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VASOPRESSIN ANALGESIA: SPECIFICITY OF ACTION AND NON-OPIOID EFFECTS

*City University of New York* PH.D. 1984

# VASOPRESSIN ANALGESIA: SPECIFICITY OF ACTION AND NON-OPIOID EFFECTS

by

JEFFREY H. KORDOWER

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

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ABSTRACT

Vasopressin Analgesia: Specificity of Action and Non-Opioid Effects

by

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Vasopressin (VP) has been localized in brain and spinal regions that are involved in nociceptive and stress-related behaviors. Behavioral studies have indicated that modulation of VP content, either by drug administration or with the use of a VP deficient rat strain, produce alterations in nociceptive thresholds. In addition, rats which are devoid of VP (Brattleboro strain) display an impaired analgesic response to stress. The present series of experiments aimed to determine the specificity of VP's action upon nociceptive processes, and to further assess VP's possible role in stress-induced analgesia in rats. Intracerebroventricular (icv) administration of arginine vasopressin (AVP) and desamino-D-AVP (DDAVP), in the ng range, significantly elevated pain thresholds on the tail-flick test. Oxytocin, a peptide which is synthesized in the same nuclei as VP, possesses a similar anatomical distribution as VP, and only differs from VP in two of its nine amino acids, produced seizure activity, suggesting that VP actions are

not due to a general magnocellular neurosecretory effect. Central pretreatment with a VP antagonist, dPTyr(me)AVP, but not naloxone, eliminated AVP and DDAVP analgesia. Conversely, icv pretreatment with naloxone, but not dPTyr(me)AVP attenuated morphine analgesia. dPTyr(me)AVP had no effect upon basal pain threholds. These data indicate that VP induced analgesia through interactions with its own receptors and independently of the endogenous opioids. Inhibition of ACTH, through systemic pretreatment with the synthetic glucocorticoid dexamethasone, potentiated icv VP analgesia, demonstrating that ACTH release is not necessary for the expression of VP analgesia. Lastly, icv pretreatment with dPTyr(me)AVP attenuated the analgesic response to prolonged intermittent foot shock and potentiated the analgesic response following brief continuous foot-shock. Therefore, it appears that VP plays a role in stress-induced analgesia with the direction of its effect determined by the parameters of the stress employed.

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# INTRODUCTION

The purpose of this dissertation is two-fold. First, to determine the relative specificity of action of vasopressin (VP) upon nociceptive processes, by comparing its effectiveness upon pain thresholds with that of oxytocin (OXY), a peptide with similar anatomical and biochemical properites, and by challenging VP's effects with various peptide antagonists. Secondly, VP's role in stress-induced analgesia is evaluated.

Vasopressin (VP) and oxytocin (OXY) are nonapeptides that are found in the magnocellular neurosecretory system. In addition, VP is an important peptide in the parvocellular neurosecretory system, and both peptides have similar and extensive neuroanatomical projections outside the hypothalamic pituitary actions. In the magnocellular neurohypophyseal system, VP and OXY act as neurohormones. VP secretion reduces extracellular fluid loss and modulates blood pressure. OXY secretion produces uterine contractions and is involved in the milk ejection reflex of lactation. In the parvocellular neurosecretory system, VP is secreted into the hypothalamo-hypophyseal system and is believed to be involved in the release of adrenocorticotrophic hormone (ACTH) in response to stressful stimulation. The terminal loci of the extrahypothalamic projection systems suggest a neuromodulatory role for VP and OXY in addition to their

neuroendocrine functions. The pathways to forebrain limbic areas lend anatomical support to behavioral evidence implicating both VP and OXY in learning and memory processes. Most importantly for the present study, VP and OXY project to caudal brain and spinal regions that are involved in the perception of noxious stimuli. VP increases pain thresholds, an effect that appears to be independent of the endogenous opioid pain-inhibitory system, providing evidence for endogenous opiate and non-opiate pain-inhibitory systems. However, it has yet to be demonstrated whether VP modulates nociceptive thresholds through action upon its own receptors or whether it activates other systems through some non-specific neuromodulatory process. Thus, the first aim of this dissertation research is to examine the relative specifity of action of VP by determining whether it exerts its influence upon nociceptive thresholds through an intrinsic VP system, or through interactions with other endogenous pain-inhibitory systems.

The interaction of VP and ACTH in the parvocellular neurosecrtory system suggests that VP might modulate coping responses to stressful stimuli. Since decreased sensitivity to noxious stimuli occurs in resppnse to stress, the influence of VP upon stress-induced analgesia was also investigated. VP's modulation of nociceptive processes is discussed following reviews of: a) the neuroanatomical localization, projection systems, and

functions of VP and OXY; b) the neuromodulation of VP and OXY with respect to learning and memory processes; and c) the evidence for opioid and non-opioid forms of pain inhibition.

I) Neuroanatomy and Function of VP and OXY Neurons

a) Cell Body Localization: VP and OXY are synthesized in anatomically distinct regions of the paraventricular (PVN) and supraoptic nuclei (SON) of the hypothalamus. VP neurons primarily occupy the ventral and lateral aspects of these nuclei, while OXY neurons are restricted to the dorsal and medial region of the SON and to a lesser extent to the PVN in the monkey (Zimmerman, 1976; Zimmerman, Stillman, Recht, Michaels, and Nilaver, 1978) although some species differences exist between the monkey, rat, cow, pig, and human (Defendini and Zimmerman, 1978; Vandesande and Dierickz, 1975; Zimmerman, Robinson, Husain, Acosta, Franz, and Sawyer, 1974). In addition, VP has been reported to be present in the smaller neurons of the suprachiasmatic nucleus (SCN) of the hypothalamus (Buijs, 1978) and in the various accessory hypothalamic nuclei, including the nucleus circularis, preoptic nucleus, zona incerta, and substantia innominata (Wiegand and Price, 1980; see review: Silverman and Zimmerman, 1983). While VP and OXY do not co-exist in the same cells, other peptides co-exist with VP in the hypothalamus, including dynorphin (Watson, Akil, Fischili,

Goldstein, Zimmerman, Nilaver, and van Wimersma Greidanis, 1982) and leu-enkephalin (Martin and Voigt, 1981). OXY also co-exists with cholecystokinan (CCK) (Vanderhaeghen, Lotstra, and Dierickx, 1981). Dissociation between the localization of VP and OXY is observed in the Brattleboro rat which, due to an autosomal defect, totally lacks VP and its neurophysin carrier protein in the PVN, SON, and SCN, although OXY levels are normal (Sokol and Valtin, 1965; Valtin, 1962, Valtin, 1976; Valtin, 1982; Valtin, Sawyer, and Sokol, 1965).

b) Neuroanatomy of the Magnocellular Neurosecretory System : Injections of horseradish peroxidase (HRP) into the posterior lobe of the pituitary gland produces retrograde labeling of the medial and lateral PVN (Hosoya and Matsushita, 1979), the dorsomedial PVN (Ono, Sasako, Muramoto, Yano, and Simpson, 1978), the PVN at the level of the anterior commissure (Armstrong, Warach, Haton, and McNeil, 1980; Rhodes, Morrell, and Pfaff, 1981; Swanson and Sawchenko, 1980) and the SON (Swanson and Sawchenko, 1980; Wiegand and Price, 1980, Zimmerman, 1981). While both the PVN and SON send neural projections containing VP and OXY to the posterior lobe of the pituitary gland, these projections do not join into a single fiber system Rather, axons from the SON decussate at the zona interna of the median eminance and continue through the infundibular stalk to the neurohypophysis. In contrast, the PVN fibers course ipsilaterally through the zona

interna of the median eminence and infundibular stalk to the posterior lobe of the pituitary gland (Alonso and Assenmacher, 1981).

c) Function of the Magnocellular Neurosecretory System: In this neurosecretory system, VP is secreted into the circulation from the posterior lobe of the pituitary gland in response to decreased fluid in extracellular space. This results in increased permeability to water in the long-collecting tubules of the kidney (Brownstein, Russell, and Gainer, 1980). VP administration also raises blood pressure although higher supra-physiological doses are needed, as compared to the VP dose necessary to induce antidiuresis (Sawyer, 1961). Since VP is absent in the Brattleboro rat, this animal it is unable to concentrate its urine and displays marked polyuria and polydipsia (Valtin, 1967; 1976). Like VP, OXY is also released from the neurohypophysis into the circulation where it interacts with those target organs involved in lactation and uterine contractions (Brownstein et al, 1980; Marshall, 1974).

d) Neuroanatomy of the Parvocellular Neurosecretory System: The suggestion that VP and its neurophysin are present in granules of axon terminals on or near portal capillaries in the zona externa of the median eminence (Bargman and Scharrer, 1951), has been confirmed by immunoelectronmicroscopy (Silverman and Zimmerman, 1975).

These capillaries form a specialized microcirculation that delivers high concentrations of neurohormones directly to the anterior lobe of the pituitary gland. The parvocellular VP-containing cells arise from the ipsilateral and medial PVN (Sofroniew, Weindl, Schinko, and Wetzstein, 1979). Although this system contains predominantly VP, OXY and its neurophysin have also been observed in the zona externa of the median eminence (Wiegand and Price, 1980).

e) Function of VP in the Parvocellular Neurosecretory System: The postulated role for VP in an organism's response to stress appears to be mediated through its interaction with corticotropin releasing factor (CRF) (Bloom, Battenberg, Rivier, and Vale, 1982; Gilles, Linton, and Lowry, 1982; Gilles and Lowry, 1979) and with ACTH (see review: McCann, 1980). These responses, in turn, appear to be influenced by the adrenal gland since adrenalectomy results in increased sprouting of VP-containing axons from the PVN to the zona externa of the median eminence, an effect that is prevented by glucocorticoid pretreatment (Silverman and Zimmerman, 1983).

f) Neuroanatomy of the Extrahypothalamic Projections: Initial neuroanatomical tracing studies of hypothalamic efferents failed to discern projections beyond the midbrain (Nauta and Haymaker, 1974; Lemmers and Lohmer,

1974). However, recent neuroanatomical techniques, such as histofluorescence, immunocytochemistry, and HRP, have indicated projections as far caudal as the spinal cord (e.g. Conrad and Pfaff, 1974). The formulation of specific antisera raised against VP and OXY and their use in immunocytochemistry has elucidated the independent systems of these peptides in the central nervous system. The site of origin for extrahypothalamic VP and OXY projections appear to be almost exclusively from the PVN and SCN, since SON cells are not labeled following HRP injections into the spinal cord and brain stem (Saper, Loewy, Swansom, and Cowan, 1976). The terminal fields of neurophysin-containing, VP-containing, and OXY-containing efferents are found in forebrain (Buijs and Swabb, 1979; Sofroniew and Weindl, 1978), hindbrain, and spinal cord (Swanson, 1977; Swanson and Sawchenko, 1980, Nilaver, Zimmerman, Wilkens, Michaels, Hoffman, and Silverman, 1980, Buijs, 1978). Forebrain VP and OXY project from the PVN and SCN to the dorsal and ventral hippocampus, ventral subiculum, the organum vasculosum of the lamina terminalis (OVLT), the medial and lateral septal nuclei, the periventricular nucleus of the hypothalamus, the lateral septal nucleus (Buijs, 1978; 1980; Sofroniew and Weidl, 1978; 1980; Hoonerman and Buijs, 1982), the subcommissural organ and the pineal gland (Buijs and Pevet, 1980) through the internal capsule and the stria terminalis (Buijs, Swabb, Dogterom, and van Leewen, 1978). Caudal PVN

projections terminate in the Edinger-Westphal nucleus of the third cranial nerve (Swanson, 1977) the locus coeruleus, the dorsal motor nucleus of the tenth cranial nerve and the nucleus tractus solitarius (Swanson, 1977; Nilaver et al, 1980). While OXY is found in all of the above sites, the latter two brain sites contain primarily VP (Nilaver et al, 1980). PVN projections also terminate in the marginal zones of the dorsal horn of the spinal cord through the dorsolateral funiculus (Swanson, 1977; Nilaver et al, 1980). Finally, another system descends to the spinal cord through the central gray (Swanson and McKeller, 1979; Nilaver, Mulhern, and Zimmerman, 1982). While most of these descending fibers contain VP and are of parvocellular origin (Swanson and Sawchenko, 1980), some contain OXY and are of magnocellular origin (Nilaver et al, 1980). Different sub-populations of PVN neurons project to the pituitary and extrahypothalamic structures (Hosoya, 1980; Hosoya and Matsushita, 1979; Ono et al, 1978), suggesting independent endocrine and neurocrine actions of VP. In addition, VP and OXY share many fiber systems with other neuropeptides, a point that will be dealt with in terms of their relevence to behavioral phenomena in the discussion section.

Appendix A provides a detailed review of the ontogeny and neurophysiology of VP and OXY neurons, and a history of the magnocellular neurosecretory system.

