which have an incident-death time interval between both peaks of peptide release (2-4 days). More violent death cases having an incident-death time interval of 2-4 days need to be examined to determine if the bimodal nociceptive response truly exists.

Ideally, the use of radioimmunoassay as a technique for peptide quantitative analysis should be replaced with ELISA methods. ELISA techniques are easily adaptable in most forensic science laboratories since ELISA methods are commonly used by forensic laboratories for the detection of P30 (prostate specific antigen). In contrast, equipment suitable for radioimmunoassay is typically only available in toxicology laboratories. Moreover, the expense for a radiation license and safety issues related to radioactivity makes the use of radioimmunoassay undesirable in forensic science laboratories. Unfortunately, the use of ELISA for this application is limited by two factors. First, ELISA is less sensitive than radioimmunoassay. Detection limits in ELISA methods are generally in the nanogram range. The

sensitivity of ELISA methods, therefore, may not be adequate since serum and midbrain nociceptive peptides were detected in concentrations in the picogram range. Second, current commercial preparations of monoclonal antisera for nociceptive peptides needed for ELISA are in limited supply and are typically not of suitable specificity. As medical interest in nociceptive peptides continues to grow, however, better preparations may become available.

Alternatively, the advent of computer automated immunohistochemistry techniques creates the possibility of immunohistochemical quantitation of these peptides. There may be value in determining the localization of peptide increases in nervous tissue when assessing the level of pain. The major drawback to any immunohistochemical method for any type of quantitative work, however, is a lack of standardization of sample size. It would be extremely difficult to determine the weight of a microtomed tissue sample mounted on a microscope slide or know the volume of a serum smear. Additionally, the concentration of each peptide would not be reported in discrete weights but rather in the number of staining "hits" in a preparation. Moreover, immunohistochemical methods are not ideal for forensic laboratories with high caseloads because they are labor intensive and time consuming.

The influence that death has on peptide levels and nociception must be further studied. This can be best studied by determining peptide levels at intervals between

injury and death. Although the study would obviously be limited to serum, the effect that death has on nociception can be determined by comparing postmortem levels with premortem levels. This data will also be able to clearly deduce the precise interval after incident that peptide levels indicative of acute pain can be found in the serum.

The scope of the study can further be expanded to include the quantitative determination of levels of other neurochemicals, neuropeptides, and amino acids which play roles in nociceptive transmission. In particular, determining levels of aspartate and glutamate would be useful in distinguishing between fast and slow nociceptive transmission. Since these amino acids are believed to play the dominant role in fast nociceptive transmission (in contrast, neuropeptides are believed to play the dominant role in slow nociceptive transmission) [143], their quantitative determination may help in further elucidating differences in the speed of nociceptive transmission in violent deaths with varying amounts of tissue injury.

Further study may include the determination whether peptide levels can be quantified in dried bloodstains. Such a determination may have implications in crime scene reconstruction. If, for instance, it is found that premortem serum peptide levels change rapidly after injury, then testing a series of bloodstains left at a crime scene may be used to determine sequentially the movements of an injured victim or suspect.

Finally, both serum and serum/midbrain peptide studies were presented. It is

apparent that data from both the midbrain and serum are needed to completely discern the state of nociception and antinociception. Data from both are especially needed when nociception occurs and the incident-death time interval is of short duration. Although it is likely that midbrain levels will be suggestive of nociception in this case, serum levels may not be. Similarly, serum peptide levels may not reflect increased pain experienced just prior to death in a victim having a long incident-death time interval and where serum peptide levels are indicative of chronic pain. This was best observed in the examination of peptide levels in deaths due to renal disease.

Several cases in the study with similar manner of death show very divergent peptide levels. For instance, not every cancer victim demonstrates peptide levels indicative of severe chronic pain. Moreover, such divergent values are observed in homicide victims of all examined mode and manner even when the incident-death time interval is similar. Although trends in peptide levels become evident during compilation of data, the range of each subset of data is normally very large as evidenced by large standard deviations commonly found in the study. The reasons for this are unclear and may be due to normal biological variation. Perhaps cases producing anomalous and unexpected data are the result of disease states or medicinal treatments unknown to the study. Additionally, the effect that comatose conditions have on the sensation of pain and ultimately on peptide levels is not

known in most of cases in this study since this condition is often not part of the medical examiner or police reports. It is the fact that similar cases can differ which makes this study very important. During the subjective determination of pain for legal purposes, it may be erroneously assumed by lawyers, judges, and jurors that the existence of pain always occurs in violent death and that the pain is always the same in similar types of cases. Although pain may always exist on some level in violent death, this study has shown that it can differ. By examining serum and midbrain levels of peptides which play different but central roles in the nociceptive process, the study shows that pain can differ both qualitatively and quantitatively in similar types of cases. The results from this study may aid in the recognition of this fact and ultimately change how premortem pain is assessed by the criminal justice system.

XX. Appendix A

Diagrams

Diagram I - Schematic indicating the locations of laminae in cervical (C5), thoracic (T4), and lumbar (L7) segments of the spinal cord (Modified from Rexed B: *Journal of Comparative Neurology*, Vol. 96, 1952, pp.415-495).

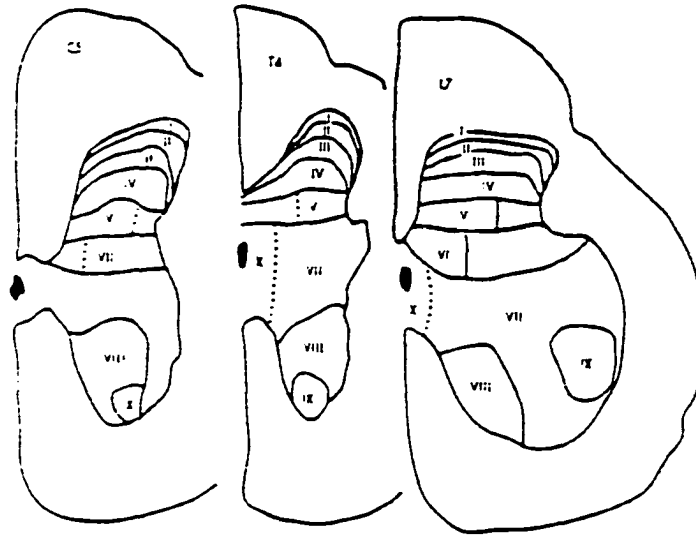


Diagram II - The peripheral activation of primary afferent fibers after tissue injury. Solid lines - initial responses: dashed lines - responses that occur after activation of peripheral nociceptors; SP - substance P (Modified from Sinatra, R.S., et al. editors: Acute Pain, Mechanisms and Management, Mosby, St. Louis, 1992).

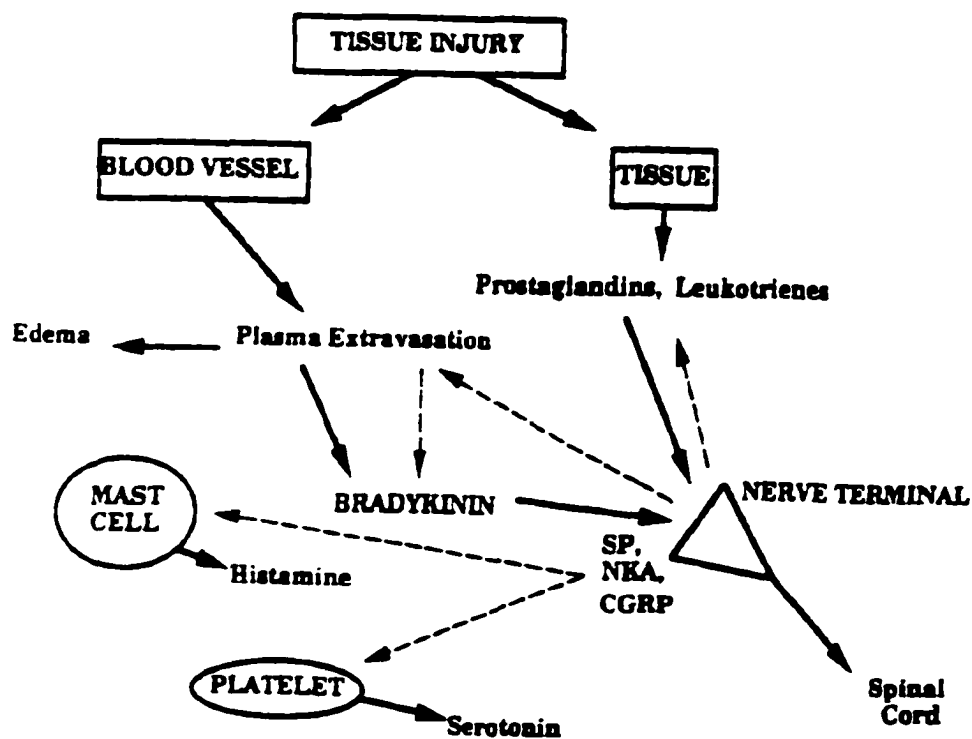


Diagram III - Summary of transmitting and modulation of nociceptive information (Modified from Sinatra, R.S., et al., editors: Acute Pain, Mechanisms and Management, Mosby, St. Louis, 1992).

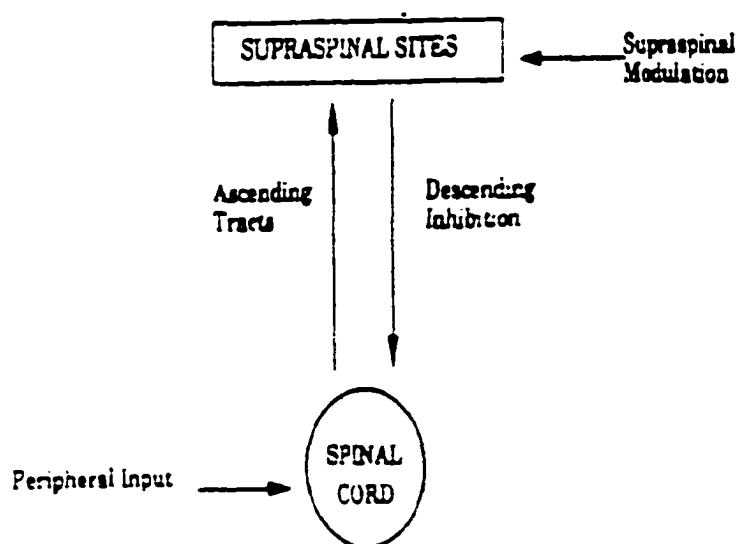


Diagram IV - Effects of neuropeptides on inflammation and tissue repair. PGE_2 - prostaglandin E_2 ; FAF - fibroblast-activating factor; SP - substance P (Modified from Payan, D.G., et al., *Advances in Immunology*, Vol. 39, 1986, pp.299-323).