## II Role of VP and OXY in Learning and Memory Processes

There is controversy as to the effects of VP and OXY upon learning and memory function. Evidence supporting a role for VP and OXY in memory consolidation and retrieval has been derived primarily from the experiments conducted by De Wied and colleagues. They found that removal of the neurohypophysis results in facilitation of extinction in shuttle-escape performance (De Wied, 1965), while systemic or intracerebroventricular (icv) administration of arginine VP (AVP) and VP fragments increased resistance to extinction in a pole jump task (De Wied, 1976). In both of these tasks, a neutral stimulus (light or buzzer) is presented prior to a noxious stimulus (electric shock). In the shuttle-escape task, the animal can escape the noxious stimulus by moving to the no-shock portion of the testing chamber. In the pole-jump task, the animal can escape by jumping on a pole. After acquisition of the proper escape behavior, shock is no longer presented following the neutral stimulus. Memory function is defined as the number of trials it takes for either escape response to be extinguished. DeWied (1976) found that a higher systemic, as compared to intraventricular (icv), VP dose was necessary to inhibit extinction of the pole-jump response, suggesting either a central, or circumventricular, site of action. This hypothesis is supported by the observation that VP, in minimal amounts, crosses the blood-brain

barrier. Intravenous infusion of either VP or its neurophysin fail to alter their respective levels in the cerebrospinal fluid (Robinson and Zimmerman, 1973; Voherr, Bradbury, Hoghoughi, and Kleeman, 1968). Moreover, while peripherally administered tritiated lysine VP (LVP) accumulates in the pituitary gland and kidney, only 0.04% of total amount of tritium is found in the central nervous system (Janaky, Laczi, and Laszlo, 1982).

In addition to its effects upon active avoidance tasks, VP appears to modulate passive avoidance performance. In one such task, a rat is placed on an exposed lighted platform with a darkened chamber attached. If the door to the chamber is opened, untrained rats will escape inside, whereupon the chamber door is closed and an intense electrical shock is administered. Increased latency to enter the chamber in subsequent trials is interpreted as a continuing memory trace. Both systemic and icv routes of administration of VP and VP analogues enhance retention on passive avoidance tasks (Bohus, Adler, and De Wied, 1972; Bohus, Gispen, and De Wied, 1973), as does a VP metabolite at icv doses as little as 100 pg (Burbach, Kovacs, De Wied, Gispen, and Greven, 1983). Conversely, central administration of an antiserum raised specifically against arginine vasopressin (AVP) decreases retention of passive avoidance (van Wimersma Greidanis, Dogterom, and De Wied, 1976; van Wimersma Greidanis and De Wied, 1976). Similarly,

Brattleboro rats display total impairments in passive avoidance functioning, a condition that is restored to within normal levels following VP administration (De Wied, Bohus, and van Wimersma Greidanis, 1975; Bohus, van Wimersma Greidanis, and De Wied, 1975).

There is further evidence of a role for VP as a modulator of memory function. VP antagonizes the amnestic states induced by either puromycin (Lande, Flexner, and Flexner, 1972), pentylenetetrazol (Bookin, and Pfeifer, 1977), carbon dioxide (Rigter and Reizen, 1978), or electroconvulsive shock (Pfeifer and Bookin, 1978). Potentiation of extinction responses on the pole-jump task are observed following systemic administration of the potent VP antagonist, 1-deamino-penacillamine-2(O-methyl)tyrosine AVP (dPTyr(me)AVP) (Koob, Le Moal, Gaffori, Manning, Rivier, and Bloom, 1981). Moreover, pretreatment with this antagonist prevents the inhibition of pole-jump extinction following systemic or central injections of VP (Le Moal, Koob, Koda, Bloom, Manning, Sawyer, and Rivier, 1981; Le Moal, Koob, Mormede, Dantzer, and Bloom, 1982).

The initial investigations into the role of VP in learning and memory processes led to the idea that these alterations in behavior were being mediated by its hormonal actions. However, the subsequent discovery of the extensive extrahypothalamic distribution of VP

supported the view that it acts as a central neuromodulator, in addition to its well described neurohormonal functions. Similar multifunctional roles have been ascribed to such peptides as CCK, neurotensin, bombesin, substance P, and the enkephalins (see review: Buchanan, 1982). The main anatomical support for a neuromodulatory, as opposed to neuroendocrine, role for VP in learning and memory processes is the projections from the PVN to those forebrain limbic areas (Buijs, 1978) believed to be involved in memory consolidation and retrieval (Scoville and Milner, 1957). Indeed, improvement in passive avoidance performance has been reported following microinjections of VP into the hippocampal dentate gyrus and dorsal septal nucleus, but not the central amygdaloid nucleus (Kovacs, Bohus, Versteeg, De Kloet, and De Wied, 1979). VP's effects upon both passive and active avoidance behavior has been linked to its interaction with the noradrenergic system. The elongation of extinction by VP on passive avoidance tasks can be prevented by administration of catecholiminergic antagonists (Kovacs, Vecsei, Szabo, and Telegdy, 1977) or destruction of the ascending dorsal noradrenergic bundle (Kovacs, Bohus, Versteeg, 1979).

Recently the hypothesis that VP enhances memory function has been questioned. Gold and Burskirk(1976) failed to replicate the finding of an improvement in passive avoidance behavior induced by systemic VP

administration. Buresova and Skopkova (1982) found that des-gly NH desamino-D-AVP significantly, but minimally, improved performance in rats of various ages, on a 24 arm radial maze. In contrast, AVP, or a longer lasting synthetic VP analogue, desamino-D-AVP (DDAVP), failed to alter performance on this task. Furthermore, others have reported effects upon memory opposite to those described previously for the Brattleboro rat. While homozygous Brattleboro rats display impaired passive avoidance behavior relative to their heterozygous littermates, which have decreased but not absent VP content, both groups performed better than intact Long-Evans controls (Bailey and Weiss, 1978; Bailey and Weiss, 1979). These data suggest that the alterations in memory processes displayed by the Brattleboro rat may not be due to the absence of VP. Still others (Brito, 1983; Carey and Miller, 1982) found no differences between Brattleboro and control rats in passive avoidance behavior.

OXY has been evaluated for its role in memory function since it also projects from the PVN to limbic structures believed to be important in learning and memory processes. Both systemic and icv injections of OXY prolong extinction on pole-jump and shuttle escape performance (De Wied and Gispen, 1977; Schulz, Kovacs, and Telegdy, 1976; (Bohus, Urban, Greidanis, and De Wied, 1978). However, systemic OXY administration reliably retards passive avoidance performance, an effect opposite

to that of VP (Bohus, Kovacs, and De Wied, 1979; Kovacs et al, 1978). OXY appears to possess behavioral activity opposite to that of VP (see review: Bailey, 1982). However, this hypothesis should be regarded as tentative until opposing effects of these peptides have been demonstrated over a wide range of doses (see review: Bailey, 1982) and a number of behaviors.

In the above-mentioned investigations, electrical foot shock was used as the contingent stimulus with retention demonstrated through avoidance or escape behavior. Recently it has been reported that the memory enhancing properties of VP may depend upon its aversive properties (Ettenberg, Van Der Kooy, Le Moal, Koob, and Bloom, 1983). Since electrical foot shock is known to activate endogenous pain-inhibitory systems (see review: Bodnar, 1983), it is conceivable, that the differences in behavior observed between groups with differing VP or OXY levels, may not be due to differences in memory function, but rather to differential perceptions of the noxious stimulus (Bailey, 1982).

III. Endogenous Pain-inhibitory Systems

a) Opiate Analgesia (OA): The advent of gate-control theory provided a framework within which descending control of noxious input was proposed as a primary means of alleviating pain (Melzak and Wall, 1965), and a means by which analgesics such as opiates exert their actions.

With the discovery of the opiate receptor (Pert and Snyder, 1973; Simon, Hiller, and Edelman, 1973; Terenius, 1973), and the subsequent isolation of the endogenous ligands that bind to these receptors, (Hughes, Smith, Kosterlitz, Fothergill, Morgan, and Morris, 1975; Goldstein, 1976, Guilleman, Ling, and Burgus, 1976) the means by which opiates might activate pain-inhibitory pathways was established. Administration of either beta-endorphin (Jacquet and Marks, 1976; Bloom and Segal, 1976) or the enkephalins (Belluzi, Grant, Garsky, Sarantakis, Wise, and Stein, 1976; Pert, Pert, Chang, and Fong, 1976), like morphine, significantly increases nociceptive thresholds, an effect which is completely eliminated by administration of the opiate antagonist naloxone (Belluzi et al, 1976; Jacquet et al, 1976). The localization of opiate receptors in regions involved in pain modulation, such as the thalamus, periaquaductal gray (PAG), raphe magnus (nRM), and dorsal horn of the spinal cord, (Pert and Snyder, 1973; Kuhar, Pert, and Snyder, 1973), supported the view that there was an endogenous system that mediates opiate analgesia through a descending bulbospinal pathway from the PAG to the nRM. In turn, nRM axons descend to laminae II and V of the dorsal horn of the spinal cord through the dorsolateral funiculus (see review: Fields and Basbaum, 1978). Electrical stimulation of the PAG, or opiate microinjection into the PAG, produces activation of nRM neurons (Oleson and Liebeskind,

1975; Oleson, Twombly, and Liebeskind, 1974) which in turn produce a profound inhibition of dorsal horn neurons in the spinal cord (LeBars, Menetrey, Conseiller, and Besson, 1974). Additionally, microinjections of opiates into the PAG, nRM, (see review: Yaksh and Rudy, 1978) and subarachnoid space of the spinal cord (Yaksh, 1978) potently elevate pain thresholds. The neurochemical substrates of this pathway appear to include serotonin, since pharmacological depletion of serotonin, or lesion placed in serotonin-rich areas, attenuate or abolish opiate analgesia (Lytle, Phebus, Fischer, and Mesing, 1976; Samanin, Ghezzi, Mauron, and Valzeli, 1973; Tenen, 1968). Conversely, either electrical stimulation of 5-HT brain foci, or administration of 5-HT agonists, potentiate the analgesic efficacy of opiates (Samanin and Valzeli, 1971; Messing, Fischer, Phebus, and Lytle, 1976; Messing, Phebus, Fischer, and Lytle, 1975).

b) Opiate and Non-Opiate Pain Inhibition: Administration of opiates and non-opiate analgesics are not the only method for increasing pain thresholds. Stimulation of a number of brain loci, including the PAG and nRM, increases pain thresholds, as measured by a number of reflexive and non-reflexive responses (Mayer, Wolfle, Akil, Carder, and Liebeskind, 1971; Reynolds, 1969). Stimulation produced analgesia (SPA) and OA share similar mechanisms since they develop partial cross-tolerance with each other (Mayer and Hayes, 1975), and can

be elicited from similar brain sites (Sharpe, Garnett, and Cicero, 1974; but see Mayer and Liebeskind, 1974). Additionally, both forms of analgesia are interrupted following lesions in the dorsolateral funiculus (Basbaum, Marley, O'Keefe, and Clanton, 1977). Like morphine analgesia, administration of serotonergic antagonists attenuate SPA (Akil and Liebeskind, 1975; Akil and Mayer, 1972). Some studies (Akil, Mayer, and Liebeskind, 1976; Oliveras, Hosobuchi, Redjemi, Guilbaud, and Besson, 1977) found that naloxone reversed SPA, but others failed to observe this effect (Pert and Walter, 1976; Yaksh, Yeung, and Rudy, 1976). These apparently contradictory findings were reconciled by the discovery that SPA could be elicited from opiate-sensitive and opiate-insensitive sites. Stimulation of the dorsal or ventral PAG both produce analgesia, only the analgesia from the latter brain site is reversed by naloxone (Cannon, Prieto, Lee, and Liebeskind, 1982) or disrupted by nRM lesions (Prieto, Cannon, and Liebeskind, 1983). Similarly, only stimulation of the ventral PAG reliably excites nRM neurons. SPA can also be elicited from nuclei that are not involved in this descending bulbospinal system. These nuclei include the lateral hypothalamus (Balgura and Ralph, 1973), medial forebrain bundle (Yunger, Harvey, and Lorens, 1973), caudate nucleus, and septum (Schmidek, Fohanno, Ervin, and Sweet, 1971), it appears that there

are both opioid and non-opioid endogenous pain inhibitory systems.