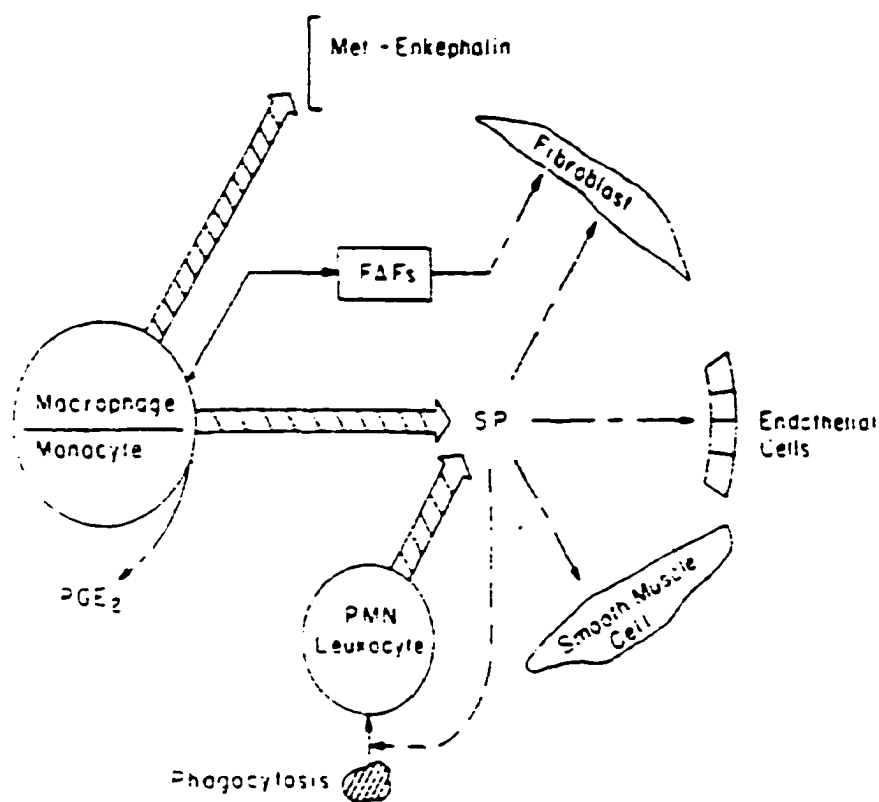
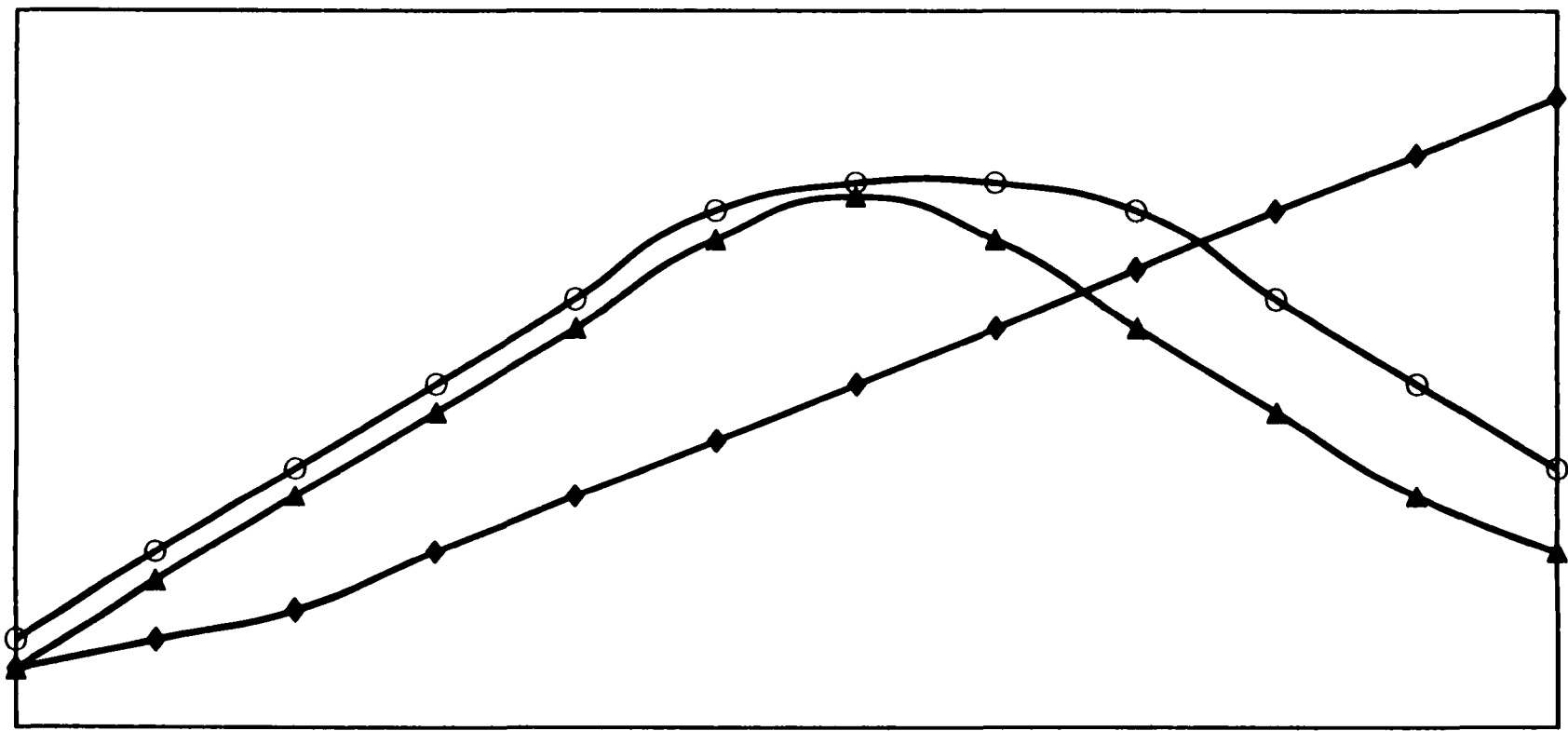


Diagram V - Theoretical Serum Peptide Levels
v. Duration Between Incident And Death

Peptide Levels

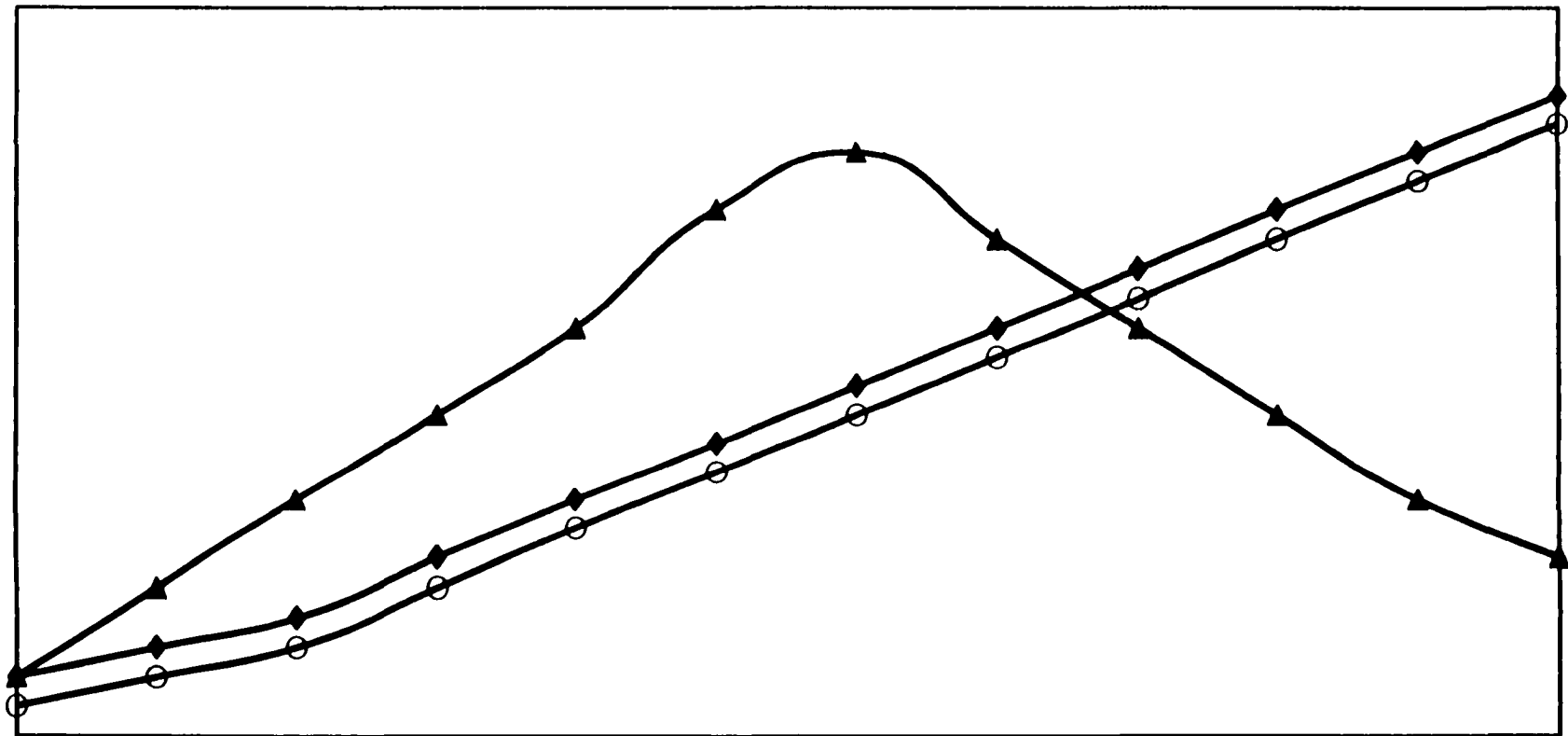


Duration Between Incident And Death

▲ Substance P ○ Bradykinin ◆ Met-Enkephalin

Diagram VI - Theoretical Brain Peptide Levels
v. Duration Between Incident And Death

Peptide Levels



Duration Between Incident And Death

▲ Substance P ○ Bradykinin ◆ Met-Enkephalin

XXI. Appendix B

Case Summaries

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml)	Bradykinin (pg/ml)	Met-Enkephalin (pg/ml)	Contributing Factors to Death and Comments
1	Cardiovascular Disease	1 hour	11.6	15.9	5.3	
2	Cardiovascular Disease	0	8.6	12.9	6.0	heavy smoker
3	Accidental Drug Overdose	0	15.5	3.8	3.3	chronic IV drug user on methadone; cardiovascular disease
4	Pulmonary Embolus Due To Leg Thrombosis	30 minutes	8.9	1.4	3.9	
5	Accidental Blunt Trauma To Head, Torso, And Extremities	1 day	86.8	129.9	2.6	73 year old man struck by train
6	Suicide - Drowning	0	16.4	13.1	5.7	jumped from bridge; trauma to head and torso
7	Suicide - Drug Overdose	0	10.1	14.8	4.7	asphyxiation; found with bag over head

232	Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml)	Bradykinin (pg/ml)	Met-Enkephalin (pg/ml)	Contributing Factors to Death and Comments
	8	Cardiac Arrest	1 hour	3.8	13.5	1.3	collapsed while arguing
	9	Suicide - Hanging	0	9.5	9.3	4.9	manic depressive
	10	Accidental Head Trauma	5 hours	82.4	236.7	4.3	67 year old struck by car; ethanol abuser
	11	Allergic Reaction To Sulfa Drugs; Sepsis	3 days	5.3	1.4	5.5	80 year old with anemia and hypothyroidism
	12	Accidental Blunt Trauma To Head, Torso, And Extremities	5 minutes	6.1	2.7	5.4	hit by car
	13	Cardiac Hypertrophy; Fibrofatty Hepatomegaly	0	7.6	3.4	2.9	obesity
	14	Encephalopathy; Hepatic Steatosis	2 days	8.1	6.1	2.3	schizophrenic; dementia

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml)	Bradykinin (pg/ml)	Met-Enkephalin (pg/ml)	Contributing Factors to Death and Comments
15	Suicide - Hanging	0	12.2	9.5	5.7	history of depression; pulmonary tuberculosis
16	Pneumonia Complicating Blunt Trauma To Head	8 months	7.3	1.7	51.3	subdural hematoma from fall; 80 year old with dementia and hypothyroidism
17	Stroke	4 days	6.9	2.9	3.3	rheumatic heart disease
18	Suicide - Blunt Trauma To Head, Torso, And Extremities	0	7.4	7.2	3.3	jumped from height; psychiatric history
19	Hypovolomic Complications During Surgical Repair Of Femur Due To Accidental Blunt Trauma To Extremities, Head, And Torso	3 days	8.8	7.9	7.4	fell from roof; cocaine and ethanol abuser

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml)	Bradykinin (pg/ml)	Met-Enkephalin (pg/ml)	Contributing Factors to Death and Comments
20	Metastatic Cervical Cancer	11 days	16.4	7.9	58.5	
21	Cardiac Arrest From Car Accident	30 minutes	22.4	10.4	2.3	
22	Cardiovascular Disease 0		18.3	14.3	1.0	schizophrenic; chronic alcohol abuser
23	Accidental Blunt Trauma To Head	3½ hours	142.9	86.2	1.7	fell down stairs
24	Cardiovascular Disease;0 Valvular Heart Disease		8.5	13.0	3.6	hypertrophy of right kidney
25	Acute And Chronic Alcoholism	0	21.2	12.7	2.7	cardiovascular disease
26	Suicide - Blunt Trauma To Head, Torso, And Extremities	0	14.4	5.4	4.9	jumped from height; acute and chronic alcoholism

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml)	Bradykinin (pg/ml)	Met-Enkephalin (pg/ml)	Contributing Factors to Death and Comments
27	Homicide - Stab Wound To Right Thigh	4 minutes	9.9	8.3	3.5	
28	Accidental Blunt Trauma To Head, Torso, And Extremities	4½ hours	95.5	8.9	2.1	fell from height
29	Acute And Chronic Alcoholism; Fatty Liver	0	11.0	7.5	2.6	cardiovascular disease; psychiatric history
30	Cardiovascular Disease	10 minutes	43.1	15.9	2.6	unilateral oophorectomy; thyroid with goiter; adrenal gland with cortical adenoma
31	Gastric Mucosal Defect	0	12.9	194.7	58.1	cardiovascular disease; schizophrenic; chronic pyelonephritis; obesity

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml)	Bradykinin (pg/ml)	Met-Enkephalin (pg/ml)	Contributing Factors to Death and Comments
32	Hypertensive Heart Disease; Fibromyxomatous Change Of Mitral Valve	1 hour	8.7	21.7	2.4	
33	Homicide - Gunshot Wound To Head	0	9.5	12.0	4.2	
34	Accidental Blunt Trauma To Head	21 hours	94.1	6.4	1.1	fell and struck head; chronic alcoholism; hepatitis C infection
35	Meningioma	29 hours	15.1	141.1	123.8	cardiovascular disease
36	Accidental Blunt Trauma To Head, Torso, And Extremities	10 minutes	13.3	25.8	5.9	fell from fire escape; acute cocaine intoxication
37	Accidental Blunt Trauma To Head, Torso, And Extremities	4 days	10.2	24.4	8.5	struck by bicycle; liver hemangioma