Yet another means to increase pain thresholds is to acutely expose animals to a wide variety of physical stressors (see review: Bodnar, 1983) that activate adrenocortical and sympathomedullary systems. Some stressors, but not others, appear to activate endogenous OA systems in a similar fashion to SPA. For instance, acute exposure to inescapable foot shock has been reported to produce a naloxone-sensitive (Akil, Madden, Patrick, and Barchas, 1976; Madden, Akil, Patrick, and Barchus, 1977) or naloxone-insensitive analgesia (Hayes, Bennett, Newlon, and Mayer, 1978). The reason for such discrepant effects appears to be due differences in the parameters of shock utilized. While prolonged intermittent foot shock analgesia (PIFS) is reversed by naloxone, and is cross-tolerant with morphine, brief continuous foot shock analgesia (BCFS) is not (Lewis and Liebeskind, 1980; Lewis, Sherman, and Liebeskind, 1981). The number of shocks administered is also an important factor. Presentation of 80, but not 20, shocks results in a naloxone sensitive analgesia (Grau, Hyson, Maier, Madden, and Barchus, 1982). Other stressors which also elevate pain thresholds have been categorized on the basis of the apparent mechanisms mediating there expression (Watkins and Mayer, 1982). The analgesic responses following cold-water swim stress and insulin administration are

classified as hormonally and non-opiate mediated. Cold-water swim analgesia is not reversed by naloxone (Bodnar, Kelly, Spiaggia, Ehrenberg, and Glusman, 1978), is not cross-tolerant with morphine (Bodnar, Steiner, Kelly, and Glusman, 1978), is attenuated following hypophysectomy (Bodnar, Glusman, Brutus, Spiaggia, and Kelly, 1979; Bodnar et al, 1979) and is enhanced by adrenalectomy (Glusman, Bodnar, Mansour, and Kelly, 1980). Similarly, the analgesic response following insulin adminstration is reversed by hypophysectomy (Bodnar, Kelly, Mansour, and Glusman, 1979). Neural and non-opiate factors are involved in the analgesic response following 2-deoxy-D-glucose (2-DG) administration, in that this glucoprivic stressor exhibits only partial cross-tolerance with morphine, is not reversed by naloxone (Bodnar, Kelly, and Glusman, 1979) and is potentiated following hypophysectomy (Bodnar et al, 1979). Based upon these data, it is evident that both opiate and non-opiate endogenous pain-inhibitory systems play a role in the modulation of nociceptive stimuli. However, it should be noted that stress is neither a necessary nor sufficient condition to increase pain thresholds in that horizontal oscilation and ether inhalation, both stressors, fail to elevate pain thresholds (Hayes, Bennet, Newlon, and Mayer, 1976), and tail-pinch stress decreases pain thresholds on some pain tests (Simone and Bodnar, 1982)

## RATIONALE

The extrahypothalamic anatomical distribution of VP and OXY to brain regions that modulate noxious imput, including the sensory nuclei of the trigeminal and vagus nerves, and the spinal cord dorsal horn (Buijs, 1978, Swanson, 1977, Nilaver et al, 1980). suggest a role for these peptides in pain perception. Supporting this view, tail-flick latencies are increased following either central (Berntson and Berson, 1980; Kordower, Sikorzky, and Bodnar, 1982) or systemic (Berntson and Berson, 1980) injection of LVP and following systemic injection of AVP (Berson, Berntson, Zipf, Torello, and Kirk, 1983). While systemic administration of AVP increases writhing thresholds and hot plate latencies in mice (Berkowitz and Sherman, 1982), DDAVP only produces a marginal analgesia. In contrast, Brattleboro rats are typically hyperalgesic, a condition that can be reinstated to within littermate control levels following three days of repeated AVP or DDAVP injections (Bodnar, Zimmerman, Nilaver, Mansour, Thomas, Kelly, and Glusman, 1980). In like fashion, central administration of an antiserum raised against AVP, decreases tail-flick latencies in normal rats (Bodnar, Wallace, Kordower, Nilaver, Cort, Zimmerman, 1982).

VP analgesia appears to act independently of the endogenous opioids since it is unaffected by opiate antagonists (Berkowitz and Sherman, 1982, Berntson and

Berson, 1980, Berson et al, 1983), and chronic morphine treatment (Berson et al, 1983). Furthermore, neither peripheral VP injections in normal rats (Schmidt, Holiday, Loh, and Way, 1978), nor diabetes insipidus itself (Bodnar et al, 1980) alter morphine analgesia. VP analgesia is also unaffected by hypophysectomy (Berson et al, 1983) and appears to act independently of its pressor actions (Berkowitz and Sherman, 1982; Berson et al, 1983).

VP may also play a role in stress-induced analgesia. VP projects from the PVN to the zona externa of the median eminance (Vandesande, Dierickx, and DeMey, 1974). It is a known releaser of ACTH (see review: McCann, 1980) and potentiates CRF's release of ACTH (Gilles, Linton, and Lowry, 1982), a peptide released in response to stress (Selye, 1953). In addition, while the Brattleboro rat displays normal morphine analgesia, it has an impaired analgesic response to cold-water swim stress (Bodnar et al, 1980).

The focus of the present series of experiments concerns a) the relative specificity of VP analgesia; and b) the role of VP in stress-induced analgesia. As stated previously, AVP (Berkowitz and Sherman, 1982), has been shown to increase pain thresholds, as has LVP (Berntson and Berson, 1980; Berson et al, 1983; Kordower et al, 1982) and desglycinamide-LVP (DGLVP)(Kordower et al, 1982), an LVP analogue. Since the majority of descending

extrahypothalamic magnocellular fibers contain OXY, as compared to VP (Nilaver et al, 1980), and given OXY's similar neurochemical structure to VP, OXY might also modulate noxious imput. Systemic administration of OXY is without effect upon pain thresholds (Berkowitz and Sherman, 1982) but its central effects have yet to be explored. Thus, the first experiment will compare the dose and temporal properties of centrally administered AVP, DDAVP, and OXY, respectively upon tail-flick latencies. The tail-flick test measures responsivity to radiant heat. This behavioral assay was chosen because it is the most reliable correlate of acute animal models of pain perception with opiates in humans (Grumbach, 1966). In addition, the VP and OXY projections to the marginal zone of the dorsal horn suggest that these peptides might be preferentially sensitive to afferents mediating thermal noxious stimulation (Kozlowski et al, 1983). Indeed, VP has been shown to alter pain thresholds on tests utilizing heat (Berntson and Berson, 1980; Berson et al, 1983; Berkowitz and Sherman, 1982; Kordower et al, 1982) rather than noxious electrical shocks (Kordower et al, 1982) as the noxious stimulus. This test also appears to be sensitive to manipulations involving VP administration (Berntson and Berson, 1980; Berson et al, 1983; Kordower et al, 1982). All peptides will be centrally administered since this route of drug infusion has been shown to elevate tail-flick latencies at much lower doses than is

the case following systemic administration. We (Kordower et al, 1982) have demonstrated that central VP injections in the ng range elevate pain thresholds, while systemic administration requires doses in the ug range (Berkowitz and Sherman, 1982; Berntson and Berson, 1980; Berson et al, 1983) The dose range (Kordower et al, 1982) and time course of testing (Berntson and Berson, 1980; Kordower et al, 1982) to be employed were chosen in the basis of past effectiveness.

VP analgesia appears not to be mediated by the endogenous opioids (Berkowitz and Sherman, 1982; Berntson and Berson, 1980; Berson et al, 1983; Schmidt et al, 1978), although it is not known whether it acts through its own binding sites. Recently, specific pharmacological antagonists to AVP have been developed (Bankowski, Manning, Haldar, and Sawyer, 1978), including 1-deamino-penacillamine-2(O-methyl)tyrosine-AVP (dPTyr(me)AVP), which antagonizes the pressor but not the antidiuretic activity of AVP (Bankowski et al, 1978). Additionally, dPTyr(me)AVP possesses behavioral effects opposite to those of VP in active avoidance paradigms (Koob et al, 1981) and it reverses the facilitory effects of VP in pole-jump avoidance (Le Moal et al, 1981; Le Moal et al, 1982) and appetitive learning and memory tasks (Ettenberg, Le Moal, Koob, and Bloom, 1983). The second experiment will investigate whether central pretreatment with dPTyr(me)AVP blocks AVP and DDAVP analgesia respectively.

This experiment will also compare naloxone's effects upon AVP and DDAVP analgesia when the opiate antagonist is administered centrally. Finally, this experiment will examine whether VP antagonists effects are due to changes in basal pain thresholds rather than to the analgesia induced by VP.

VP fails to exhibit synergy with morphine in normal animals (Schmidt et al, 1978) and Brattleboro rats display normal morphine analgesia (Bodnar et al, 1980). To extend and confirm these findings, the third experiment addresses the question of whether central injections of naloxone, but not dPTyr(me)AVP, would attenuate systemic morphine analgesia.

Since VP releases ACTH (see review: McCann, 1980) and potentiates CRF's release of VP (Gilles et al, 1982), and since ACTH increases pain thresholds under certain circumstances (Amir, 1981; Walker, Akil, and Watson, 1980; Walker, Berntson, Sandman, Kastin, and Akil, 1981), it is conceivable that VP analgesia is mediated through ACTH release. To test this possibility, VP analgesia was tested in animals pretreated with dexamethasone (DEX). Dex is a potent synthetic glucocorticoid that is effective in suppressing ACTH secretion (Kreiger and Hughes, 1980).

VP projects to brain and spinal sites that are important for the modulation of stress-related behaviors. These sites include the median eminence (Swanson et al,

1980) and the thoracic segments of the spinal cord (Swanson and McKeller, 1979). In fact, VP is a releaser of ACTH (see review: McCann, 1980) and potentiates CRF activity (Gilles et al, 1979; Gilles and Lowry, 1982). Moreover, the Brattleboro rat displays an impaired analgesic response to cold water stress (Bodnar et al, 1980). The fifth experiment assessed directly the role of VP upon opioid and non-opioid forms of stress-induced analgesia. Rats received icv pretreatment with dPTyr(me)AVP prior to either the opiate-sensitive PIFS or opiate-insensitive BCFS (Lewis and Liebeskind, 1980; Lewis et al, 1981). This temporal parameter paradigm was selected since PIFS analgesia appears to be mediated through both hormonal (lewis, Tordoff, Sherman, and Liebeskind, 1982) and neuronal (Lewis, 1982) factors.

## GENERAL METHOD

Subjects: Female Sprague Dawley rats (250-350 g), bred at Queens College but derived from Charles River Breeding Laboratories, were used in all experiments. Animals of one gender were used to avoid steroid interactions, although it should be noted that both males and females display VP analgesia (e.g. Berntson and Berson, 1980; Kordower et al, 1982). Females were chosen as they have been shown to be sensitive on nociceptive measures to VP manipulations (Berntson and Berson, 1980; Kordower et al, 1982). They were maintained on a 12h

light: 12h dark cycle, and were housed individually with food (Purina Rat Chow) and water available ad libitum.

Surgery: Rats were premedicated with chlorpromazine HCl (3mg/ml normal saline/kg body weight, IP) and anesthetized 20 min later by Ketamine HCl (95mg/ml sterile water/kg body weight, IM). A stainless steel 22 gauge guide cannula (Plastic Products) was then stereotaxically (Kopf) placed so that its tip was positioned 0.3 mm above the left lateral ventricle. With the incisor bar set at +5 mm, coordinates were 0.5 mm anterior to the bregma suture, 1.3 mm lateral to the sagittal suture, and 3.6 mm from the top of the skull. The cannula was secured to three stainless steel anchor screws with dental acrylic. All animals were allowed 10 days to recover from surgery before behavioral testing began.

Tail-Flick Latencies: All rats were tested for their responsiveness to radiant heat in a modification of the procedure of D'Amour and Smith (1941). A radiant heat source (IITC Company) was mounted 8 cm above the dorsum of the tail of a lightly restrained animal, 6 cm proximal to the tail tip. Upon onset of the thermal stimulus, a timer was started. When the rat flicked its tail away from the stimulus, a photocell was broken, automatically stopping the timer. The pain thresholds were expressed as the latency for the animal to flick its tail away from the thermal stimulus. The intensity of the thermal stimulus

was set to produce stable basal tail-flick latencies between 2.0 and 3.0 sec. In each experiment, rats were matched into groups of 8 rats each based upon baseline tail-flick latencies unless otherwise stated. Except where noted, eight blocks of tail-flick latencies were determined 10, 5, and 0 min before, and 5, 15, 30, 45, and 60 min following drug treatment. Each block consisted of two latency determinations, separated by a 20 sec intertrial interval, and trials were automatically terminated if a response did not occur within 6 sec (to avoid tissue damage). A minimum of 72h elapsed between each experimental condition. To eliminate redundancy, it should be noted that in all experiments, pre-injection latencies failed to differ across time or treatment conditions and latencies failed to differ in vehicle treated animals at any time.

Drugs: Doses of AVP (Penninsula Labs), DDAVP, (Penninsula Labs), dPTyr(me)AVP, (Dr. M. Manning, Medical College of Ohio), and naloxone (Endo Laboratories) were dissolved in a 5 ul volume and were infused into the lateral ventricle through a 28 gauge internal cannula, at a rate of 1ul every 20 sec. The agonists were dissolved in 0.5% chlorbutanol and 0.05M acetic acid in normal saline while the antagonists were dissolved in normal saline. DEX (Sigma) was dissolved in 20% ethanol. Morphine sulfate (Pennick) was dissolved in normal saline. These solvents served as the vehicle solutions for their

respective solutes. Except where noted, all injections were made icv.

Histology: Following experimental testing, animals were anesthetized with sodium pentobarbital (100mg/2ml normal saline/kg body weight, ip) and perfused through transcardiac puncture with 0.9% saline followed by 10% buffered formalin. Each brain was removed, blocked, sliced into 40 um sections, mounted and stained with cresyl violet for cell body visualization. Coronal sections through the lateral ventricle were analyzed under an ordinary light microscope for cannula placement. Only animals with cannula tips located in the lateral ventricle were included in the data analysis.

Statistical Analysis: Two-way analyses of variance (ANOVA) were used to discern significant main and interaction effects. Where appropriate, the Dunnett test was employed to compare experimental and control conditions. Additionally, Tukey comparisons were carried out when comparisons between two experimental conditions were necessary. Comparisons were made relative to pre-injection levels to ascertain whether alterations in pain thresholds occurred. Additionally, comparisons were made between experimental and vehicle conditions to determine the relative contribution of the vehicle to the behavioral response.

## Experiment 1: Tail-Flick Latencies After AVP, DDAVP, and OXY Administration

### Method

Insert Table 1 about here

Table 1 summarizes the experimental protocol. Rats received injections of either AVP (0, 75, 150, 500 ng), DDAVP (0, 150, 500 ng) or OXY (0, 75, 150, 500, 1000 ng) respectively, with the injection order determined according to an incompletely conterbalanced design (D'Amato, 1970).

### Results

AVP and Tail-Flick Latencies: Significant effects were observed across the time of testing ($F(7,35)=8.50;p<.001$) and for the interaction between doses and time ($F(21,105)=1.89;p<.019$), but not among doses ($F(3,15)=1.12$), between injection orders ($F(1,5)=.01$) or for any other interaction term. Table 2 shows that AVP significantly elevated tail-flick latencies above pre-injection levels at 15 min following the 75ng dose, at 5 and 15 min following the 150ng dose, and at 5 and 15 min following the 500ng dose. AVP also significantly elevated tail-flick latencies above corresponding vehicle values at 15 min following the 75ng dose, at 5, 15, and 30 min following the 150ng dose, and at 5 and 15 following the 500ng dose.

Table 1: Experimental Protocol For Experiment 1

Group 1:

Pretest-------AVP (0, 75, 150, 500 ng)------Posttest

10-5-0 (min) 5, 15, 30, 45, 60 (min)

Group 2:

Pretest------DDAVP (0, 150, 500 ng)---------Posttest

10-5-0 (min) 5, 15, 30, 45, 60 (min)

Group 3:

Pretest------OXY (0, 150, 500, 1000 ng)-----Posttest

10-5-0 (min) 5, 15, 30, 45, 60 (min)

Insert Table 2 about here

DDAVP and Tail-Flick Latencies: Significant effects were observed across the time of testings ($F(7,42)=2.36;p<.04$), but not among doses ($F(2,12)=.95$), between injection orders ($F(1,6)=.28$), or for any interaction effects. Table 3 shows that DDAVP significantly elevated tail-flick latencies above pre-injection levels at 5 min following the 150ng dose, and at 5, 15, 30, and 45 min following the 500ng dose. DDAVP also significantly elevated tail-flick latencies above corresponding vehicle values at 5 min following the 150ng dose, and at 15 min following the 500ng dose.

Insert Table 3 about here

OXY and Tail-Flick Latencies: Significant effects were observed among doses ($F(3,18)=5.24;p<.009$), among the time of testing ($F(7,42)=5.95;p<.0001$), and for the interaction between doses and time ($F(21,126)=2.04;p<.009$), but not between injection orders ($F(1,6)=4.11$) or any other interaction effects. Table 4 shows that OXY significantly elevated tail-flick latencies above pre-injection levels and above corresponding vehicle values at 5 min following both the 150 and 500ng doses, and at 5, 15, and 30 min following the 1000ng dose. However, profound "barrel roll" seizure activity was

Table 2. Alterations in tail-flick latencies following intracerebroventricular (icv) administration of arginine vasopressin (AVP).

| | | | Post-Injection (min) | | | | |
|---|---|---|---|---|---|---|---|
| Dose (ng) | | PRE | 5 | 15 | 30 | 45 | 60 |
| 0 | X | 2.68 | 2.41 | 2.53 | 2.30 | 2.78 | 2.43 |
| | SEM | 0.19 | 0.16 | 0.50 | 0.18 | 0.36 | 0.25 |
| 75 | X | 2.48 | 3.27+ | 3.33+ | 2.64 | 3.20 | 2.79 |
| | SEM | 0.17 | 0.35 | 0.37 | 0.19 | 0.41 | 0.17 |
| 150 | X | 2.66 | 3.85* | 3.35+ | 3.01+ | 2.75 | 2.35 |
| | SEM | 0.18 | 0.31 | 0.36 | 0.46 | 0.43 | 0.21 |
| 500 | X | 2.32 | 4.47* | 4.35 | 2.66 | 2.99 | 2.40 |
| | SEM | 0.15 | 0.63 | 0.31 | 0.25 | 0.70 | 0.49 |

Note 1: Since in this and all subsequent experiments, the pre-injection values failed to differ from each other across doses or across times, these values are pooled (PRE).

Note 2: Significant differences from corresponding vehicle value-Dunnett comparison ($p<05$)+ and ($p<.01$)*.

Table 3. Alterarions in tail-flick latencies following icv administration of 1-deamino-8-D-arginine vasopressin (DDAVP).

| | | Post-Injection (min) | | | | | |
|---|---|---|---|---|---|---|---|
| Dose (ng) | | PRE | 5 | 15 | 30 | 45 | 60 |
| 0 | X | 3.07 | 3.03 | 2.81 | 3.19 | 3.03 | 2.94 |
| | SEM | 0.32 | 0.22 | 0.19 | 0.41 | 0.42 | 0.23 |
| 150 | X | 2.75 | 3.84+ | 3.02 | 3.05 | 2.87 | 2.96 |
| | SEM | 0.27 | 0.60 | 0.39 | 0.38 | 0.46 | 0.31 |
| 500 | X | 2.63 | 3.65+ | 3.91* | 3.58+ | 3.61+ | 3.22 |
| | SEM | 0.22 | 0.40 | 0.50 | 0.45 | 0.55 | .018 |

Note 1: Significant difference from corresponding vehicle value-Dunnett comparison ($p<.05$)+ and $p<.01$)*.

observed in five rats following the 1000 ng dose and in one rat following the 500 ng dose.

Insert Table 4 about here

## Discussion

Similar doses of AVP and DDAVP significantly elevated tail-flick latencies. However, it is known that the route of administration plays a crucial role as to whether, to what extent, and at what dose, a particular form of VP or its analogues produce analgesia. The present study showed that central administration of AVP (75-500 ng) increased rats tail-flick latencies for 15 min. Berkowitz and Sherman (1982) reported that subcutaneous (400 ug) but not intravenous (120 ug) AVP administration increased tail-flick latencies, while lower systemic doses (30 ug, sc) of AVP increased hot-plate latencies for up to 60 min and writhing thresholds for up to 15 min in mice. The present study showed that central administration of DDAVP (150-500 ng) increased tail-flick latencies for up to 45 min in rats. Others (Berson et al, 1983) have found that subcutaneous DDAVP (128 ug) injections produce a marginal, but significant analgesia on this measure. Moreover, while central LVP (500 ng) injections increased tail-flick latencies, but not flinch-jump thresholds for up to 15 min in rats (Kordower et al, 1982), subcutaneous LVP injections increased tail-flick latencies only at a doses ranging between 16-128 ug (Berntson and Berson, 1980;

Table 4. Alterations in tail-flick latencies following icv administration of oxytocin.

| | | Post-Injection (min) | | | | | |
|---|---|---|---|---|---|---|---|
| Dose (ng) | | PRE | 5 | 15 | 30 | 45 | 60 |
| 0 | X | 2.10 | 2.10 | 2.27 | 2.16 | 2.09 | 2.44 |
| | SEM | 0.12 | 0.17 | 0.22 | 0.08 | 0.16 | 0.21 |
| 150 | X | 2.23 | 2.91* | 2.04 | 1.84 | 2.07 | 1.80 |
| | SEM | 0.10 | 0.30 | 0.16 | 0.14 | 0.06 | 0.10 |
| 500 | X | 2.16 | 2.75+ | 2.51 | 2.43 | 2.08 | 1.87 |
| | SEM | 0.14 | 0.29 | 0.22 | 0.42 | 0.12 | 0.06 |
| 1000 | X | 2.24 | 3.96* | 3.04* | 2.70+ | 2.23 | 2.50 |
| | SEM | 0.14 | 0.47 | 0.43 | 0.33 | 0.12 | 0.40 |

Note 1: Significant difference from corresponding vehicle values ($p<.05$)+ and $p<.01$)*.

Berson et al, 1983) and not lower (Kordower et al, 1982). Finally, while central administration of DGLVP (500 ng) increased tail-flick latencies, but not flinch-jump thresholds for up to 60 min (Kordower et al, 1982), systemic administration of DGLVP (128 ug) is ineffective (Berson et al, 1983).

It appears that the threshold dose of various VP peptide forms and analogues necessary to elicit significant analgesia is lower following central administration, as compared to systemic VP injections. In contrast, the magnitude of VP analgesia is larger following the larger systemic, as compared to central, VP dose. Thus parametric variables are important in analyzing VP analgesia, just as they are in the analysis of analgesia induced by other peptides. This is apparent for two peptides that have been studied extensively, substance P and neurotensin. While central administration of low doses of substance P produce analgesia, similar administration of higher doses produce hyperalgesia (Frederickson, Burgis, Harrell, and Edwards, 1978). Additionally, the same dose of substance P is capable of elicitiing analgesia in rats with low basal pain thresholds and hyperalgesia in rats with high basal pain thresholds (Oehme, Hilse, Morgenstern, and Gores, 1980). While intrathecal administration of capsaicin, a substance P antagonist, produces a prolonged thermal analgesia (Yaksh, Farb, Leeman, and Jessell, 1979), icv

administration of capsaicin produces hyperalgesia to electric shock (Bodnar, Kirchgessner, Nilaver, Mulhern, and Zimmerman, 1982). Moreover, neurotensin, administered intrathecally (Yaksh, Schmauss, Micevyvh, Abay, and Go, 1982), intracisternally, and intraventricularly (Clineschmidt, Martin, and Veber, 1982; Clineschmidt, McGuffin, and Bunting, 1979) but not intravenously (Clineschmidt et al, 1982) elevate pain thresholds on the hot plate test, but not the tail-flick test.

OXY is synthesized in the same brain nuclei as VP (see: Kozlowski et al, 1983), projects to similar hypothalamo-hypophyseal (see: Silverman and Zimmerman, 1982) and extrahypothalamic brain sites (see: Kozlowski et al,1983) and differs structurally from VP only in two of the nine amino acids of its peptide chain. Though the present study showed that central administration of OXY elevates tail-flick latencies, this effect appears not to be a direct consequence of activation of a pain-inhibitory system, but rather an epiphenomenon of concommittant seizure activity. These seizures resembled closely those reported previously following central OXY administration (Kruse, Van Wimersma Greidanis, and De Weid, 1978). Soon after drug infusion, rats exhibit forelimb and trunk rigidity followed by ipsilaterally tilting toward the injected side. This was followed by rapid "barrel roll" seizures in which the rat maintains a rigid body posture and quickly rotates ipsilaterally to the injection site.

Such seizures last for a few minutes after which the rat's trunk becomes slightly flaccid. While the animals in the present study appeared to have recovered from the barrel-roll seizures prior to the start of behavioral testing, the increase in tail-flick latencies following icv OXY administration might best be interpreted in terms of the analgesic properties of seizures (Holiday and Belenky, 1980; Lewis, Cannon, Chudler, and Liebeskind, 1981) and rotation (Hayes, et al, 1978) rather than a direct effect of OXY itself upon pain-inhibitory mechanisms. That OXY fails to alter pain thresholds is further supported by the fact that systemic OXY administration fails to alter hot-plate latencies in mice (Berkowitz and Sherman, 1982). Thus, while VP and OXY share neuroanatomical and biochemical properties, they appear to possess dissociable effects upon nociceptive processes.

Experiment 2: Effects of dPTyr(me) and Naloxone upon AVP and DDAVP analgesia

Method

Table 5 summarizes the experimental protocol. Four groups of rats received pairs of injections, with a 10 min inter- injection interval within each pair. The first group of rats received four series of injections: a)saline-vehicle; b) saline-AVP (500 ng); c) naloxone (1ug)-AVP (500 ng); and d) dPTyr(me)AVP (500 ng)-AVP (500 ng). A second group received naloxone (10 ug) followed by

AVP (500 ng) to determine if a higher dose of the opiate antagonist woulds alter VP analgesia. A third group of 13 rats received an identical injection sequence as the first group except that DDAVP (500 ng) was administered in lieu of AVP. A larger number of rats were used in this group to better discern significant differences. A fourth group received dPTyr(me)AVP (0,500 ng) followed by an injection of vehicle.