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml)	Bradykinin (pg/ml)	Met-Enkephalin (pg/ml)	Contributing Factors to Death and Comments
38	Cardiovascular Disease	0	38.7	1.4	5.8	cortical adenoma; interstitial tumor of both kidneys
39	Acute Esmolal Hydrochloride Intoxication Due To Inadvertant Overdose Following Heart Bypass Surgery	15 days	10.5	16.8	12.1	
40	Accidental Blunt Trauma To Neck, Head, And Extremities	0	13.9	22.5	25.8	fell from ledge; chronic alcoholism
41	Cardiovascular Disease	20 minutes	10.9	27.3	11.6	hemorrhagic gastritis
42	Accidental Head Trauma	9 days	210.5	41.9	32.8	motor vehicle accident
43	Homicide - Blunt Trauma To Head And Torso	2 hours	84.9	57.9	33.0	dragged by car

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml)	Bradykinin (pg/ml)	Met-Enkephalin (pg/ml)	Contributing Factors to Death and Comments
44	Homicide - Gunshot Wound To Chest	25 minutes	60.2	18.6	31.2	
45	Homicide - Stabbing To Chest	8 days	245.6	164.8	56.6	
46	Accidental Drug Overdose	4 minutes	13.1	6.7	43.7	acute and chronic cocaine and opiate abuse
47	Pulmonary And Hepatic Complications Of AIDS	11 days	97.6	70.8	129.2	chronic intravenous drug use
48	Acute Cocaine Intoxication	0	13.0	10.3	36.9	
49	Cardiovascular Disease	30 minutes	3.9	10.1	9.6	
50	Intracerebral Hemorrhage	15 minutes	48.3	5.1	75.3	overnight headache

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml)	Bradykinin (pg/ml)	Met-Enkephalin (pg/ml)	Contributing Factors to Death and Comments
51	AIDS	6 months	98.0	33.4	41.5	wasting, pneumonia, intravenous drug abuser
52	Suicide - Blunt Trauma 0 To Torso		7.5	33.8	9.8	fell from height; Prosac user
53	Myocardial Infarct; Coronary Artery Vasoplasm	20 hours	10.1	6.6	8.3	
54	Complications Of Coronary Artery Bypass Grafting For Treatment Of Hypertensive And Arteriosclerotic Heart Disease	2 weeks	29.4	8.9	14.8	
55	Myocarditis Of Viral Etiology	10 minutes	11.4	6.0	11.2	
56	Homicide - Gunshot Of Chest	10 minutes	43.5	32.6	21.2	

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml)	Bradykinin (pg/ml)	Met-Enkephalin (pg/ml)	Contributing Factors to Death and Comments
57	Complications Of Acute And Chronic Opiate Abuse	6 days	25.6	27.7	16.6	
58	Intracerebral Hemorrhage	9 days	19.7	22.3	24.0	chronic alcoholism
59	Accidental Drug Overdose	20 minutes	17.1	9.1	26.9	acute intoxication of cocaine and opiates
60	Hypertensive Cardiovascular Disease	0	81.7	16.7	128.3	osteoarthritic
61	Metastatic Carcinoma Of Tongue	15 days	24.8	27.4	136.6	
62	Suicide - Blunt Trauma To Head, Torso, And Extremities	9 days	16.7	5.0	100.3	jumped from height

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml)	Bradykinin (pg/ml)	Met-Enkephalin (pg/ml)	Contributing Factors to Death and Comments
63	Deep Venous Thromboses With Pulmonary Embolization Due To Cardiovascular Disease	0	10.3	40.1	27.4	diabetes mellitus
64	Suicide - Blunt Trauma To Head, Torso, And Extremities	0	18.6	28.0	27.7	hit by train
65	Homicide - Gunshot Wound To Head, Neck, And Torso	4 months	107.6	24.5	31.2	AIDS, hepatitis C
66	Homicide - Stabbing To Abdomen	30 minutes	91.2	39.2	20.5	
67	Renal Disease Of Unknown Etiology	15 minutes	9.0	5.3	20.9	pericarditis
68	Accidental Blunt Trauma To Head, Torso, And Extremities	0	37.5	20.0	13.8	hit by train while sleeping

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml)	Bradykinin (pg/ml)	Met-Enkephalin (pg/ml)	Contributing Factors to Death and Comments
69	Homicide - Gunshot Wound To Shoulder	0	48.5	31.3	7.9	
70	Cardiovascular Disease	10 minutes	6.1	11.5	7.9	
71	Hemopericardium With Cardiac Tamponade From Aortic Dissection	0	23.8	3.5	40.2	
72	Homicide - Gunshot Wound To Head	16 hours	162.5	101.4	90.1	
73	Homicide - Stab Wound To Chest	15 minutes	67.0	53.6	60.4	
74	Pulmonary Embolization From Immobilization Following Drainage Of Subdural Hematoma Due To Blunt Trauma To Head As Result Of Fall	1 hour	70.8	22.6	98.4	fall 2 months ago; diabetes mellitus; cardiovascular disease

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml)	Bradykinin (pg/ml)	Met-Enkephalin (pg/ml)	Contributing Factors to Death and Comments
75	Suicide - Blunt Trauma To Head, Torso, And Extremities	1 hour	21.4	8.9	42.6	jumped from height
76	Intracranial Hemorrhage	23 days	20.1	23.0	71.5	.
77	Accidental Blunt Trauma To Torso	6 hours	15.6	177.7	19.3	motor vehicle accident
78	Accidental Blunt Trauma To Head	5 days	101.1	31.7	29.8	fall from ladder
79	Complications Of Cardiovascular Disease Due To Diabetes Mellitus	0	10.1	31.5	6.9	
80	Living Subject #1	----	12.3	22.4	94.3	76 year old woman with arthritic history
81	Living Subject #2	----	66.8	31.8	1.2	77 year old man with acute pancreatitis (symptomatic for 1 month)

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml)	Bradykinin (pg/ml)	Met-Enkephalin (pg/ml)	Contributing Factors to Death and Comments
82	Homicide - Gunshot Wound To Face	0	27.8	10.9	36.7	
83	Homicide - Multiple Stab Wounds To Chest	0	207.0	123.4	4.3	
84	Homicide - Multiple Gunshot Wounds To Torso	0	10.9	15.5	10.2	
85	Homicide - Gunshot To Torso	0	16.7	31.6	18.6	
86	Suicide - Incised Wound To Arm	15 minutes	47.7	53.3	24.3	schizophrenic; chronic emphysema
87	Homicide - Gunshot Wound To Torso	15 minutes	17.8	31.4	3.9	
88	Homicide - Blunt Trauma To Head	18 hours	170.8	130.2	46.5	hit in head with bat
89	Homicide - Gunshot Wound To Torso	5 minutes	13.2	24.8	7.7	

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml)	Bradykinin (pg/ml)	Met-Enkephalin (pg/ml)	Contributing Factors to Death and Comments
90	Suicide - Stab Wound To Chest	0	11.1	18.7	9.9	
91	Carcinoma of Colon	months	4.0	117.4	12.6	osteoarthritis
92	Homicide - Stab Wound To Neck	1 hour	70.8	42.5	30.8	
93	Homicide - Gunshot Wound To Head	9 hours	54.6	77.8	34.5	
94	Homicide - Stab Wound To Chest, Blunt Trauma To Head And Torso	20 minutes	78.5	72.6	9.8	
95	Complications Due To Chronic Narcotism	0	3.3	66.9	34.0	AIDS; cardiovascular disease
96	Suicide - Gunshot To Mouth	10 minutes	15.6	11.8	5.3	
97	SIDS	30 minutes	17.9	16.2	17.5	5 month old

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml)	Bradykinin (pg/ml)	Met-Enkephalin (pg/ml)	Contributing Factors to Death and Comments
98	Cardiac Tamponade Due To Rupture Of Myocardial Infarct	6 hours	31.9	36.9	14.1	
99	Streptococcal Meningitis; Sickle Cell Anemia	10 minutes	13.9	16.7	22.9	3 year old ,
100	Suicide - Blunt Trauma To Head And Torso	2 hours	50.6	48.4	3.5	chronic alcoholism; fatty liver
101	SIDS	25 minutes	20.2	4.2	15.1	3 week old baby found dead by mother; may have been lodged between mother and sofa
102	Brain Stem Astrocytoma	1½ months	11.1	51.2	151.8	
103	Homicide - Gunshot Wound To Head	0	56.9	42.1	15.5	

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml)	Bradykinin (pg/ml)	Met-Enkephalin (pg/ml)	Contributing Factors to Death and Comments
104	Homicide - Gunshot Wound To Head	0	22.0	10.4	21.9	
105	Smoke Inhalation; Carbon Monoxide Poisoning	0	73.1	59.5	6.3	
106	Homicide - Gunshot Wound To Torso	15 minutes	81.7	77.0	5.2	
107	Asphyxia By Aspiration	5 hours	12.1	11.0	14.1	
108	Cardiovascular Disease	25 minutes	11.0	11.5	6.6	obesity
109	Homicide - Ligature Strangulation	5 minutes	11.0	3.9	9.0	mother wrapped umbilical cord around neck of newborn
110	Living Subject #3	----	3.1	46.7	46.7	chronic neurogenic pain
111	Living Subject #4	----	9.9	26.0	9.2	periodic back pain

248	Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml)	Bradykinin (pg/ml)	Met-Enkephalin (pg/ml)	Contributing Factors to Death and Comments
	112	Living Subject #5	----	19.9	22.5	3.9	no health problems
	113	Living Subject #6	----	17.0	25.5	3.7	no health problems
	114	Living Subject #7	----	14.8	19.9	9.6	no health problems
	115	Asthma Related Heart Disease	0	27.6	48.1	14.5	76 year old woman
	116	Homicide - Blunt Trauma To Head	1½ months	10.4	74.6	21.8	hit in head with bat; AIDS; emphysema
	117	Homicide - Gunshot Wound To Arm And Chest	2 minutes	18.3	20.8	12.1	
	118	SIDS	0	18.6	22.2	36.6	2 year old baby
	119	Cardiovascular Disease	4 days	4.9	76.8	84.2	diabetes mellitus; urinary tract infection

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml)	Bradykinin (pg/ml)	Met-Enkephalin (pg/ml)	Contributing Factors to Death and Comments
120	Accidental Drug Overdose; Acute Intoxication Of Cocaine, Opiates, And Methadone	10 minutes	30.7	8.4	16.3	bronchial asthma
121	Homicide - Gunshot Wound To Head	0	109.9	58.2	38.1	
122	Acute And Chronic Alcoholism With Hemorrhagic Gastritis	0	18.4	7.6	14.0	
123	Acute And Chronic Asthmatic Bronchitis	30 minutes	26.1	17.4	35.6	
124	Homicide - Blunt Trauma To Head, Torso, And Extremities	0	131.2	72.2	41.1	crushed to death by bed; history of spousal abuse
125	Homicide - Stab Wound To Thigh	40 minutes	122.9	35.5	40.6	

250	Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml)	Bradykinin (pg/ml)	Met-Enkephalin (pg/ml)	Contributing Factors to Death and Comments
	126	Cardiac Tamponade Due To Rupture Of Aorta	1 hour	53.1	14.0	7.8	
	127	Cerebral Hemorrhage	4 hours	22.3	31.2	33.5	.
	128	Metastatic Prostate Cancer	1 month	4.6	197.6	125.7	stroke; diabetes mellitus
	129	Homicide - Gunshot Wound To Head, Arm, And Torso	4 days	121.9	66.6	99.6	
	130	Liver Carcinoma	1 month	3.3	196.6	193.9	hepatitis B, C
	131	AIDS	3 weeks	5.7	152.1	140.8	chronic opiate abuse
	132	AIDS	1 month	7.4	160.5	148.3	hepatic cirrhosis; chronic alcoholism and intravenous drug use

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml)	Bradykinin (pg/ml)	Met-Enkephalin (pg/ml)	Contributing Factors to Death and Comments
133	Colitis With Peritonitis	10 days	10.3	27.4	121.4	lupus; hepatitis; open reduction internal fixation of left femur due to blunt impact (fall); 75 year old woman
134	Complications Due To Acute And Chronic Drug Abuse	0	30.5	5.9	14.9	hypertrophy of heart
135	AIDS	3 weeks	4.3	118.0	175.2	chronic pancreatitis; chronic intravenous drug user
136	Accidental Blunt Trauma To Head	13 days	110.7	86.2	128.2	fell and struck head
137	Bronchopneumonia Complicating Seizures Due To Acute Cocaine Intoxication	31 hours	23.1	6.1	32.4	

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml)	Bradykinin (pg/ml)	Met-Enkephalin (pg/ml)	Contributing Factors to Death and Comments
138	Cardiovascular Disease	0	27.5	17.2	22.2	pulmonary emphysema; leiomyoma of stomach
139	Accidental Drug Overdose; Acute Intoxication Of Cocaine, Alcohol, And Amitriptyline	0	6.8	180.9	121.6	AIDS
140	AIDS	2 months 1 week	5.7	215.0	175.1	chronic substance abuse
141	SIDS	0	8.5	7.3	16.9	5 month old
142	Infective Endocarditis With Cerebral Infarction	10 days	99.0	13.3	110.7	chronic intravenous drug abuse
143	Suicide - Hanging	0	47.2	64.5	30.2	
144	Epilepsy	0	15.2	7.9	19.3	pulmonary vascular congestion; edema