Insert table 5 about here

## Results

dPTyr(me)AVP, Naloxone, and AVP Analgesia: Significant effects were observed across treatments ($F(3,35)=6.64;p<.0004$), across the time course of testing ($F(7,28)=13.18;p<.0001$), and for the interaction between treatments and time ($F(28,245)=2.94;p<.0001$), but not among injection orders. Table 6 shows that AVP itself significantly elevated latencies at 5, 15, and 30 min following injection relative both to pre-injection levels and corresponding saline-vehicle values. When dPTyr(me)AVP was paired with AVP, tail-flick latencies failed to differ from those elicited by the saline-vehicle condition, and were significantly lower than the saline-AVP condition an 5, 15, and 30 min after injection. Pairing the 1 or 10 ug naloxone doses with AVP yielded latencies that failed to differ from those elicited by

Table 5: Experimental Protocol For Experiment 2

Group 1:
Pretest---Saline---10 min---Vehicle---------Posttest
10-5-0 (min) 5, 15, 30, 45, 60
(min)

Pretest---Saline---10 min---AVP (500 ng)----Posttest
10-5-0 (min) 5, 15, 30, 45, 60
(min)

Pretest--Naloxone--10 min---AVP (500 ng)----Posttest
(1 ug)
10-5-0 (min) 5, 15, 30, 45, 60
(min)

Pretest--dPTyr(me)AVP-10 min-AVP (500 ng)---Posttest
(500 ng)
10-5-0 (min) 5, 15, 30, 45, 60
(min)

Group 2:
Pretest---Naloxone---10 min---AVP (500 ng)---Posttest
(10 ug)
10-5-0 (min) 5, 15, 30, 45, 60
(min)

Group 3:
Pretest--dPTyr(me)AVP-10 min--Vehicle--------Posttest
(500 ng)
10-5-0 (min) 5, 15, 30, 45, 60
(min)

the saline-AVP condition, and that were significantly higher than the saline-vehicle condition at 5, 15, and 30 min after injection. Latencies were also significantly elevated above pre-injection levels 60 min following the the naloxone(1ug)-AVP condition.

Insert Table 6 about here

dPTyr(me)AVP, Naloxone and DDAVP analgesia: Significant effects were observed across the time course of testing ($F(8,88)=7.64;p<.0001$), and for the interaction between treatment and time ($F(24,264)=1.67;p<.036$), but not among treatments ($F(3,33)=2.24$), between orders ($F(1,6)=.28$) or for any other interaction effects. Table 7 shows that DDAVP itself significantly elevated latencies at 5 and 15 min following injection relative to pre-injection and corresponding saline-vehicle values. When dPTyr(me)AVP was paired with DDAVP, tail-flick latencies failed to differ from those following the saline-vehicle condition and were significantly lower than the saline-DDAVP condition at 5 and 15 min after injection. Again, pairing naloxone(1ug) with DDAVP yielded latencies that failed to differ from those elicited by the saline-DDAVP condition and were significantly higher than the saline-vehicle condition at 5 and 15 min after injection.

Insert Table 7 about here

Table 6. Reversal of AVP (500 ng) analgesia by 1-deamino-penicillamine-2(O-methyl) tyrosine AVP (dPTyr(me)AVP: 500 ng) but not naloxone (NAL:1, 10 ug).

| | | | Post-Injection (min) | | | | |
|---|---|---|---|---|---|---|---|
| Condition | | PRE | 5 | 15 | 30 | 45 | 60 |
| SAL-VEH | X | 2.42 | 2.36 | 2.32 | 2.24 | 2.48 | 2.33 |
| | SEM | 0.10 | 0.15 | 0.15 | 0.13 | 0.14 | 0.13 |
| SAL-AVP | X | 2.54 | 3.84* | 3.96* | 3.59+ | 2.86 | 2.23 |
| | SEM | 0.14 | 0.36 | 0.19 | 0.46 | 0.11 | 0.13 |
| NAL 1-AVP | X | 2.51 | 3.59* | 3.77* | 3.23* | 2.93 | 3.12 |
| | SEM | 0.11 | 0.57 | 0.42 | 0.26 | 0.23 | 0.38 |
| NAL 10-AVP | X | 2.48 | 3.41* | 3.89* | 3.95* | 2.64 | 2.63 |
| | SEM | 0.08 | 0.45 | 0.36 | 0.45 | 0.15 | 0.22 |
| dPTyr(me)AVP-AVP | X | 2.30 | 2.57 | 2.28 | 2.48 | 2.31 | 2.43 |
| | SEM | 0.07 | 0.22 | 0.13 | 0.19 | 0.15 | 0.27 |

Note 1: Significant differences from corresponding saline-vehicle values (SAL-VEH)-Dunnett comparison ($p<.05$)+ and ($p<.01$)*.

Table 7. Reversal of DDAVP (500 ng) analgesia by dPTyr(me)AVP (500 ng), but not by NAL (1 ug).

| | | | Post-injection (min) | | | | |
|---|---|---|---|---|---|---|---|
| Condition | | PRE | 5 | 15 | 30 | 45 | 60 |
| SAL-VEH | X | 2.37 | 2.72 | 2.46 | 2.24 | 2.59 | 2.60 |
| | SEM | 0.05 | 0.28 | 0.24 | 0.34 | 0.47 | 0.23 |
| SAL-DDAVP | X | 2.45 | 3.55* | 3.22+ | 2.92 | 2.90 | 2.73 |
| | SEM | 0.12 | 0.37 | 0.27 | 0.27 | 0.18 | 0.24 |
| NAL-DDAVP | X | 2.70 | 3.62* | 3.33+ | 2.85 | 2.79 | 3.07 |
| | SEM | 0.15 | 0.30 | 0.33 | 0.18 | 0.17 | 0.17 |
| dPTyr(me)AVP-DDAVP | X | 2.62 | 2.93 | 2.90 | 3.08 | 2.82 | 3.07 |
| | SEM | 0.17 | 0.36 | 0.21 | 0.24 | 0.23 | 0.28 |

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Note 1: Significant difference from corresponding saline-vehicle values (SAL-VEH)-Dunnett comparison ($p<.05$)+ and ($p<.01$)*.

dPTyr(me)AVP and Tail-Flick Latencies: Significant differences were observed between dPTyr(me)AVP-vehicle and saline-vehicle treatments ($F(1,5)=6.65$; $P<.05$), but not across the time course of testing ($F(7,35)=.96$), or for the interaction between treatment and time ($F(7,39)=.61$). However, post-injection values failed to differ from corresponding pre-injection levels (data not shown).

## Discussion

Previous evidence has shown that interactions occur between peripheral VP and the endogenous opioids since beta-endorphin and morphine each inhibit plasma VP release following either electrical stimulation to the medial basal hypothalamus (Knepel and Reiman, 1982), exposure to foot shock (Knepel, Nutto, and Hertting, 1982) or isoprenaline administration (Knepel, Nutto, and Hertting, 1981). Anatomical support for VP-opioid interactions appears to be two-fold. First, VP co-exists with dynorphin in the PVN (Watson et al, 1980) and with leu-enkephalin in the neurohypophysis (Martin and Voigt, 1981). Moreover, the VP projections to the marginal zones of the spinal cord are through the dorsolateral funiculus (Nilaver et al, 1980; Swanson et al, 1980), the fiber bundle integral to the full expression of OA (see review:

Fields and Basbaum, 1978). Despite the fact that this evidence suggests an interaction between the endogenous opioids and analgesic processes, the present experiment indicates that VP analgesia is mediated independently of the endogenous opioids. Central pretreatment with either 1 or 10 ug doses of naloxone failed to alter the analgesic effects of AVP or DDAVP even though the higher naloxone dose is capable of eliminating the analgesic effects induced by a systemic 75 mg/kg morphine dose (Yeung and Rudy, 1980). These data support and extend previous findings showing that a) administration of either naloxone (Berntson and Berson, 1980; Berson et al, 1983) or naltrexone (Berkowitz and Sherman, 1982) fail to alter VP analgesia; b) morphine-tolerant rats display normal VP analgesia (Berson et al, 1983); c) Brattleboro rats exhibit normal morphine analgesia (Bodnar et al, 1980); and d) systemic VP administration had no effect upon morphine analgesia (Schmidt et al, 1978).

VP analgesia appears to be dependent upon interactions with its own binding sites. The VP antagonist dPTyr(me)AVP blocks the pressor but not the antidiuretic responses of AVP (Bankowski et al, 1978) and exerts effects opposite to those of VP on the pole-jump task (Koob et al, 1981). It also reverses VP's effects upon appetitive (Ettenberg et al, 1982) and aversive learning and memory paradigms (Le Moal et al, 1981; Le Moal et al, 1982), and blocks the isoprenaline-induced

release of VP (Knepel, Benner, and Hertting, 1982). The present data demonstrate that central pretreatment with an equimolar dose of dPTyr(me)AVP completely blocked the analgesia following both icv AVP and DDAVP. This elimination of AVP and DDAVP analgesia was not due to a compensatory decrease in basal tail-flick latencies since the dose sufficient to block VP analgesia failed to alter tail-flick latencies. Berkowitz and Sherman (1982) have reported that systemic administration of another VP antagonist, 1-(B-Mercapto-B,B-cyclopentamethylene proprionic acid) 2-O-ethyl-tyrosine 4 valine) AVP, reversed systemic VP analgesia in mice.

Receptors and receptor sub-types have been characterized for the endogenous opioids (see: Martin, 1981) and for neurotensin (Fox, Sakai, Jury, McLean, Daniel, 1982; Jolicoeur, Barbeau, Quirion, Rioux, and St-Pierre, 1982) through bio-assay and competitive binding assay procedures. However, the receptor for VP, as well as for other peptides, have yet to be fully characterized. The use of VP agonists and antagonists that modulate some, but not all, of VP's actions suggests that different subpopulations of VP receptors, with different functions, exist. This issue will be explored in more detail in the general discussion.

Experiment 3: Effects of dPTyr(me)AVP and Naloxone upon Morphine analgesia

## Method

Table 8 summarizes the experimental protocol. Three groups of rats received a single injection of either dPTyr(me)AVP (1ug), naloxone (1ug), or saline respectively 10 min prior to a subcutaneous injection of morphine (10mg/kg body weight). Tail-flick latency determinations were made as before, except for an additional determination at 120 min following morphine administration.

Insert table 8 about here

## Results

Significant effects were observed across treatments ($F(2,21)=6.59;p<.05$), across the time course of testing ($F(8,168)=129.57;p<.0001$), and for the interaction between treatments and time ($F(16,168)=2.44;p<.0025$). Table 9 shows that morphine significantly elevated tail-flick latencies, at all post-injection test intervals, following pretreatment with either saline or dPTyr(me)AVP. In contrast,, while the naloxone-morphine group displayed analgesia at 15, 30, 45, 60, and 120 min after injection, these effects were significantly less than the saline-morphine group at 15, 30, 45, and 60 min following injection.

Insert Table 9 about here

Table 8: Experimental Protocol For Experiment 3

Group 1:

Pretest--dPTyr(me)AVP--10 min-Morphine-----Posttest

(1 ug) (10 mg/kg)

10-5-0 (min) 5, 15, 30, 45, 60, 120 (min)

Group 2:

Pretest---Naloxone-----10 min-Morphine-----Posttest

(1 ug) (10 mg/kg)

10-5-0 (min) 5, 15, 30, 45, 60, 120 (min)

Group 3:

Pretest---Saline----10 min---Morphine------Posttest

(10 mg/kg)

10-5-0 (min) 5, 15, 30, 45, 60, 120 (min)

Table 9. Reversal of morphine (MOR: 10 mg/kg) analgesia by NAL (1 ug), but not dPTyr(me)AVP (1 ug).

| | | Post-Injection (min) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Condition | | PRE | 5 | 15 | 30 | 45 | 60 | 120 |
| VEH-MOR | X | 2.32 | 3.02 | 4.44 | 5.66 | 5.82 | 6.00 | 5.55 |
| | SEM | 0.15 | 0.40 | 0.47 | 0.18 | 0.15 | 0.00 | 0.30 |
| NAL-MOR | X | 2.25 | 2.46 | 2.71* | 4.63* | 5.17* | 5.46 | 4.73* |
| | SEM | 0.16 | 0.38 | 0.36 | 0.34 | 0.34 | 0.29 | 0.49 |
| dPTyr(me)AVP-MOR | X | 2.24 | 2.79 | 4.85 | 5.74 | 6.00 | 5.98 | 5.69 |
| | SEM | 0.13 | 0.31 | 0.46 | 0.13 | 0.00 | 0.02 | 0.22 |

---

Note 1: Significant difference from corresponding value in VEH-MOR condition: Dunnett comparison ($p<.05$)+ and ($p<.01$).

## Discussion

As described previously, central VP analgesia appears to act independently of the endogenous opioid system despite, the presence of biochemical and neuroanatomical interactions between these two peptides. The present data support this view. Central pretreatment with the VP antagonist dPTyr(me)AVP, at a dose twice as large as that needed to eliminate AVP or DDAVP analgesia, failed to alter the antinociceptive response of a 10 mg/kg systemic injection of morphine. This extends the finding that peripheral manipulations of VP fail to alter VP analgesia (Schmidt et al, 1978) to include central manipulations. However, icv pretreatment with 1 ug of naloxone, a dose that failed to alter AVP or DDAVP analgesia, significantly attenuated the analgesic effect of morphine.