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml)	Bradykinin (pg/ml)	Met-Enkephalin (pg/ml)	Contributing Factors to Death and Comments
145	Homicide - Stab Wound To Chest	1 hour	110.9	54.8	100.6	
146	Accidental Blunt Trauma Causing Multiple Lacerations To Torso And Extremities	0	109.7	145.8	96.2	pit bull attack
147	Suicide - Hanging	0	32.4	71.5	16.6	
148	Metastatic Prostate Cancer	2 weeks	2.0	187.5	175.1	77 year old man
149	Ventricular Arrhythmia	30 minutes	119.1	147.0	119.3	
150	Acute Viral Myocarditis	1 hour	28.8	17.4	2.6	
151	Homicide - Blunt Trauma To Head	4 hours	197.3	184.7	51.4	hit in head with rock
152	Living Subject #8	----	24.1	3.5	4.7	healthy individual

254	Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml)	Bradykinin (pg/ml)	Met-Enkephalin (pg/ml)	Contributing Factors to Death and Comments
	153	Living Subject #9	----	31.4	18.2	2.1	healthy individual
	154	Living Subject #10	----	26.4	12.3	1.5	healthy individual
	155	Living Subject #11	----	32.2	15.7	4.4	healthy individual
	156	Living Subject #12	----	44.5	22.2	7.6	healthy individual
	157	Bleeding Duodenal Ulcer	3 days	195.5	205.8	49.3	dehydration
	158	Homicide - Blunt Trauma To Head	6 days	190.4	245.3	53.1	hit head on sidewalk during altercation
	159	Accidental Blunt Trauma To Head	4 days	202.3	185.3	55.0	13 month old; knocked from pedestrian's arms by automobile
	160	SIDS	20 minutes	6.2	77.1	15.9	1 month old
	161	Asphyxia Due To Tracheal Obstruction By A Bean	3 hours	28.4	28.8	29.8	23 month old

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml)	Bradykinin (pg/ml)	Met-Enkephalin (pg/ml)	Contributing Factors to Death and Comments
162	Hypoglycemia	0	28.7	12.7	8.4	10 week old
163	Acute Laryngitis, Bronchitis, And Pneumonia	10 minutes	28.8	41.0	25.8	2 month old
164	Congenital Heart Disease With Atrial Septal Defect	30 minutes	24.2	9.4	9.6	9 day old
165	Complications Of Prematurity	35 minutes	14.6	6.3	13.1	3 month old
166	Acute Chorioamnionitis And Funisitis With Intrauterine Pneumonitis	0	23.3	19.3	20.9	stillborn; 6 month pregnancy
167	Klebsiella Pneumoniae Septicemia	30 minutes	26.5	108.7	22.0	3 month old
168	Homicide - Stab Wound To Neck	50 minutes	24.0	95.3	10.5	

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml)	Bradykinin (pg/ml)	Met-Enkephalin (pg/ml)	Contributing Factors to Death and Comments
169	Suicide - Blunt Trauma To Head And Torso	0	28.3	26.4	13.7	jumped from height
170	Accidental Blunt Trauma To Torso And Extremities	5 minutes	22.1	93.2	7.2	fell from height
171	Hypertensive And Atherosclerotic Cardiovascular Disease	0	32.3	52.9	10.5	chronic alcoholic
172	Suicide - Hanging	1 year	24.2	22.8	18.7	comatose state for 1 year
173	Suicide - Gunshot Wound To Head	0	28.1	34.2	8.6	
174	Accidental Blunt Trauma To Head	21 hours	28.3	101.7	25.2	pedestrian hit by car
175	Drowning In Bathtub Due To Seizure	1 hour	31.2	23.9	26.6	11 year old; mental retardation

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml)	Bradykinin (pg/ml)	Met-Enkephalin (pg/ml)	Contributing Factors to Death and Comments
176	Suicide - Blunt Trauma To Head, Torso, And Extremities	1½ hours	24.8	28.9	17.6	hit by train
177	Ruptured Dissecting Aneurysm Of Thoracic Aorta	21 hours	33.1	34.8	12.1	.
178	Suicide - Gunshot Wound To Head	0	25.7	34.1	22.9	history of psychiatric illness
179	Accidental Drug Overdose; Acute Opiate Intoxication	30 minutes	4.8	15.5	2.3	bronchial illness
180	Metastatic Carcinoma Of Endometrium	24 days	8.6	134.3	166.1	
181	Thrombophlebitis With Pulmonary Embolism Due To Immobilization Due To Diabetic Ketoacidosis	4 days	11.9	7.1	39.0	morbid obesity

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml)	Bradykinin (pg/ml)	Met-Enkephalin (pg/ml)	Contributing Factors to Death and Comments
182	Suicide - Blunt Trauma To Neck, Torso, And Extremities	0	19.9	23.0	12.1	jumped from height
183	Suicide - Gunshot Wound To Head	0	21.3	5.8	11.9	.
184	Multiple Complications Of Anoxic Encephalopathy Following Resuscitation From Cardiac Arrest	10 months	25.9	25.9	9.3	hit by car; multiple skeletal fractures; micronodular cirrhosis and steatosis
185	Accidental Drug Overdose; Acute Opiate Intoxication	0	20.5	4.4	5.6	diabetes mellitus
186	Accidental Drug Overdose; Acute Intoxication With Opiates, Cocaine, And Ethanol	0	21.7	10.8	6.7	pulmonary congestion and edema

259	Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml)	Bradykinin (pg/ml)	Met-Enkephalin (pg/ml)	Contributing Factors to Death and Comments
	187	Accidental Drug Overdose; Acute Intoxicationn With Methadone, Cocaine, Amitriptyline, Mortriptyline, And Diazepam	15 hours	29.5	35.4	8.1	sepsis, cirrhosis
	188	Sickle Cell Anemia	3 minutes	24.4	14.0	10.9	8 month old
	189	Sickle Cell Anemia With Deep Vein Thrombosis With Pulmonary Embolism And Venous Statis Ulcers In Ankles	1 day	22.7	66.2	15.6	
	190	Anaphylactoid Reaction Due To Injection Of Intravenous Radiologic Contrast Medium During Abdominal CT Scan For Abdominal Pain	5 minutes	66.1	30.3	74.9	AIDS; Kaposi's Sarcoma

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml)	Bradykinin (pg/ml)	Met-Enkephalin (pg/ml)	Contributing Factors to Death and Comments
191	Vaginal Hemorrhage Due To Caesarian Section And Hysterectomy	10 minutes	56.7	43.4	3.8	36 week pregnancy
192	Suicide - Asphyxia Due To Plastic Bag Over Head And Acute Intoxication Of Diazepam, Chlordiazepoxide, And Meprobamate	0	18.9	19.6	4.6	history of depression
193	End Stage Renal Disease	2 days	16.4	9.5	2.6	cardiovascular disease
194	Acute And Chronic Viral Interstitial Pneumonitis Complicating Premature Delivery	15 minutes	21.0	16.4	17.1	4 month old

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml)	Bradykinin (pg/ml)	Met-Enkephalin (pg/ml)	Contributing Factors to Death and Comments
195	Suicide - Blunt Trauma To Head And Torso	0	24.8	106.4	24.7	jumped from bridge
196	Drowning In Bathtub Due To Seizure Disorder	30 minutes	17.4	29.0	40.8	
197	Hypertensive Cardiovascular Disease	0	18.7	17.2	13.1	obesity; diabetes mellitus
198	Homicide - Blunt Trauma To Head And Torso; Dislocation Of Upper Cervical Vertebrae	6½ hours	152.8	87.6	7.6	
199	Suicide - Blunt Trauma To Torso And Extremities	0	21.8	7.0	8.9	jumped from height; intravenous drug abuse
200	Homicide - Gunshot Wound To Abdomen	5 hours	21.3	91.7	54.1	

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml)	Bradykinin (pg/ml)	Met-Enkephalin (pg/ml)	Contributing Factors to Death and Comments
201	Homicide - Complications Of Pneumonia And Sepsis Due To Gunshot Wounds To Chest And Abdomen	8 months	9.1	15.2	216.0	
202	Homicide - Blunt Trauma To Head	47 hours	64.1	13.2	3.1	head banged against surface; alcoholism
203	Homicide - Blunt Trauma To Head	3 months	70.1	106.1	311.8	hit in head with bat
204	Sepsis And Bronchopneumonia Complicating Thermal Burns Of Groin And Legs From Steam Bath	1 week	166.8	170.1	57.5	cardiovascular disease; 75 year old
205	Second And Third Degree Burns Over 75% Of Body	1 day	92.9	92.8	65.2	cooking accident

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml)	Bradykinin (pg/ml)	Met-Enkephalin (pg/ml)	Contributing Factors to Death and Comments
206	Pneumonia; Sepsis	12 hours	19.2	3.0	246.5	
207	Acute Pulmonary Thromboembolism Due To Phlebothrombosis Of Lower Extremities Following Above Ankle Amputation Of Left Foot And Right Toes For Infected Frostbites	30½ hours	133.7	178.9	121.1	
208	Fatal Child Abuse Syndrome And Neglect With Starvation, Malnutrition, And Multiple Blunt Trauma Of Head, Torso, Extremities And Thoracic Spine	15 minutes	104.1	32.4	117.7	5 year old
209	Suicide - Hanging	0	37.4	17.9	48.1	HIV positive; heroin abuser
210	End Stage Renal Disease	27 days	100.3	83.9	83.9	femur bypass groin infection

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml)	Bradykinin (pg/ml)	Met-Enkephalin (pg/ml)	Contributing Factors to Death and Comments
211	Accident - Seizure Disorder, Post-Traumatic, Complicating Blunt Trauma To Head	10 minutes	75.9	41.8	14.6	8 year old; hemophiliac
212	Accidental Blunt Trauma To Head	10 days	223.4	113.1	39.4	hit by bicyclist; 74 year old man
213	Accidental Drug Overdose; Acute Intoxication Of Opiates And Methadone	10 minutes	53.4	16.5	48.0	acute and chronic bronchial asthma
214	Smoke Inhalation	0	71.8	48.0	49.7	fire at residence
215	Arteriosclerotic Cardiovascular Disease	0	60.0	92.0	40.4	burn victim; chronic pulmonary emphysema

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml)	Bradykinin (pg/ml)	Met-Enkephalin (pg/ml)	Contributing Factors to Death and Comments
216	Smoke Inhalation	0	68.3	83.5	66.3	body removed from rubble of fire-destroyed building after demolition.
217	Smoke Inhalation	0	33.6	82.6	52.7	fire at residence
218	Smoke Inhalation	0	58.2	87.7	72.2	fire at residence
219	Accident - Drowned In Bathtub	1 hour	40.4	37.8	47.8	5 year old
220	Bronchopneumonia	0	63.5	22.5	66.8	chronic alcoholism
221	Carcinoma Of Colon	24 days	98.2	93.3	84.7	chronic illness
222	Intracerebral Hemorrhage Following Anticoagulant Therapy For Ischemia Of Lower Extremity	2 days	55.9	26.4	54.3	cardiovascular disease

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml)	Bradykinin (pg/ml)	Met-Enkephalin (pg/ml)	Contributing Factors to Death and Comments
223	Acute Bronchial Asthma	0	73.8	24.9	77.8	cardiovascular disease
224	Gastrointestinal Hemorrhage Due To Gastroenteritis	1 week	32.2	59.3	73.5	cardiovascular disease
225	Homicide - Ligature Strangulation	0	120.7	80.1	62.5	
226	Sustained Head Trauma Due To Seizure Disorder	1 day	46.8	37.5	131.8	
227	Diabetes Mellitus Complicating Hypertensive And Arteriosclerotic Cardiovascular Disease	11 days	17.8	11.6	119.2	79 year old
228	Prematurity With Perinatal Asphyxia Due To Cocaine And Phenobarbital Toxicity	1 day	48.3	1.9	71.6	12 hour old; maternal drug abuse

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml)	Bradykinin (pg/ml)	Met-Enkephalin (pg/ml)	Contributing Factors to Death and Comments
229	Accidental Drug Overdose; Acute Cocaine Intoxication	0	65.4	20.5	118.1	
230	Sepsis And Dehydration; Intestinal Obstruction; Strangulated Inguinoscrotal Hernia	2 days	50.5	19.4	48.6	2 month old
231	Suicide - Blunt Trauma To Head And Torso	0	149.7	93.8	86.3	jumped from height in hospital; admitted for abdominal pain (10 days)
232	Chronic Alcoholism	48 hours	15.3	52.4	31.4	infected laceration to thumb
233	Recent Myocardial Infarct Due To Arteriosclerotic And Hypertensive Cardiovascular Disease	0	45.3	33.6	62.5	
234	Smoke Inhalation	0	60.2	34.9	45.7	house fire