Data dissociating VP from opiates and endogenous opioids in analgesic processes have been derived primarily from work using naloxone, naltrexone, and morphine. Just as there are different sub-types of opiate receptors (see review: Wood, 1982), there are different classes of endogenous opioids, including the enkephalins (Hughes et al, 1975), the endorphins (Li and Chung, 1976) and dynorphin (Goldstein, Fischili, Lowner, Hunkapiller, and Hood, 1981). Each class of endogenous opioids has different affinities for the receptor sub-types (see

review: Wood, 1982). The present study and others which have used naloxone, naltrexone, and morphine, are only investigating one receptor sub-type, the mu receptor (Martin, 1981). Dynorphin, which co-exists with VP in the PVN (Watson et al, 1981) appears to be acting through another receptor sub-type, the kappa receptor (Chaukin, and Goldstein, 1981) and therefore could conceivably still modulate VP analgesia despite the ineffectiveness of mu ligands. However, dynorphin immunoreactivity is present in the PVN Brattleboro rat (Watson et al, 1981), which displays decreased basal pain thresholds but normal morphine analgesia (Bodnar et al, 1980). Future studies might preferably employ other opiate antagonists, such as naloxonazine, which block the high affinity binding sites of all opiate receptor sub-types (Hahn, Carroll-Buatti, and Pasternak, 1982).

## Experiment 4: Effect of Dexamethasone Upon AVP Analgesia

### Method

Table 10 summarizes the experimental protocol. Four groups of rats served as experimental subjects. The first and second groups received DEX injections 24 h (0.4mg/kg body weight, IP) and 1 h (0.2mg/kg body weight, IP) prior to an injection of either AVP (500ng) or vehicle respectively. This dose regimen of DEX has been shown to eliminate the rise in ACTH following intense foot-shock stimulation (French, Bloom, Rivier, Guillemin, and

Rossier, 1978). Groups three and four received identical drug treatment except that vehicle injections were administered in lieu of the dexamethasone. Tail-flick determinations were made as described earlier, except for the addition of a tail-flick determination 24h after peptide administration.

Insert Table 10 about here

## Results

DEX and AVP analgesia: Significant effects were observed across treatments ($F(1,28)=4.47;p<.011$), across the time course of testing ($F(9,252)=8.84;p<.0001$), and for the interaction between treatments and time ($F(27,252)=4.82;p<.0001$). Significant decreases in latencies were observed relative to baseline levels at 15 and 30 min following the DEX-vehicle condition and at 30 and 45 min following the vehicle-vehicle condition. However, latencies of these groups failed to differ when one compared the pre-injection and post-injection values. Table 11 shows that AVP treated rats displayed significantly elevated latencies at 5 and 15 min after injection relative to to pre-injection and baseline levels and at 5, 15, 30, and 45 min after injection relative to corresponding vehicle-vehicle latencies. The DEX-AVP group displayed significantly elevated latencies at 5, 15 and 30 min after injection relative to the pre-injection,

Table 10: Experimental Protocol For Experiment 4

Group 1:

DEX----23h------DEX---1h----Pretest---AVP----Posttest

(0.4 mg) (0.2 mg) (500 ng)

Pretest 10-5-0 min Posttest 5, 15, 30, 45, 60 min; 24h

Group 2:

DEX----23h------DEX---1h----Pretest---Saline--Posttest

(0.4 mg) (0.2 mg)

Pretest 10-5-0 min Posttest 5, 15, 30, 45, 60; min 24h

Group 3:

SAL----23h------SAL---1h----Pretest---AVP-----Posttest

(500 ng)

Pretest 10-5-0 min Posttest 5, 15, 30, 45, 60 min; 24h

Group 4:

Sal----23h------Sal---1h----Pretest---SAL-----Posttest

Pretest 10-5-0 min Posttest 5, 15, 30, 45, 60 min; 24h

baseline, and corresponding DEX-vehicle levels. Tukey comparisons showed that DEX significantly potentiated the magnitude of AVP analgesia at 5, 15, and 30 min after injection.

Insert Table 11 about here

## Discussion

VP releases ACTH from the adenohypophysis into the general circulation,(see review: McCann, 1980) and potentiates CRF-induced ACTH release (Gilles and Lowry, 1979; Gilles et al, 1982). The site of action for the latter effect probably occurs at the median eminence, since PVN neurons containing either VP or CRF project to the zona externa of the median eminence (Bloom et al, 1982; see: Kozlowski et al, 1983). Such a relationship suggests that VP analgesia is dependent upon ACTH release, especially in light of the analgesic properties of ACTH (Amir, 1981; Walker et al, 1980) and ACTH analogues (Walker et al, 1981). Therefore, any inhibition of ACTH activity, including activation of the adreno-hypothalamic negative feedback loop with the synthetic glucocorticoid, DEX (e.g. Krieger and Hughes, 1980), should attenuate or eliminate VP analgesia. Yet the present data show that dexamethasone pretreatment potentiats VP analgesia, suggesting an alternative role for ACTH. In the model previously suggested, ACTH mediated VP analgesia through VP synapses upon CRF-containing neurons in the medial

Table 11. Potentiation of AVP (500 ng) analgesia by dexamethasone (DEX) pretreatment.

| | | Post-Injection (min) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Condition | | PRE | 5 | 15 | 30 | 45 | 60 | 24h |
| VEH-VEH | X | 2.11 | 2.18 | 1.98 | 1.86 | 1.81 | 1.94 | 2.66 |
| | SEM | 0.14 | 0.18 | 0.13 | 0.17 | 0.17 | 0.23 | 0.34 |
| VEH-AVP | X | 1.97 | 3.18* | 3.27* | 2.45+ | 2.40+ | 2.09 | 2.10 |
| | SEM | 0.15 | .056 | 0.49 | 0.44 | 0.38 | 0.32 | 0.15 |
| DEX-VEH | X | 1.91 | 1.90 | 1.80 | 1.66 | 2.06 | 2.17 | 2.21 |
| | SEM | 0.17 | 0.17 | 0.11 | 0.19 | 0.26 | 0.25 | 0.12 |
| DEX-AVP | X | 2.06 | 4.56# | 4.20# | 3.47# | 2.14 | 2.11 | 2.43 |
| | SEM | 0.16 | 0.52 | 0.51 | 0.61 | 0.25 | 0.31 | 0.23 |

Note 1: Significant difference from corresponding VEH-VEH values: Dunnett comparison ($p<.05$)+ and ($p<.01$)*. Significant difference from corresponding VEH-AVP values: Tukey comparison ($p<.01$).

basal hypothalamus (Saper et al, 1976; Zimmerman, 1981). Two other models might better explain the present results. The first model would focus upon the synapse of ACTH-containing neurons upon VP-containing cells in the PVN (Sawchenko, Swanson, and Joseph, 1982). This synapse would have to be inhibitory, and DEX would thereby disinhibit VP, potentiating VP analgesia. Since systemic AVP analgesia is unaffected by hypophysectomy (Berson et al, 1983), any proposed ACTH-VP interaction would therefore involve central processes. The second alternative model assumes direct inhibition of central VP by dexamethasone pretreatment with the resulting potentiation of VP analgesia due to analgesia due to receptor supersensitivity. This hypothesis is supported by the quick onset of dexamethasone-induced alterations of peptide activity, with the 24 gap between dexamethasone and VP administration providing a window for the physiological actions to occur. To differentiate among the models, other experiments including altering the interval between dexamethasone and VP should be carried out. In summary, while VP analgesia does not appear to be dependent upon ACTH neurons for its effects, but it appears that ACTH, or at least dexamethasone, is capable of modulating VP analgesia.

Experiment 5: Effects of dPTyr(me)AVP Upon PIFS and BCFS Analgesia

## Method

Table 12 summarizes the experimental protocol. Nine groups of rats were tested for their responsiveness to either PIFS or BCFS stress as described by Lewis and collegues (1981). Except where noted, the thermal stimulus was automatically terminated if the animal failed to respond within 7 sec. Rats were placed into one of three experimental conditions. Groups 1-4 received PIFS consisting of 1 sec shocks delivered every 5 sec, at an intensity of 2.5mA, for 20 min. Groups 4-8 received BCFS consisting of a 17 min rest period followed by 3 min of continous foot shock at an intensity of 2.5mA. Group 9 was a 20 min no-shock control condition. Prior to the shock, five tail-flick trials, of one latency determination each, were conducted at 1 min intervals. After stress, similar determinations resumed 1 min later, continuing at 1 min intervals for 9 min, and subsequently at 2 min intervals until 15 min had elapsed. Group 1 received dPTyr(me)AVP (1ug) just prior to PIFS. Group 2 received an identical treatment except that saline was administered in lieu of the dPTyr(me)AVP. After pre-stress testing, group 3 received an injection of dPTyr(me)AVP followed immediately by a subcutaneous (sc) injection of naloxone (10mg/kg). An additional naloxone (10 mg/kg, sc) injection was administered immediately after the foot-shock. Group 4 was treated in an identical fashion as group 3 except that saline was administered in

lieu of the dPTyr(me)AVP. After pre-stress testing, group 5 was administered an injection of dPTyr(me)AVP (1ug) while group 6 received an injection of saline. These two groups then received BCFS treatment. To eliminate any ceiling effects potentially observed following BCFS, groups 7 and 8 underwent identical treatment and testing as groups 5 and 6 respectively, except that the thermal stimulus was now terminated if the animal failed to respond within 12 sec. Additionally, to minimize tisue damage, post-stress determinations were made at every other min following stress, for 15 min. Lastly, group 9 received an injection of saline followed by a 20 min no-shock period. This was followed by an identical testing sequence as described for groups 1-6.

Insert table 12 about here

## Results

dPTyr(me)AVP, naloxone, and PIFS analgesia: Significant effects were observed across treatments ($F(4,35)=5.58$; $p<.002$), across the time course ($F(16,560)=22.86$; $p<.0001$), and for the interaction between treatments and time ($F(64,560)=2.44$; $p<.0001$). Figure 1 shows that PIFS significantly elevated latencies when compared to the no shock condition. In addition, latencies of the saline-treated rats were were significantly elevated as compared to their respective pre-stress levels. Latencies of dPTyr(me)AVP-treated rats

Table 12: Experimental Protocol For Experiment 5

Group 1:
Pretest------dPTyr(me)AVP--------PIFS-----Posttest
(1 ug)
4-3-2-1-0 (min) min 1-9; 11, 13, 15

Group 2:
Pretest------Saline--------------PIFS-----Posttest
4-3-2-1-0 (min) min 1-9; 11, 13, 15

Group 3:
Pretest-dPTyr(me)AVP-0 min-NAL-PIFS-0 min-NAL-Posttest
(1 ug) (10 mg) (10 mg)
4-3-2-1-0 (min) min 1-9; 11, 13, 15

Group 4:
Pretest-Saline-------0 min-NAL-PIFS-0 min-NAL-Posttest
(10 mg) (10 mg)
4-3-2-1-0 (min) min 1-9; 11, 13, 15

Group 5:
Pretest----dPTyr(me)AVP--------BCFS (7 sec)----Posttest
(1 ug)
4-3-2-1-0 (min) min 1-9; 11, 13, 15

Group 6:
Pretest-------Saline-----------BCFS (7 sec)----Posttest
4-3-2-1-0 (min) min 1-9; 11, 13, 15

Group 7:
Pretest----dPTyr(me)AVP--------BCFS (12 sec)---Posttest
(1 ug)
4-3-2-1-0 (min) (min) 1, 3, 5, 7, 9, 11, 13, 15

Group 8:
Pretest----Saline--------------BCFS (12 sec)---Posttest
4-3-2-1-0 (min) (min) 1, 3, 5, 7, 9, 11, 13, 15

Group 9:
Pretest----Saline---------no shock-------------Posttest
4-3-2-1-0 (min) min 1-9; 11, 13, 15

were elevated over pre-stress levels for the first six and then at 8 and 13 min following PIFS, but were significantly reduced relative to saline treated animals at 1, 3, 4, 5, 7, 9, and 15 min following the foot-shock relative to saline treated animals.

Insert Figure 1 about here

Figure 2 shows that tail-flick latencies were elevated by PIFS following the administration of saline-naloxone, or dPTyr(me)AVP-naloxone. Latencies were reduced in the dPTyr(me)AVP-naloxone group 3, 4, 5, 6, and 15 min following PIFS relative to both the saline and saline-naloxone conditions. In contrast, the saline-naloxone treated animals displayed latencies which were elevated relative to the saline group 8 and 13 min following the stressor.

Insert Figure 2 about here

dPTyr(me)AVP and BCFS: Significant effects were observed across treatments ($F(2,21)=41.82$; $p<.0001$), across the time course of testing ($F(16,336)=53.25$; $p<.0001$), and for the interaction between treatments and time ($F(32,336)=12.47$; $p<.0001$), when the stimulus was terminated if a response did not occur within 7 sec. As summarized in figure 3, latencies were elevated following BCFS across all intervals relative to the no-shock

Figure 1: Attenuation of PIFS analgesia following administration of dPTyr(me)AVP (1 ug). The x denotes a significant difference from saline treated rats (Dunnett comparison, $p<.05$). Latencies failed to differ in the no shock condition at any time interval. Open squares = no shock condition; open circles = vehicle treatment; closed circles = dPTyr(me)AVP treatment.