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml)	Bradykinin (pg/ml)	Met-Enkephalin (pg/ml)	Contributing Factors to Death and Comments
235	SIDS	30 minutes	63.8	25.6	52.4	5 month old
236	Homicide - Blunt Trauma To Torso	30 minutes	103.3	36.0	54.6	3 year old; child abuse syndrome
237	Fire Victim	0	41.6	42.2	47.3	2 nd and 3 rd degree burns from house fire
238	Fire Victim	0	19.2	52.1	143.9	body charred from house fire
239	Homicide - Blunt Trauma To Head	10 days	111.5	44.6	61.2	mugging; fever and sepsis; low blood pressure
240	Bilateral Deep Venous Thrombi And Pulmonary Emboli Due To Term Pregnancy	2 days	35.1	78.3	35.8	
241	Myocardial Infarction	50 days	156.6	107.2	137.1	spinal stenosis; 77 year old

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml)	Bradykinin (pg/ml)	Met-Enkephalin (pg/ml)	Contributing Factors to Death and Comments
242	Accidental Blunt Trauma To Head; Anoxic Brain Injury	1 day	111.3	41.6	52.1	fall; history of drug abuse
243	Hypertensive Cardiovascular Disease	0	22.8	24.2	25.2	thyroid condition
244	Seizure Disorder	0	23.7	20.9	47.8	
245	Homicide - Stab Wound To Abdomen	0	108.3	52.7	57.3	
246	Accident - Electrocution	15 minutes	17.9	23.9	62.2	
247	Homicide - Asphyxia	0	16.9	8.4	66.9	3 week old
248	Living Subject #13	----	25.7	27.7	84.3	healthy individual
249	Living Subject #14	----	13.2	48.9	158.5	chronic neurogenic pain; history of spinal cord tumor

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml)	Bradykinin (pg/ml)	Met-Enkephalin (pg/ml)	Contributing Factors to Death and Comments
250	Living Subject #15	----	26.9	29.3	165.2	history of lymphatic cancer and scoliosis
251	Thermal Burns Over 90% Of Body	25 hours	127.0	204.1	10.8	cardiovascular disease
252	Pulmonary Thromboembolism With Right Lung Infarct	30 minutes	75.8	62.4	9.4	bedridden due to anoxic encephalopathy due to heroin abuse
253	SIDS	0	9.9	47.2	7.1	6 month old
254	Respiratory Distress - Etiology Unknown	2 hours	32.0	39.4	16.5	4 month old
255	Accidental Blunt Trauma To Head; Hypothermia	4 hours	38.2	82.2	7.8	acute and chronic alcoholism
256	Thermal Burns Over 90% Of Body	4 days 4 hours	88.2	275.6	19.3	22 month old

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml)	Bradykinin (pg/ml)	Met-Enkephalin (pg/ml)	Contributing Factors to Death and Comments
257	Acute Bronchopneumonia Due To Tetralogy Of Fallot	14 hours	28.1	37.7	13.8	mental retardation
258	Homicide - Blunt Trauma To Head, Neck, And Extremities	8 hours	44.5	65.5	11.7	14 month old; child abuse syndrome
259	Hypertensive And Arteriosclerotic Cardiovascular Disease	0	25.6	22.3	68.5	pulmonary emphysema; severe arthritis
260	Homicide - Strangulation	0	21.6	93.5	10.8	
261	Accidental Blunt Trauma To Head	5 hours	25.4	66.9	7.2	5 month old; television fell on deceased
262	Acute And Chronic Asthmatic Bronchitis	30 minutes	24.0	24.9	4.2	

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml)	Bradykinin (pg/ml)	Met-Enkephalin (pg/ml)	Contributing Factors to Death and Comments
263	Carcinoma Of Pancreas	6 days	23.6	100.3	5.6	bronchopneumonia; cirrhosis of liver; chest and abdominal pain for 2 weeks
264	Coronary Vasculitis And Myocarditis	15 minutes	34.5	27.5	10.5	asthma; chronic substance abuse
265	Suicide - Hanging	0	32.6	49.6	6.3	
266	End Stage Renal Disease	0	10.8	66.9	40.2	hypertensive cardiovascular disease
267	Bronchopneumonia Complicating Multiple Congenital Anomalies	7 hours	27.5	47.0	7.0	2 year old; dwarfism; quadriplegia; hydronephrosis; microcephaly
268	End Stage Renal Disease	30 minutes	31.6	75.5	59.2	cardiomegaly

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml)	Bradykinin (pg/ml)	Met-Enkephalin (pg/ml)	Contributing Factors to Death and Comments
269	Acute And Chronic Bronchitis/Bronchiolitis	9 hours	34.7	39.9	8.5	asthmatic; 8 month old
270	Subarachnoid Hemorrhage - Etiology Unknown	3 days	98.7	85.5	9.6	
271	Lobar Pneumonia Due To AIDS	0	17.4	88.7	61.2	chronic substance abuse; hypertensive and atherosclerotic cardiovascular disease
272	Sickle Cell Anemia	54 hours	24.1	37.9	9.3	adult; hypertensive cardiovascular disease
273	Carcinoma Of Pancreas	8 weeks	106.3	135.1	48.5	arteriosclerotic cardiovascular disease; renal failure

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml)	Bradykinin (pg/ml)	Met-Enkephalin (pg/ml)	Contributing Factors to Death and Comments
274	Diabetes Mellitus Complicated By Bronchopneumonia	0	22.0	38.4	12.8	decedent did not take insulin on day; chronic alcoholism; hypertensive and atherosclerotic cardiovascular disease
275	Atherosclerotic And Hypertensive Cardiovascular Disease With Cardiac Arrest Following Induction Anesthesia With Brevital And Lidocaine For Cataract Surgery	3 hours	29.7	33.1	10.9	hypothyroidism; diabetes mellitus
276	Carcinoma Of Lung And Liver	0	7.5	52.8	283.8	cardiac hypertrophy

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml)	Bradykinin (pg/ml)	Met-Enkephalin (pg/ml)	Contributing Factors to Death and Comments
277	Accidental Blunt Trauma To Head	4 days	108.9	206.3	15.1	fell at home; chronic alcoholism; hypertensive cardiovascular disease
278	Cerebral Infarcts; Complications Of Streptococcus Milleri Brain Abscess Following Tooth Extraction	15 days	143.7	166.1	7.5	hypertensive and arteriosclerotic cardiovascular disease
279	Smoke Inhalation - Carbon Monoxide Poisoning	1½ hours	19.4	40.5	5.2	4 year old; fire in house; no burns
280	Bronchial Asthma	8 hours	25.5	25.8	3.9	obesity
281	Accidental Blunt Trauma To Head And Torso	17 days	77.7	110.2	23.9	diabetes mellitus; hepatitis C; 76 year old in motor vehicle accident

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml)	Bradykinin (pg/ml)	Met-Enkephalin (pg/ml)	Contributing Factors to Death and Comments
282	Anoxic Encephalopathy And Bronchopneumonia Complicating Acute Cocaine Intoxication	11 days	74.2	51.8	12.1	hypertensive and arteriosclerotic cardiovascular disease
283	Bronchopneumonia Complicating Adrenal Carcinoma	1 hour	20.1	55.3	9.5	hypertensive cardiovascular disease
284	Smoke Inhalation - Carbon Monoxide Poisoning	0	62.1	79.7	8.2	house fire; thermal burns over 100% of body
285	Peritonitis And Pseudomembranous Colitis; Connective Tissue Disorder Of Unknown Etiology	2 months	55.3	50.6	58.3	necrosis of liver; splenic infarcts
286	Hepatitis	0	29.6	21.5	8.9	chronic alcoholism

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml)	Bradykinin (pg/ml)	Met-Enkephalin (pg/ml)	Contributing Factors to Death and Comments
287	Polycystic Kidney Disease With Chronic Dialysis	0	17.9	66.9	57.9	acute cocaine intoxication
288	SIDS	30 minutes	19.8	32.5	18.5	4 month old
289	Thermal Burns Over 60% Of Body	15 minutes	76.7	123.9	5.4	building fire
290	Pulmonary Thromboembolism Due To Deep Venous Thrombosis Of Lower Extremities	6 weeks	26.9	30.7	8.7	hypertensive and arteriosclerotic cardiovascular disease
291	Rheumatic Heart Disease	3 days; 15 hours	81.7	67.6	5.8	diabetes mellitus; angina
292	Acute Opiate Intoxication	20 minutes	22.5	12.9	9.9	sickle cell disease; hypertensive cardiovascular disease

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml)	Bradykinin (pg/ml)	Met-Enkephalin (pg/ml)	Contributing Factors to Death and Comments
293	Febrile Upper Respiratory Illness Of Unknown Etiology	1 hour	20.7	31.8	4.4	2 year old; SC hemoglobinopathy with anemia and cardiac hypertrophy
294	Bronchial Asthma	0	30.5	45.3	12.4	chronic alcoholism
295	Cardiac Arrhythmia Due To Hypertensive And Arteriosclerotic Cardiovascular Disease	5 minutes	39.4	36.8	23.0	diabetes mellitus
296	Accidental Blunt Trauma To Head	3½ hours	74.2	203.1	23.2	pedestrian hit by car; hypertensive and arteriosclerotic cardiovascular disease; pulmonary emphysema
297	Bronchopneumonia	25 minutes	40.4	33.1	7.4	1 month old

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml)	Bradykinin (pg/ml)	Met-Enkephalin (pg/ml)	Contributing Factors to Death and Comments
298	Cirrhosis Of Liver, Intestinal Hemorrhage, And Peptic Duodenal Ulcer Due To Chronic Alcoholism	0	96.5	41.6	6.8	
299	Bronchopneumonia	15 minutes	18.1	35.7	4.8	1 year old
300	Bronchopneumonia Complicating Cerebral Infarcts And Atrophy Due To Pneumococcal Meningitis	0	24.2	11.0	4.3	14 year old
301	Respiratory Distress Due To Acute Cocaine Intoxication	11 hours	10.9	7.0	237.8	lung carcinoma
302	Homicide - Multiple Stab Wounds To Head, Torso, And Arm	0	73.6	29.5	27.2	

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml)	Bradykinin (pg/ml)	Met-Enkephalin (pg/ml)	Contributing Factors to Death and Comments
303	Accidental Blunt Trauma To Head, Torso, And Extremities	0	47.4	26.5	20.6	hit by train; alcohol intoxication
304	Hepatic Cirrhosis And Chronic Pancreatitis Due To Chronic Alcoholism	0	19.4	3.3	9.4	atherosclerotic cardiovascular disease; pulmonary congestion
305	Accidental Blunt Trauma To Head	3 days	165.4	175.3	9.3	operator of bicycle struck by motor vehicle
306	Bronchopneumonia Complicating Acute Intoxication Of Opiates And Cocaine	15 minutes	21.1	20.1	9.7	
307	Respiratory Obstruction Due To Rigid Bronchoscopy For Attempted Biopsy Of Peribronchial Anaplastic Carcinoma Of Thymus	42 hours	9.7	5.2	231.3	cardiomegaly; heavy smoker

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml)	Bradykinin (pg/ml)	Met-Enkephalin (pg/ml)	Contributing Factors to Death and Comments
308	Cardiac Arrest Due To Arteriosclerotic And Hypertensive Cardiovascular Disease	30 minutes	11.8	5.4	28.2	pulmonary edema
309	Ruptured Abdominal Aortic Aneurysm Due To Hypertensive And Arteriosclerotic Cardiovascular Disease	5½ days	69.7	12.9	45.9	history of obstructive pulmonary disease and gastroenteritis
310	Homicide - Multiple Stab Wounds To Chest	15 days	51.2	55.6	20.9	renal cell adenocarcinoma; cardiovascular disease
311	Smoke Inhalation - Carbon Monoxide Poisoning	15 minutes	10.8	3.4	11.2	3 year old in house fire
312	Accidental Blunt Trauma To Head	5 days, 5 hours	188.7	241.7	11.5	hit in head by falling bricks from building

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml)	Bradykinin (pg/ml)	Met-Enkephalin (pg/ml)	Contributing Factors to Death and Comments
313	Atherosclerotic And Hypertensive Cardiovascular Disease	0	6.6	3.1	12.6	hepatic cirrhosis; chronic alcoholism
314	Viral Syndrome With Bronchiolitis, Hepatitis, And Acute Splenitis	0	15.6	9.5	6.3	2 month old
315	Pulmonary Embolus Due To Deep Venous Thrombosis In Legs	35 hours	69.5	62.7	29.8	schizophrenic
316	Bronchopneumonia Complicating Accidental Blunt Trauma To Head	3 weeks	114.3	212.7	72.9	fell from height
317	Hemoperitoneum Following Liver Biopsy	4 days, 9 hours	159.3	173.5	61.8	hypertensive and arteriosclerotic cardiovascular disease; diabetes mellitus

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml)	Bradykinin (pg/ml)	Met-Enkephalin (pg/ml)	Contributing Factors to Death and Comments
318	Suicide - Gunshot Wound To Chest	5 minutes	22.4	3.5	9.7	
319	Bronchopneumonia Complicating Cerebral Infarction Due To Hypertensive And Arteriosclerotic Cardiovascular Disease	5½ hours	35.6	52.9	9.8	
320	Suicide - Blunt Trauma 0 To Head, Neck, Torso, And Extremities		25.3	2.8	6.5	jumped from bridge
321	Accidental Drug Overdose Due To Acute Intoxication Of Cocaine And Ethanol	10 minutes	63.9	66.8	28.1	
322	Accidental Blunt Trauma To Head And Extremities	54 hours	51.1	58.2	11.4	pedestrian struck by vehicle; pulmonary thromboembolosis