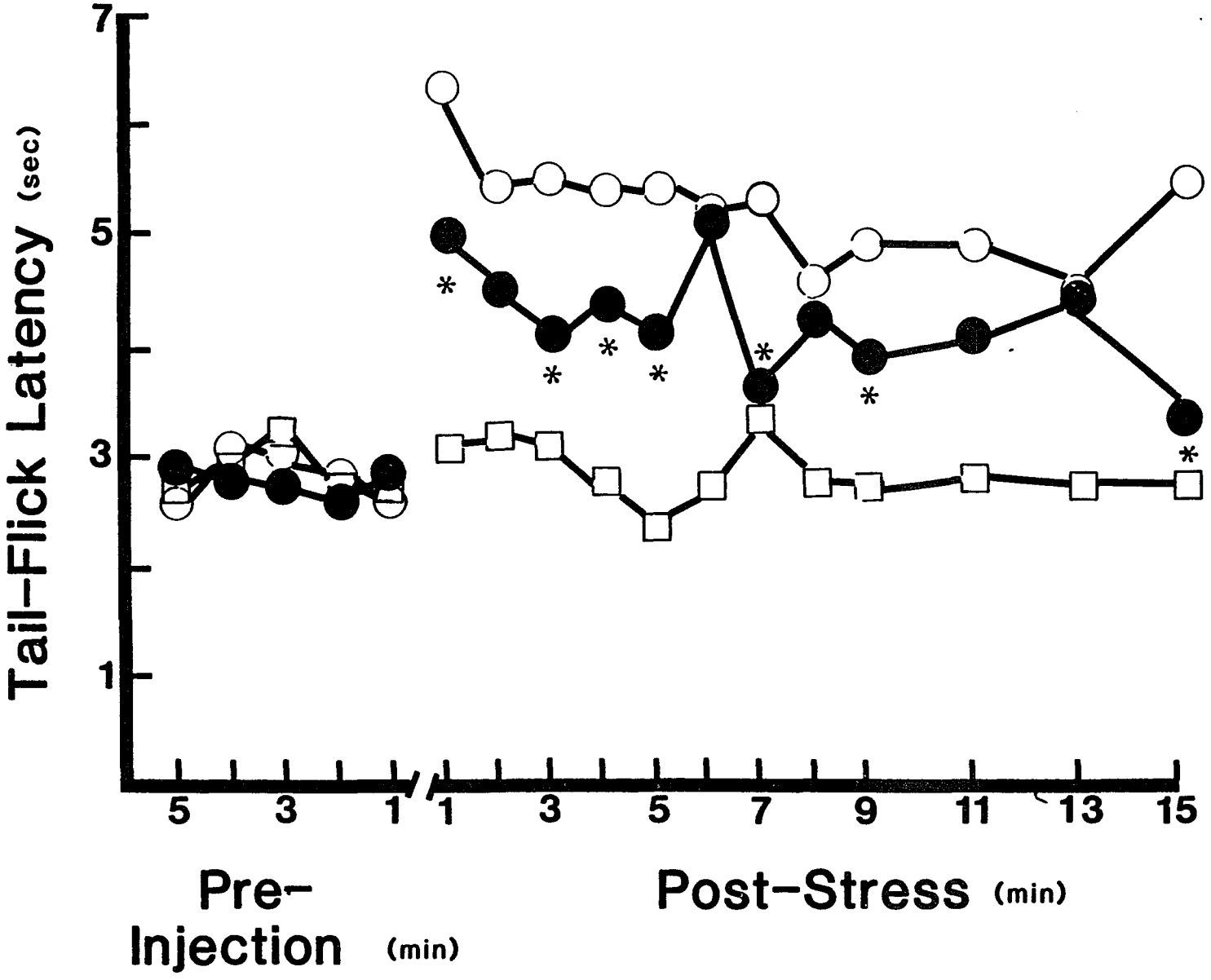
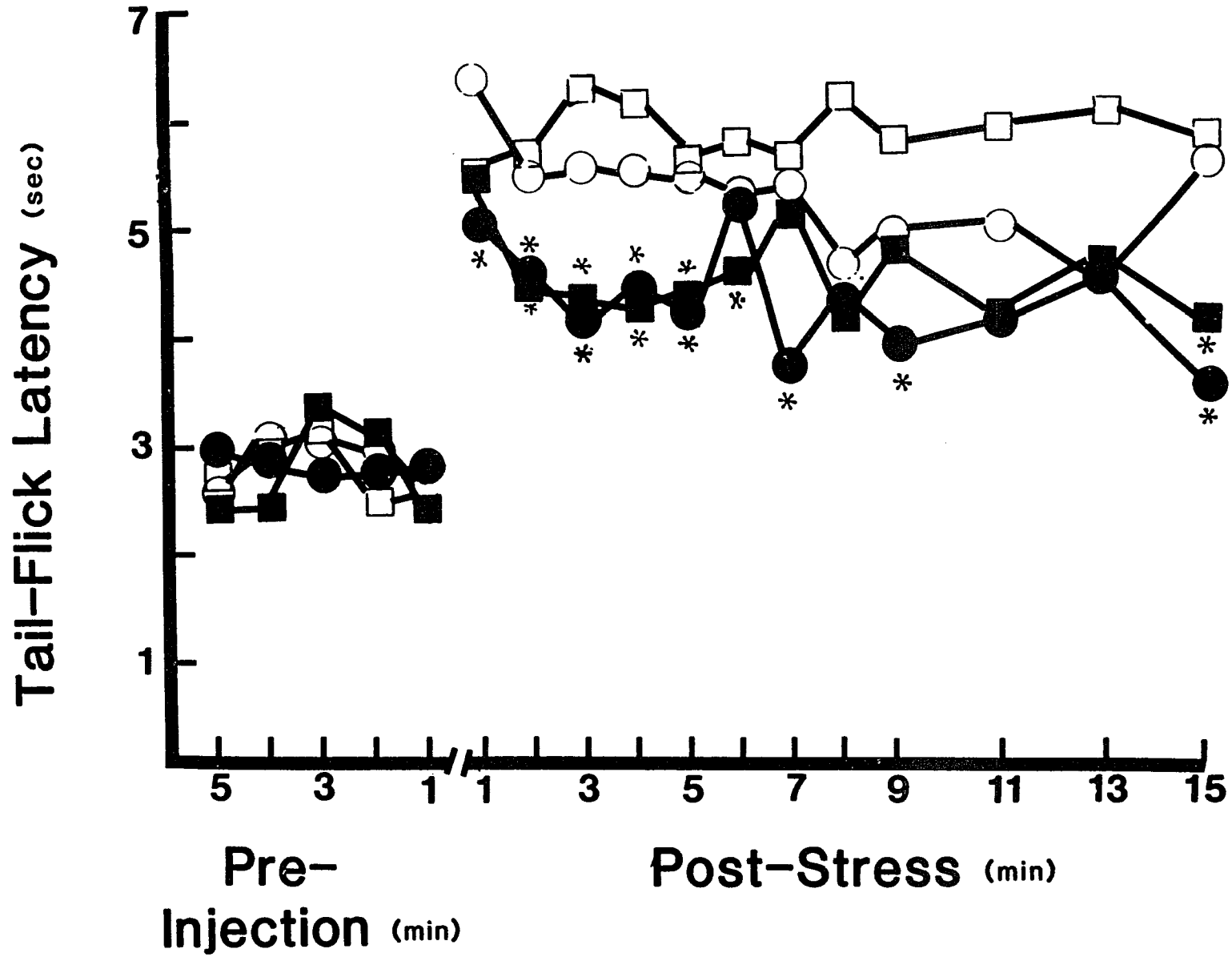

Tail-Flick Latency (sec)
7
5
3
1
5
3
1
1
3
5
7
9
11
13
15
Pre-
Injection (min)
Post-Stress (min)

Figure 2: Failure of systemic naloxone (10mg/kg; before and after stress) to attenuate PIFS analgesia. The pairing of naloxone and dPTyr(me)AVP (1 ug) failed to decrease latencies beyond that of dPTyr(me)AVP alone. Saline and dPTyr(me)AVP data are from figure 1 for comparison purposes. The x denotes a significant decrease below saline-treated rats (Dunnetts comparison, $p<.05$). Open circles = vehicle treatment; open squares = naloxone treatment; closed circles = dPTyr(me)AVP treatment; closed squares = naloxone + dPTyr(me)AVP treatment.


Tail-Flick Latency (sec)
7
5
3
1
5
3
1
1
3
5
7
9
11
13
15
Pre-
Injection (min)
Post-Stress (min)

condition. dPTyr(me)AVP treatment significantly potentiated BCFS analgesia, relative to saline treated rats, from the fourth to the ninth minute following shock.

Insert Figure 3 about here

Significant effects observed across treatments ($F(2,21)=10.74$; $p<.0006$), across the time course of testing ($F(12,252)=23.53$, $p<.0001$), and for the interaction between treatments and time ($F(24,252)=5.80$; $p<.0001$), when the stimulus was terminated if a response did not occur within 12 sec. As summarized in figure 4, latencies were elevated following BCFS relative to the no shock condition. dPTyr(me)AVP treatment significantly potentiated BCFS analgesia, relative to saline treated rats, 5 and 9 min following the foot-shock stress.

Insert figure 4 about here

## Discussion

As described previously, VP-containing neurons project to brain and endoocrine sites that are involved in mediating stress responses. One VP pathway projects from the PVN and terminates in the zona externa of the median eminence (Swanson et al, 1980) where it appears to potentiate the corticotrophic activity of CRF (Gilles et al, 1982; Gilles and Lowry, 1979). Another VP pathway descends to the intermediolateral segments of the thoracic spinal cord where it appears to act upon sympathetic

Figure 3: Potentiation of BCFS analgesia by pretreatment with dPTyr(me)AVP (1 ug). The stimulus was terminated if the animal failed to respond within 7 sec. The x denotes a significant difference from saline treated rats (Dunnett comparison, $p<.05$). Data from no-shock treated rats is the same as shown in figure 1. Open squares = no shock condition; open circles = vehicle treatment; closed circles = dPTyr(me)AVP treatment.