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml)	Bradykinin (pg/ml)	Met-Enkephalin (pg/ml)	Contributing Factors to Death and Comments
323	AIDS Related Pneumocystis Carinii Pneumonia	79 hours	8.9	2.1	158.5	pulmonary embolism
324	Kidney Failure Due To Acute And Chronic Alcohol Abuse	0	73.2	43.9	89.3	hypertensive and atherosclerotic heart disease
325	Acute Hemorrhage In Pons And Midbrain Due To Hypertensive Cardiovascular Disease	16 hours	90.5	72.7	6.1	
326	Staphylococcal Bronchopneumonia And Sepsis	72½ hours	151.2	205.8	24.3	diabetes mellitus
327	SIDS	0	16.5	14.2	5.4	6 month old; maternal history of crack use during pregnancy

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml)	Bradykinin (pg/ml)	Met-Enkephalin (pg/ml)	Contributing Factors to Death and Comments
328	Acute And Chronic Bronchial Asthma	12 hours	23.1	6.9	13.4	generalized acute visceral congestion
329	Acute Pancreatitis; Hyperlipidemia	25 hours	51.1	22.4	9.8	abdominal pain after eating
330	Suicide - Hanging	0	22.6	8.3	4.5	
331	Homicide - Complications Of Gunshot Wound To Abdomen Due To Bronchopneumonia	4 months	78.9	68.8	249.8	diabetes mellitus; degenerative joint disease
332	Homicide - Gunshot Wound To Head	2 weeks	144.3	172.9	9.8	
333	Exsanguination Following Breakage Of Indwelling Central Venous Catheter	3 days, 8 hours	11.2	8.0	33.6	81 year old

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml)	Bradykinin (pg/ml)	Met-Enkephalin (pg/ml)	Contributing Factors to Death and Comments
334	Intrapelvic And Retroperitoneal Hemorrhage Following Revision Arthroplasty Of Hip For Treatment Of Femoral Fracture Due To Fall	31 days	172.1	216.9	105.6	84 year old
335	Hypertensive Cardiovascular Disease	30 minutes	23.2	14.6	9.0	chronic cholecystitis
336	Homicide - Gunshot To Head	15 minutes	17.3	7.6	10.8	
337	Suicide - Blunt Trauma To Torso And Extremities	0	14.8	3.1	4.7	psychiatric history; jumped from height
338	Suicide - Hanging	0	30.7	16.8	5.9	

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml)	Bradykinin (pg/ml)	Met-Enkephalin (pg/ml)	Contributing Factors to Death and Comments
339	Hypertensive And Arteriosclerotic Cardiovascular Disease Complicated By Bilateral Pneumonia With Sepsis	19 days	97.6	80.8	11.0	lung carcinoma; pulmonary emphysema; chronic diverticulosis; diabetes mellitus
340	AIDS Related Pneumocystis Carinii Pneumonia	3 days, 7 hours	63.3	75.6	90.8	
341	Pulmonary Embolism Due To Deep Venous Thrombosis Complicating Cellulitis Of Lower Extremity	17½ hours	68.1	90.6	23.5	
342	Intracerebral Hemorrhage Due To Hypertensive Cardiovascular Disease	0	15.8	2.8	14.8	fatty liver

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml)	Bradykinin (pg/ml)	Met-Enkephalin (pg/ml)	Contributing Factors to Death and Comments
343	Suicide - Self-Immolation; Sepsis And Multisystem Organ Failure Complicating Thermal Burns Of 70% Of Body Surface Area	17½ days	172.9	244.2	329.4	mental illness
344	Acute Peritonitis Due To Perforation Of Appendix Due To Acute Appendicitis	0	74.1	118.2	14.9	
345	Viral Pneumonitis Due To Respiratory Syncytial Virus Infection	0	14.8	5.4	26.4	6 month old
346	Accidental Blunt Trauma To Torso Causing Multiple Pelvic And Rib Fractures With Exsanguination	4 hour	74.3	56.1	17.2	pedestrian hit by bus

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml)	Bradykinin (pg/ml)	Met-Enkephalin (pg/ml)	Contributing Factors to Death and Comments
347	Acute And Chronic Bronchial Asthma	30 minutes	15.3	3.3	4.9	2 year old; premature birth at 6 months gestation due to maternal placental rupture
348	Homicide - Gunshot Wound To Head With Perforation Of Spine And Spinal Cord	0	18.0	2.9	9.5	
349	Homicide - Gunshot Wound To Head	4 days, 2½ hours	88.8	32.9	27.4	
350	Anoxic Encephalopathy Due To Ruptured Cerebral Artery Aneurysm	11 days, 8½ hours	90.5	80.2	74.7	
376	Fetal Demise-Probable Chorioammionitis	0	3.4	29.4	5.6	stillborn fetus; 9 month gestation

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml)	Bradykinin (pg/ml)	Met-Enkephalin (pg/ml)	Contributing Factors to Death and Comments
377	Fetal Demise	0	10.4	32.6	10.2	stillborn; 9 month gestation; umbilical cord wrapped around head
378	Suicide - Acute Intoxication Due To Combined Effects Of Butalbital And Sertraline	0	8.2	94.1	24.2	
379	Complications Of Retroperitoneal Lysosarcoma And Leiomyosarcoma	1 month	106.0	145.0	45.5	hypertension; bronchitis
380	Acute And Chronic Bronchial Asthma	0	8.5	24.4	11.3	hypertensive cardiovascular disease
381	Homicide - Stab Wound To Chest With Perforation Of Heart And Liver	0	72.3	67.3	4.4	

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml)	Bradykinin (pg/ml)	Met-Enkephalin (pg/ml)	Contributing Factors to Death and Comments
382	Complications Of Prematurity Caused By Abruptive Placentae And Maternal Cocaine Use During Pregnancy	1½ hours	20.2	25.6	13.9	2 week old; born 3 months premature
383	Suicide - Incised Wound Of Upper Extremity With Transection Of Ulnar Artery	0	50.9	82.7	37.7	cholelithiasis; depressive
384	Complications Of Bronchopulmonary Dysplasia	0	12.4	8.2	16.1	9 month old; born 3 months premature

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml)	Bradykinin (pg/ml)	Met-Enkephalin (pg/ml)	Contributing Factors to Death and Comments
385	Sarcoidosis	8 hours	34.4	103.9	120.9	diabetic ketoacidosis; hyperosmolar coma due to hyperglycemia and high dose prednisone therapy for treatment of sarcoidosis
386	Complications Of Gangrene, Diabetes Mellitus, And Cardiovascular Disease	16 hours	43.6	266.9	187.1	lidocaine, diphenhydramine, pentoxifylline detected
387	Homicide - Parental Neglect; Malnutrition	5 minutes	17.5	1.1	34.1	5 week old
388	Acute Pancreatitis	2 hours, 15 minutes	156.2	187.9	84.5	abdominal pain, weakness, nausea

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml serum) (pg/g brain)	Bradykinin (pg/ml serum) (pg/g brain)	Met-Enkephalin (pg/ml serum) (pg/g brain)	Contributing Factors to Death and Comments
351	Accidental Drug Overdose Due To Acute Intoxication Of Opiates And Cocaine	0	serum: 16.9 brain: 8.5	24.0 9.5	5.6 4.5	chronic abuser
352	Suicide-Hanging	10 days	serum: 66.3 brain: 110.1	71.4 104.3	54.0 83.8	treated medicinally for depression
353	Chronic Obstructive Pulmonary Disease With Cor Pulmonale Due To Pulmonary Emphysema	15 minutes	serum: 22.8 brain: 130.9	50.5 56.9	63.3 39.3	
354	Multiple Complications Of Radiation Therapy For Carcinoma Of Left Lung	30 minutes	serum: 22.6 brain: 61.1	56.0 279.6	159.2 226.6	asthmatic
355	Cardiac Arrhythmia Due To Dilated Cardiomyopathy Of Unknown Etiology	1 hour	serum: 48.6 brain: 65.8	44.7 94.9	3.9 67.5	obesity
356	Hypertensive And Atherosclerotic Cardiovascular Disease	10 minutes	serum: 18.9 brain: 174.0	11.9 38.5	3.3 42.3	obesity, diabetes mellitus
357	Homicide-Gunshot Wound To Head	0	serum: 9.9 brain: 63.2	41.1 54.2	16.2 86.2	

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml serum) (pg/g brain)	Bradykinin (pg/ml serum) (pg/g brain)	Met-Enkephalin (pg/ml serum) (pg/g brain)	Contributing Factors to Death and Comments
358	Accidental Drug Overdose Due To Acute Intoxication Of Opiates And Cocaine	0	serum: 5.0 brain: 6.5	58.5 148.0	148.3 130.2	AIDS; wasting syndrome; end renal disease; dilated cardiomyopathy
359	Homicide-Stab Wound To Abdomen Causing Laceration Of Spleen With Hemoperitoneum	5½ hours	serum: 25.6 brain: 186.7	44.8 29.6	25.5 53.6	.
360	Cardiac Arrhythmia Due To Hypertensive Cardiovascular Disease	30 minutes	serum: 29.9 brain: 130.6	39.2 53.9	47.2 64.9	
361	Homicide-Gunshot Wound To Head	0	serum: 56.1 brain: 114.6	64.3 45.1	27.0 66.9	
362	Seizure Disorder Due To Chronic Alcoholism	0	serum: 102.1 brain: 93.6	2.5 17.9	9.1 11.7	schizophrenic
363	Accidental Blunt Trauma To Head And Torso Causing Lacerations Of Brain And Heart	0	serum: 42.3 brain: 225.4	45.3 59.1	44.8 75.3	fell from height
364	Accidental Blunt Trauma To Head, Neck, And Torso	0	serum: 118.8 brain: 317.6	52.1 72.9	60.0 89.7	pedestrian struck by motor vehicle

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml serum) (pg/g brain)	Bradykinin (pg/ml serum) (pg/g brain)	Met-Enkephalin (pg/ml serum) (pg/g brain)	Contributing Factors to Death and Comments
365	Accidental Blunt Trauma To Torso Causing Hemorrhagic Complications	32 days	serum: 109.5 brain: 91.2	55.8 187.4	191.0 153.8	pedestrian struck by motor vehicle
366	Suicide-Blunt Trauma To Head, Torso, And Extremities	0	serum: 99.7 brain: 137.8	89.9 173.7	9.5 82.0	jumped from height
367	Accidental Drug Overdose Due To Acute Intoxication Of Opiates And Cocaine	0	serum: 38.7 brain: 46.4	20.4 68.5	36.6 60.3	
368	Accidental Drug Overdose Due To Acute Intoxication Of Opiates	0	serum: 42.8 brain: 86.7	39.4 61.0	4.2 65.6	chronic substance abuser
369	Accidental Blunt Trauma To Torso Causing Lung Lacerations With Hemothorax	28 hours	serum: 158.3 brain: 72.0	134.3 206.8	38.6 187.4	fell from height
370	Seizure Disorder Of Unknown Etiology	0	serum: 30.8 brain: 66.2	54.4 126.8	26.2 126.9	generalized visceral congestion and edema
371	Hypertensive And Atherosclerotic Cardiovascular Disease	0	serum: 30.0 brain: 104.6	39.1 55.7	41.8 86.0	collapsed while getting wheelchair in car; diabetes mellitus

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml serum) (pg/g brain)	Bradykinin (pg/ml serum) (pg/g brain)	Met-Enkephalin (pg/ml serum) (pg/g brain)	Contributing Factors to Death and Comments
372	Suicide-Blunt Trauma To Head Causing Lacerations Of Brain And Spinal Cord	5 days, 18 hours	serum: 50.6 brain: 101.9	56.3 5.4	8.3 33.4	jumped from height
373	Suicide-Blunt Trauma To Head, Torso, And Extremities	0	serum: 105.5 brain: 236.7	91.7 80.8	52.9 114.4	jumped in front of train
374	Bile Peritonitis Complicating Liver Biopsy For Diagnostic Work-Up Of Viral Hepatitis C	8 days	serum: 101.2 brain: 58.8	95.4 168.8	112.2 142.3	sepsis
375	Bronchopneumonia Complicating Pancreatic Adenocarcinoma	1 week	serum: 12.0 brain: 16.7	94.3 250.7	130.2 174.2	metastasis to liver; wasting
389	Homicide-Stab Wound To Chest With Perforation Of Heart And Aorta	30 minutes	serum: 49.9 brain: 74.7	20.3 17.6	36.3 53.6	compression of neck
390	Hemoptysis With Aspiration Of Blood Due To Carcinoma Of Lung	0	serum: 8.3 brain: 3.3	56.4 103.1	197.0 192.1	
391	Acute And Chronic Bronchial Asthma	0	serum: 30.8 brain: 39.1	95.4 94.3	33.0 70.6	morbid obesity

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml serum) (pg/g brain)	Bradykinin (pg/ml serum) (pg/g brain)	Met-Enkephalin (pg/ml serum) (pg/g brain)	Contributing Factors to Death and Comments
392	Homicide-Gunshot To Neck	30 minutes	serum: 109.1 brain: 192.9	48.2 46.9	40.2 19.2	
393	Bronchopneumonia Complicating Emphysema	0	serum: 56.6 brain: 100.7	109.0 43.2	77.3 95.2	chronic alcohol abuser; heavy smoker
394	Carcinoma Of Breast	0	serum: 9.5 brain: 7.5	56.6 164.6	168.3 126.0	hypertensive , cardiovascular disease
395	Homicide-Stab Wound To Neck With Lacerations To Common Carotid Artery And Hypopharynx	30 minutes	serum: 72.2 brain: 127.7	55.1 64.9	44.4 18.9	
396	Accident-Hanging	0	serum: 21.1 brain: 50.6	62.1 39.1	36.9 37.3	autocrotic asphyxia
397	Accidental Blunt Trauma To Torso And Right Lower Extremity	2 hours	serum: 59.0 brain: 130.2	116.2 41.7	19.9 52.3	pedestrian hit by car
398	Diabetes Mellitus Complicating Cardiovascular Disease	0	serum: 20.1 brain: 8.6	140.7 151.9	170.3 138.7	gallstones
399	Diabetes Mellitus Complicating Hypertensive Cardiovascular Disease	10 months	serum: 62.3 brain: 78.1	63.9 40.6	56.3 51.1	heavy smoker; diabetic coma

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml serum) (pg/g brain)	Bradykinin (pg/ml serum) (pg/g brain)	Met-Enkephalin (pg/ml serum) (pg/g brain)	Contributing Factors to Death and Comments
400	Suicide-Gunshot Wound To Head	0	serum: 46.8 brain: 57.2	46.6 62.3	13.9 13.5	
401	Homicide-Multiple Gunshot Wounds To Chest With Perforation Of Lung And Heart	0	serum: 30.4 brain: 93.1	30.6 31.9	13.4 24.4	
402	Gangrenous Small Bowel Due To Incarceration Into Unbilical Hernial Sac	0	serum: 19.6 brain: 14.5	72.6 101.5	66.8 196.6	abdominal pain and vomiting prior to death
403	Cardiac Arrest Due To Hypertensive Cardiovascular Disease	30 minutes	serum: 95.1 brain: 160.0	111.3 33.7	30.9 31.7	gallbladder removed 2 days prior death; sickle cell disease
404	Acute And Chronic Bronchial Asthma	45 minutes	serum: 27.3 brain: 50.4	58.4 25.2	14.2 39.4	abdominal pain prior to death
405	Homicide-Gunshot Wound To Head	0	serum: 31.9 brain: 57.2	43.9 49.8	18.5 43.3	

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation		Substance P (pg/ml serum) (pg/g brain)	Bradykinin (pg/ml serum) (pg/g brain)	Met-Enkephalin (pg/ml serum) (pg/g brain)	Contributing Factors to Death and Comments
406	Septic Complications Following Dislodgement Of Gastrostomy Tube Inserted After Pulmonary Complications From Lung Nodule Biopsy Before Kidney Transplant Due To Atherosclerotic And Hypertensive Cardiovascular Disease	67 days	serum: brain:	74.8 79.3	39.9 32.0	46.4 23.0	
407	Homicide-Ligature Strangulation	0	serum: brain:	92.8 167.4	145.6 78.5	18.9 25.6	81 year old woman
408	Suicide-Hanging	0	serum: brain:	61.8 89.6	33.1 56.5	70.1 52.6	
409	Fungal Bronchopneumonia Complicating Pulmonary Emphysema	10 minutes	serum: brain:	46.8 117.3	93.5 28.5	62.0 63.1	diabetes mellitus; cardiovascular disease; chronic alcoholism
410	Upper Gastrointestinal Hemorrhage Due To Benign Duodenal Peptic Ulcer	0	serum: brain:	105.2 142.4	89.2 38.9	54.3 62.5	chronic cocaine and opiate abuse
411	Peritonitis Due To Perforated Duodenal Ulcer	0	serum: brain:	78.9 79.1	167.0 50.8	40.6 59.8	chronic alcoholism; hepatic cirrhosis; hepatic steatosis; cholelithiasis

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml serum) (pg/g brain)	Bradykinin (pg/ml serum) (pg/g brain)	Met-Enkephalin (pg/ml serum) (pg/g brain)	Contributing Factors to Death and Comments
412	Septic Complications Following Sigmoid Colon Resection Due To Sigmoid Colon Volvulus Of Unknown Etiology	11 days, 7 hours	serum: 173.3 brain: 194.3	87.7 54.0	73.5 46.0	renal failure; hypertensive and atherosclerotic cardiovascular disease; schizophrenic
413	Homicide-Stab Wound To Chest With Perforation Of Heart	30 minutes	serum: 94.1 brain: 221.6	145.3 51.9	52.3 55.3	
414	Homicide-Multiple Gunshot Wounds To Neck, Extremities, And Chest With Perforations Of Subclavian Blood Vessels And Lung	8 hours	serum: 135.9 brain: 185.5	99.6 37.4	9.9 48.3	
415	Diabetes Mellitus	0	serum: 40.8 brain: 44.9	6.5 8.2	7.2 14.1	obesity, mental retardation, gout
416	Suicide-Hanging	0	serum: 50.8 brain: 130.9	14.1 12.8	15.9 6.7	psychiatric patient
417	Acute Hemorrhagic Pancreatitis	0	serum: 101.1 brain: 111.7	102.8 59.7	5.9 9.1	chronic alcoholism; prostate cancer; hypertensive and arteriosclerotic cardiovascular disease

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml serum) (pg/g brain)	Bradykinin (pg/ml serum) (pg/g brain)	Met-Enkephalin (pg/ml serum) (pg/g brain)	Contributing Factors to Death and Comments
418	Hypertensive And Arteriosclerotic Cardiovascular Disease	20 minutes	serum: 42.1 brain: 63.2	69.7 48.9	10.3 6.9	peptic ulcer
419	Bronchopneumonia Complicating End Stage Renal Disease	11 days	serum: 20.7 brain: 7.0	50.5 146.5	72.2 248.4	systemic lupus erythematosis
420	SIDS	7 hours	serum: 33.1 brain: 28.1	77.9 33.4	11.1 9.5	4 week old
421	Homicide-Stab Wound To Chest With Perforation Of Pulmonary Artery	3 hours	serum: 119.6 brain: 183.9	216.0 24.9	19.2 49.7	
422	Homicide-Stab Wound To Chest With Perforation Of Aorta, Lung, And Heart	40 minutes	serum: 57.7 brain: 85.3	117.9 29.6	22.9 28.6	
423	Smoke Inhalation And Second And Third Degree Burns On Face, Forehead, Right Extremities, And Left Shoulder Chest	0	serum: 106.1 brain: 189.7	267.1 50.2	12.6 37.1	history of cardiac arrest

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml serum) (pg/g brain)	Bradykinin (pg/ml serum) (pg/g brain)	Met-Enkephalin (pg/ml serum) (pg/g brain)	Contributing Factors to Death and Comments
424	Septic Complications Following Bladder Perforation During Cytoscopy For The Evaluation And Treatment Of Bladder Metastasis Due To Metastatic Prostate Carcinoma	35 days	serum: 11.8 brain: 3.0	33.6 149.8	121.3 296.9	.
425	Pulmonary Thromboembolism Due To Venous Thrombosis Of Left Lower Extremity As Result Of Ankle Injury	0	serum: 153.6 brain: 179.7	243.1 35.1	20.2 17.0	recent ankle surgery
426	Acute And Chronic Bronchial Asthma	0	serum: 64.3 brain: 126.2	93.2 23.7	6.5 14.2	cocaine and ethanol abuser
427	Post-Operative (splectomy) Exsanguinating Hemorrhage	11½ hours	serum: 101.6 brain: 198.7	65.2 53.5	13.3 71.6	history of polycythemia vera/myelodysplastic syndrome
428	Homicide-Blunt Trauma To Head	0	serum: 98.6 brain: 142.8	176.8 74.9	12.2 7.0	decedent was beaten
429	Accidental Drug Overdose Due To Acute Opiate Intoxication	0	serum: 28.6 brain: 7.0	51.5 243.2	148.2 164.5	AIDS

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml serum) (pg/g brain)	Bradykinin (pg/ml serum) (pg/g brain)	Met-Enkephalin (pg/ml serum) (pg/g brain)	Contributing Factors to Death and Comments
430	Bronchopneumonia	0	serum: 60.4 brain: 147.6	226.4 12.4	14.6 32.7	chronic alcoholism; hepatic cirrhosis
431	Accidental Drug Overdose Due To Acute Toxicity Of Anti-Depressant Medication	0	serum: 31.7 brain: 40.4	50.7 20.7	52.3 82.6	psychiatric disorder
432	Smoke Inhalation Resulting In Carbon Monoxide Poisoning; Second And Third Degree Burns Over 72% Of Body	3 months, 3 weeks	serum: 18.3 brain: 3.0	122.2 202.1	171.4 236.6	
433	Homicide-Stab Wound To Chest With Perforation Of Heart	30 minutes	serum: 62.5 brain: 129.9	136.4 44.2	21.6 21.5	
434	Homicide-Gunshot Wound To Neck With Perforations Of Great Vessels And Pharynx	1½ hours	serum: 35.4 brain: 43.2	13.1 33.0	12.3 40.4	

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml serum) (pg/g brain)	Bradykinin (pg/ml serum) (pg/g brain)	Met-Enkephalin (pg/ml serum) (pg/g brain)	Contributing Factors to Death and Comments
435	Pulmonary Thromboembolism With Deep Venous Thrombosis Following Surgical Fixation Of Ankle Fracture	10½ hours	serum: 68.2 brain: 165.4	61.7 11.1	17.2 40.0	fell at home; hypertensive and atherosclerotic cardiovascular disease
436	Lobar Pneumonia With Emphysema	30 minutes	serum: 49.6 brain: 56.5	98.1 27.9	18.2 6.9	chronic alcoholism; fatty liver
437	Complications Of End Stage Renal Failure	16 days, 16 hours	serum: 37.8 brain: 190.6	181.7 15.7	15.7 6.0	chronic intravenous drug abuse
438	Suicide-Gunshot Wound To Head	0	serum: 55.1 brain: 164.0	149.9 30.3	17.5 26.3	metastatic prostate cancer
439	Chronic Pancreatitis And Hepatic Steatosis Due To Chronic Alcoholism	3 hours	serum: 35.2 brain: 29.2	32.9 41.3	100.4 132.4	hypertensive cardiovascular disease
440	Fatty Liver Due To Acute And Chronic Alcoholism	0	serum: 33.7 brain: 46.5	51.4 76.1	21.7 28.1	
441	Arteriosclerotic Cardiovascular Disease	20 minutes	serum: 23.2 brain: 33.8	35.7 90.6	74.1 106.9	chronic alcoholism with chronic pancreatitis
442	Fatty Liver And Fibrosis Of Liver Due To Chronic Alcoholism	2 hours	serum: 12.2 brain: 30.9	20.2 55.2	17.2 19.4	hypertensive cardiovascular disease

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml serum) (pg/g brain)	Bradykinin (pg/ml serum) (pg/g brain)	Met-Enkephalin (pg/ml serum) (pg/g brain)	Contributing Factors to Death and Comments
443	Hypertensive And Atherosclerotic Cardiovascular Disease	0	serum: 42.9 brain: 52.9	45.2 73.9	33.9 47.9	cardiac enlargement
444	Accidental Acute Intoxication By The Combined Effects Of Opiates And Cocaine	2 ½ hours	serum: 13.4 brain: 16.6	130.3 203.7	80.6 127.3	AIDS related pneumonia; chronic intravenous drug abuse
445	Thrombosis Of Left Anterior Descending Coronary Artery Due To Arteriosclerotic Disease	20 minutes	serum: 44.7 brain: 85.5	47.0 47.5	62.5 70.1	peptide ulcer disease
446	AIDS	20 minutes	serum: 30.0 brain: 41.6	53.1 187.8	64.0 125.5	chronic alcoholism with fatty liver; history of chronic seizure disorder
447	Hypertensive And Arteriosclerotic Cardiovascular Disease With Diabetes Mellitus	10 minutes	serum: 61.9 brain: 83.0	39.3 46.2	21.5 43.4	bronchial asthma with chronic obstructive pulmonary disease
448	Rupture Of Aortic Aneurysm With Hemoperitoneum Due To Atherosclerotic Cardiovascular Disease	1 hour	serum: 78.0 brain: 145.7	154.1 44.1	27.9 48.6	obesity

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation		Substance P (pg/ml serum) (pg/g brain)	Bradykinin (pg/ml serum) (pg/g brain)	Met-Enkephalin (pg/ml serum) (pg/g brain)	Contributing Factors to Death and Comments
449	Pulmonary Thromboembolus Due To Deep Venous Thrombosis Due To Immobility Following Left L4-5 Laminotomy And Discectomy For Herniated Intervertebral Disk	20 minutes	serum: brain:	62.9 85.1	73.9 39.4	30.4 41.7	pulmonary emphysema
450	Anaphylactic Reaction Following Administration Of Taxel For Metastatic Breast Cancer	14 hours	serum: brain:	14.7 5.2	130.7 134.0	105.1 156.8	bilateral breast carcinoma; obesity; hypertensive and atherosclerotic cardiovascular disease
451	Accidental Acute Cocaine Intoxication	0	serum: brain:	12.2 12.9	33.3 28.9	39.9 51.3	hypertensive and atherosclerotic cardiovascular disease
452	Accidental Blunt Trauma To Head, Neck, And Torso	20 minutes	serum: brain:	49.1 157.2	71.9 40.4	47.4 57.3	81 year old passenger of vehicle struck by another vehicle
453	Chronic Pancreatitis And Cirrhosis Of Liver As A Result Of Chronic Alcoholism	0	serum: brain:	19.2 16.4	69.8 67.6	82.7 133.9	

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation		Substance P (pg/ml serum) (pg/g brain)	Bradykinin (pg/ml serum) (pg/g brain)	Met-Enkephalin (pg/ml serum) (pg/g brain)	Contributing Factors to Death and Comments
454	Suicide - Gunshot Wound To Head	0	serum: brain:	23.5 32.6	43.5 62.7	10.8 16.4	78 year old male
455	Rhabdomyolysis With Multiple Complications Due To Postural Compression Of Lower Extremities While Intoxicated With Accidental Overdose Of Heroin And Cocaine	4 days	serum: brain:	8.6 17.7	70.8 81.2	110.2 120.8	AIDS
456	Hepatosteatosis And Cirrhosis Of Liver As A Result Of Chronic Alcoholism	15 ½ hours	serum: brain:	33.1 50.8	55.0 144.4	76.7 135.6	osteoporosis; atherosclerotic cardiovascular disease
457	Suicide - Asphyxiation Caused By Plastic Bag Over Head	0	serum: brain:	21.5 70.4	70.5 47.0	48.7 53.5	intoxication due to combined effects of zolpidem, propoxyphene, and sertraline; history of depression and diabetes
458	Homicide - Gunshot Wound Of Left Chest Causing Perforation Of Heart And Lungs	40 minutes	serum: brain:	49.8 155.1	73.3 40.5	56.5 58.9	atherosclerosis of coronary arteries
459	Atherosclerotic Cardiovascular Disease	4 ½ hours	serum: brain:	46.3 110.7	50.5 59.3	25.7 32.2	bronchopneumonia

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation		Substance P (pg/ml serum) (pg/g brain)	Bradykinin (pg/ml serum) (pg/g brain)	Met-Enkephalin (pg/ml serum) (pg/g brain)	Contributing Factors to Death and Comments
460	Multiple Complications Of Hypertensive Cardiovascular Disease With End Stage Renal Failure Treated With Peritoneal Dialysis Including Bacterial Peritonitis	16 days	serum: 87.0 brain: 94.1	145.8 59.7	101.8 134.6		cholelithiasis
461	Suicide - Drowning	0	serum: 26.5 brain: 76.1	12.7 58.6	30.9 66.2		fractures of ribs and spine
462	Arteriosclerotic Cardiovascular Disease	0	serum: 6.2 brain: 20.3	155.9 119.9	169.7 150.7		carcinoma of stomach; 87 year old female
463	Accidental Acute Intoxication By The Combined Effects Of Opiates And Cocaine	0	serum: 21.6 brain: 40.5	25.3 64.9	43.4 66.7		chronic substance abuse
464	Complications Of Bowel Perforation Due To Diverticulitis	6 weeks	serum: 94.7 brain: 253.1	103.0 109.9	66.1 111.1		diabetes mellitus; obesity; hypertensive and atherosclerotic cardiovascular disease
465	Gastroesophageal Hemorrhage And Multiple Cirrhosis As A Result Of Chronic Alcoholism	11 ½ days	serum: 78.9 brain: 214.7	76.0 140.2	94.9 104.5		weakened renal function; chronic pancreatitis; pleural effusions; splenomegaly

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml serum) (pg/g brain)	Bradykinin (pg/ml serum) (pg/g brain)	Met-Enkephalin (pg/ml serum) (pg/g brain)	Contributing Factors to Death and Comments
466	Smoke Inhalation	0	serum: 29.0 brain: 63.0	50.9 48.1	45.5 50.8	no burns; apartment fire
467	Acute And Chronic Asthma	7 minutes	serum: 63.6 brain: 95.9	56.9 36.4	28.5 31.0	accidental acute cocaine intoxication; crack user; hepatic steatosis
468	Homicide - Gunshot Wounds Of Head, Chest, And Arm With Perforations Of Lung, Heart, And Aorta	15 minutes	serum: 17.5 brain: 104.7	28.7 42.0	15.9 28.3	
469	Homicide - Gunshot Wound Of Chest With Perforations Of Left Subclavian Artery, Left Lung And Penetration Of Spinal Cord	1 day 14 ½ hours	serum: 138.3 brain: 341.3	104.2 57.7	29.9 33.1	
470	Homicide - Gunshot Wound Of Head With Penetration Of Skull And Brain	15 minutes	serum: 61.2 brain: 117.4	83.4 24.8	20.0 26.9	
471	Homicide - Stab Wound To Right Thigh With Perforations Of Femoral Vessels	10 hours	serum: 152.4 brain: 228.7	158.6 36.9	27.2 45.0	

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml serum) (pg/g brain)	Bradykinin (pg/ml serum) (pg/g brain)	Met-Enkephalin (pg/ml serum) (pg/g brain)	Contributing Factors to Death and Comments
472	SIDS	20 minutes	serum: 10.3 brain: 15.4	13.8 27.3	17.1 22.3	5 month old; prematurely born at 34 weeks
473	Small Bowel Obstruction And Gangrene Due To Herniation Below Fibrous Intraabdominal Band	0	serum: 81.4 brain: 296.7	76.8 38.8	60.2 68.5	colonic diverticula
474	Homicide - Gunshot Wound Of Eye Penetrating Brain	24 hours	serum: 247.8 brain: 317.1	157.2 42.2	11.7 27.9	
475	Chronic Alcoholism With Cirrhosis Of The Liver And Alcoholic Cardiomyopathy	0	serum: 43.9 brain: 34.4	36.7 29.3	18.5 40.8	
476	Chronic Alcoholism With Hepatic Cirrhosis And Portal Hypertension	0	serum: 16.7 brain: 27.2	67.4 25.8	32.6 38.9	
477	Homicide - Gunshot Wound To Chest Perforating Carotid Arteries	4 ½ hours	serum: 75.8 brain: 253.4	96.8 48.4	25.5 48.3	

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation		Substance P (pg/ml serum) (pg/g brain)	Bradykinin (pg/ml serum) (pg/g brain)	Met-Enkephalin (pg/ml serum) (pg/g brain)	Contributing Factors to Death and Comments
478	Congenital Heart Abnormality, Tetralogy Of Fallot With Pulmonary Atresia	15 minutes	serum: 14.7 brain: 60.2	29.5 21.4	21.8 21.2	6 week old	
479	Acute And Chronic Bronchial Asthma	15 minutes	serum: 31.4 brain: 107.4	90.9 23.9	27.2 58.6		
480	Hypertensive And Atherosclerotic Cardiovascular Disease	0	serum: 37.5 brain: 47.6	14.0 18.5	22.8 23.8	chronic alcoholism with hepatosteatosi	
481	Accidental Drug Overdose Due To Acute Cocaine Intoxication	0	serum: 17.1 brain: 21.6	107.6 106.4	144.2 144.0	AIDS; chronic alcoholism with cirrhosis of the liver; crack user	
482	Stroke	0	serum: 47.5 brain: 103.0	37.0 45.2	20.9 35.5	history of alcohol abuse	
483	Acute Bronchopneumonia Due To Chronic Alcoholism	0	serum: 40.9 brain: 153.2	45.6 52.2	17.5 25.7	hypertensive heart disease; hepatic steatosis	
484	Cardiac Arrhythmia Of Unknown Etiology And Edema Of Lungs	0	serum: 18.1 brain: 102.6	59.7 55.4	18.1 36.9	obesity; hepatomegaly; splenomegaly	

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation		Substance P (pg/ml serum) (pg/g brain)	Bradykinin (pg/ml serum) (pg/g brain)	Met-Enkephalin (pg/ml serum) (pg/g brain)	Contributing Factors to Death and Comments
485	Homicide - Child Abuse Syndrome Due To Blunt Trauma To Torso With Laceration Of Heart And Liver	30 minutes	serum: brain:	187.2 322.4	357.4 70.0	54.3 75.8	2 year old
486	Bronchial Asthma	24 hours	serum: brain:	20.5 111.5	84.9 59.2	23.7 38.2	mild bronchopneumonia; peritoneal adhesions
487	SIDS	30 minutes	serum: brain:	45.9 50.9	34.9 44.3	11.4 60.6	5 year old; full term delivery
488	Intestinal Hemorrhage Following Total Gastrectomy For Recurrent Gastric Carcinoma	4 days	serum: brain:	42.9 69.9	267.5 100.5	106.7 194.3	hypertensive and arteriosclerotic cardiovascular disease; pulmonary emphysema
489	Accidental Drowning	0	serum: brain:	44.1 90.9	86.9 65.7	8.7 14.2	mass lesions of thyroid
490	Intraperitoneal Hemorrhage Complicating Abdominal Peritoneal Resection For Renal Cancer	9 days	serum: brain:	13.2 40.4	330.3 249.6	109.1 179.2	end stage renal disease; hypertensive and atherosclerotic heart disease
491	Bronchopneumonia Due To AIDS	0	serum: brain:	10.6 15.2	202.0 294.6	119.3 190.9	chronic intravenous narcotism

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml serum) (pg/g brain)	Bradykinin (pg/ml serum) (pg/g brain)	Met-Enkephalin (pg/ml serum) (pg/g brain)	Contributing Factors to Death and Comments
492	Hypertensive Cardiovascular Disease With Congestive Heart Disease	0	serum: 72.8 brain: 56.9	97.0 71.3	26.4 56.7	edema of lungs; severe allergies
493	Accidental Electrocution	45 minutes	serum: 25.2 brain: 52.4	58.5 66.1	15.6 53.9	electrical burns of mouth, chest, and arm; 16 month old chewing on an electric cord
494	Accidental Asphyxiation Caused By Compression Of Chest	40 minutes	serum: 36.1 brain: 172.3	42.3 65.6	20.9 64.8	6 week old born premature; decedent found with 3 year old sibling sleeping across upper body; congestion of all internal organs
495	Multiple Complications Of Pancreatic Adenocarcinoma With Metastasis, Status Post Cholecystojejunostomy And Gastrojejunostomy	22 days	serum: 19.9 brain: 24.2	338.0 300.9	150.4 163.1	renal failure
496	Hypertensive And Atherosclerotic Cardiovascular Disease	0	serum: 82.9 brain: 136.9	85.6 57.3	15.8 51.4	cirrhosis of liver due to chronic alcoholism

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml serum) (pg/g brain)	Bradykinin (pg/ml serum) (pg/g brain)	Met-Enkephalin (pg/ml serum) (pg/g brain)	Contributing Factors to Death and Comments
497	Multiple Complications Of Graft vs. Host Disease Due To Allogeneic Bone Marrow Transplant For Chronic Myelogenous Leukemia	2 weeks	serum: 279.6 brain: 310.2	267.9 175.2	177.8 217.3	bronchopneumonia; sepsis; renal insufficiency
498	Meconium Aspiration Due To Maternal Asphyxia Preeclampsia And Ruptured Uterus	2 weeks	serum: 20.9 brain: 48.7	47.4 75.6	28.6 39.8	severe axonic encephalopathy; 2 week old
499	Smoke Inhalation	0	serum: 55.3 brain: 140.9	66.7 70.6	28.0 65.5	thermal burns of body surfaces; accidental fire at home
500	Diffuse Large Cell Lymphoma	8 ½ hours	serum: 10.4 brain: 39.5	354.8 238.8	186.6 234.2	fever; respiratory distress
501	Acute Pulmonary Thromboembolism Due To Deep Vein Phlebothrombosis Of Lower Extremities Of Undetermined Etiology	13 ½ hours	serum: 78.4 brain: 196.8	58.3 84.3	31.4 49.5	history of alzheimer's disease

Case #	Primary Manner of Death	Duration of Medical Therapy and Resuscitation	Substance P (pg/ml serum) (pg/g brain)	Bradykinin (pg/ml serum) (pg/g brain)	Met-Enkephalin (pg/ml serum) (pg/g brain)	Contributing Factors to Death and Comments
502	Homicide - Child Abuse Syndrome Due To Multiple Complications Of Second And Third Degree Burns Of Approximately 67% Of Body Surface Including Head, Torso, And Extremities	4 months 15 days	serum: 13.8 brain: 23.4	375.6 295.3	166.2 183.1	6 month old immersed in hot water

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