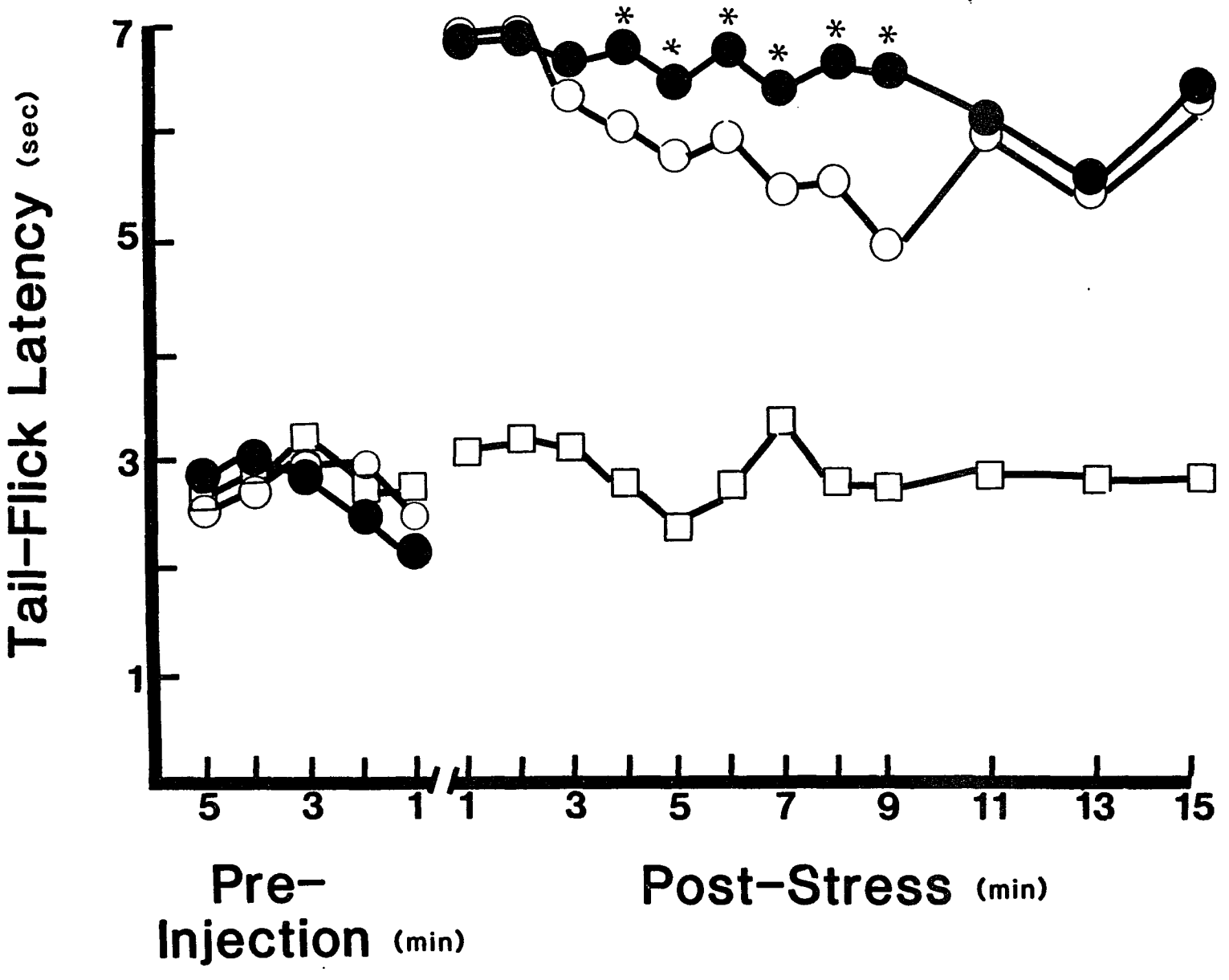
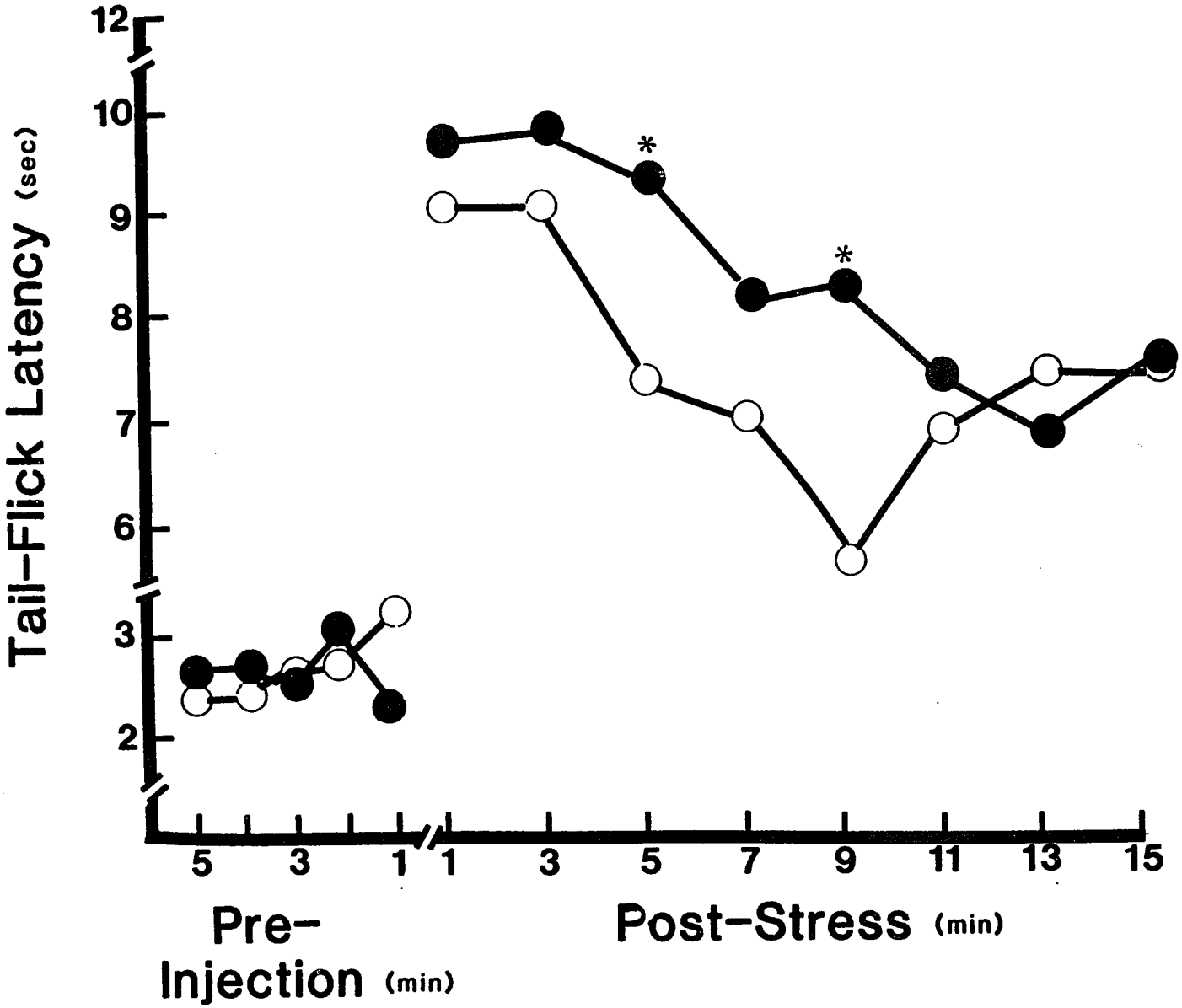

Tail-Flick Latency (sec)
7
5
3
1
*
*
*
*
*
*
5
3
1
1
3
5
7
9
11
13
15
Pre-
Injection (min)
Post-Stress (min)

Figure 4: Potentiation of BCFS analgesia following pretreatment with dPTyr(me)AVP (1 ug). The stimulus is now terminated if the rat fails to respond within 12 sec. The x denotes a significant difference from saline treated animals. Open circles = vehicle treatment; closed circles = dPTyr(me)AVP treatment.


Tail-Flick Latency (sec)
12
10
9
8
7
6
3
2
*
*
5
3
1
1
3
5
7
9
11
13
15
Pre-
Injection (min)
Post-Stress (min)

preganglionic fibers (Swanson and McKeller, 1979). Acute exposure to a wide variety of physical stimuli that activate stress responses significantly elevate pain thresholds as measured by a number of different animal pain tests (see review: Bodnar, 1983). Acute exposure to one such stressor, inescapable foot shock, appears to activate either opioid or non-opioid pain-inhibitory systems, depending upon the temporal parameters of the shock (Lewis et al, 1980), the body region shocked (Watkins and Mayer, 1982), or the number of shocks administered (Grau et al, 1981). The procedures described by Lewis and colleagues (1980) employing PIFS and BCFS parameters of shock were chosen for investigation because, like VP, the analgesia resulting from its application appears to work through both pituitary-adrenal and neuronal mechanisms. The analgesic response following PIFS is reduced following hypophysectomy (Lewis, Chudler, Cannon, and Liebeskind, 1981) and enhanced by adrenalectomy and adrenaldemedullation (Lewis, Tordoff, Sherman, and Liebeskind, 1982). In contrast, BCFS analgesia is enhanced by hypophysectomy (Lewis et al, 1981) and unaffected by adrenal manipulations (Lewis et al, 1982). Moreover, PIFS analgesia is reduced following either destruction of the nRM or transection of the dorsolateral funiculus, while BCFS is reduced by the latter, and not former, manipulation (Lewis, 1982) The analgesia induced by PIFS is thought to be opiate

mediated since it is attenuated in naloxone-treated (Lewis et al, 1980), and morphine-tolerant rats (Lewis et al, 1981). In contrast, analgesia induced by BCFS is unaffected by these manipulations (Lewis et al, 1980; Lewis et al, 1981).

The present experiment demonstrates that VP is involved in both PIFS and BCFS analgesia. Central pretreatment with the VP antagonist, dPTyr(me)AVP, significantly attenuated the analgesic response following PIFS, yet potentiated the analgesic effects of BCFS. However, these results cannot presently be interpreted in terms of possible modulation by VP of both opioid vs non-opioid forms of stress-induced analgesia because this study failed to replicate the findings that naloxone reduces PIFS analgesia (Lewis et al, 1980). Indeed, administration of dPTyr(me)AVP and naloxone together produced similar reductions in pain thresholds as dPTyr(me)AVP alone, suggesting that the reduction observed in the former group was due solely to the administration of the VP antagonist. The divergent results do not appear to be due to gender differences since others (Marek, 1983) have reported that naloxone reverses PIFS analgesia in female rats. Other factors, such as possible differences in the amount and intensity of the shock might better explain the discrepant effect. The analgesic response following both PIFS and BCFS reported by Lewis (Lewis et al, 1981) was both lower in magnitude and of

shorter duration than that found in the present study. Lewis and co-workers (1981) found PIFS analgesia lasting for up to 11 min following shock, with an average magnitide of 1.9 sec for those intervals eliciting a significant effect. The present study, found PIFS analgesia lasting for the full 15 min time course with an average magnitiude of 2.7 sec. Lewis and colleagues (1981) demonstrated BCFS analgesia lasting for 4 min with an average magnitude of 2.7 sec. The present study found BCFS analgesia lasting for the full 15 min with an average magnitude of 3.2 sec. Indeed, in our experiment, latencies were still elevated by 2.75 sec and 3.58 sec following the PIFS and BCFS stressors respectively, at the last test interval, suggesting that the analgesic effects probably would have persisted longer. The difference in analgesic potencies suggest that the stimuli in the two experiments were different. The basis for using foot-shock in this investigation centered around the knowledge that different parameters to activate different pain-inhibitory systems. Indeed, the mechanisms mediating some forms of antinociception have been reported to depend upon the magnitude of the analgesic response. Both opioid and non-opioid mechanisms mediate the analgesic response following cold-water swim stress for those animals that display a large analgesic effect, while only non-opioid mechanisms appear to mediate the analgesic response following swims for those animals displaying a weaker

analgesic effect (Bodnar and Sikorzky, 1983). Therefore, if the actual intensity of the shock was different across the two studies, as the differing time course and magnitude of analgesia would imply, then different, as yet undetermined, pain-inhibitory pathways might be involved. In fact, it has recently been reported that a non-opioid analgesic effect can be elicited following PIFS with alterations in the intensity or duration of the shock administered (Cannon, Maro, Telep, and Carty, 1983;Terman and Liebeskind, 1983).

The fact that dPTyr(me)AVP potentiates BCFS suggests that VP modulates this form of analgesia in a fashion opposite to that of PIFS. It has been proposed that the multiple pain-inhibitory systems do not act totally independently, but rather influence each other through a form of collateral inhibition (Kirchgessner, Bodnar, and Pasternak, 1982). In this model, activation of one pain-inhibitory system by those endogenous or exogenous stimuli that maximally interact with it, inhibits the activation of other pain-inhibitory systems, with the magnitude of the effect dependent upon each system's tonic activational state and the magnitude of the activating stimuli. The very existence of multiple pain-inhibitory systems suggests that they would respond differentially to incoming environmental stimuli. It would seem maladaptive if all systems designed to inhibit pain were to be activated in response to a particular stimulus. Rather,

the suppression of system A by system B would serve to protect the organism should system B fail or be overextended. In those cases, system A would be released from inhibition, thus being able to take over the pain-inhibitory role. In the present studym dPTyr(me)AVP administration potentiated BCFS, probably because it blocked VP from its binding sites. This suggests that a VP pathway typically acts to inhibit pain-inhibitory systems mediating BCFS. As would be predicted from the collateral inhibition model, dPTyr(me)AVP decreased PIFS analgesia, suggesting that VP synapses is important in activating the pain-inhibitory system mediating PIFS. Further support for this model of mutually inhibiting pain-inhibitory systems has been found using this foot-shock paradigm. Reserpine, a catecholamine depletor, eliminates PIFS analgesia while potentiating BCFS analgesia (Lewis et al, 1982). Conversely, hypophysectomy attenuates the analgesic response to PIFS while potentiating the analgesic response to BCFS (Lewis et al, 1981). Support for the hypothesis is not limited to data acquired with foot-shock stress. Manipulations that reduce cold water swim analgesia, such as hypophysectomy (Bodnar et al, 1979), potentiate morphine analgesia (Bodnar, Kelly, Mansour, and Glusman, 1979; Holiday, Law, Tseng, Loh, and Li, 1980). Conversely, manipulations that potentiate morphine analgesia, such as d-phenylalanine administration decrease cold water swim analgesia (Bodnar,

Lattner, and Wallace, 1980). Finally, manipulations that decrease morphine analgesia, such as naloxazone administration, potentiate cold water swim analgesia (Kirchgessner et al, 1982).

In the present study, PIFS produced an analgesia that appeared to act independently of the endogenous opioids. The analgesia following BCFS has been previously reported to be mediated through non-opioid mechanisms (Lewis et al, 1980). Additionally, the Brattleboro rat displays an impaired analgesic response to cold-water stress (Bodnar et al, 1980), an non-opioid analgesic manipulation (see:Bodnar, 1983). These data, therefore support the hypothesis that VP exerts its influence upon nociceptive processes through intrinsic VP pathways and not through an endogenous opioid pain-inhibitory system. However, it should be noted that naloxone reversibility is not the sole criterion for the determination of opioid involvement in PIFS. Additional experiments using cross-tolerance and lesion techniques should be performed to assess further a role for VP in the mechanisms underlying these stressors.

## GENERAL DISCUSSION

VP elevates nociceptive thresholds by interacting with its own binding sites, and acting independently of the endogenous opioid and ACTH systems. In addition, VP plays a modulatory role in the expression of analgesic responses following acute exposure to foot-shock stress,

with the direction of effect dependent upon the differential systems activated by the parameters of the shock. The implications of these findings will be the subject of this section

The action of VP upon pain thresholds appears to be a specific one. It is not simply a general magnocellular effect because low doses of VP elevate pain thresholds, while OXY induced elevations in tail-flick latencies occurred only at higher doses and were accompanied by seizure activity. It was demonstrated that VP-induced elevations of pain thresholds were due to interactions of VP with its receptors since the VP antagonist, dPTyr(me)AVP, eliminated VP analgesia. Furthermore, VP and endogenous opioid analgesia appeared independent since each respective antagonist failed to alter the other system's analgesic response. Two interpretations can be offered regarding VP analgesia: a) VP alters pain thresholds through direct actions upon nociceptive processes; or b) VP analgesia is an epiphenomenon of some other response system that is activated by VP. dPTyr(me)AVP has many actions, including decreasing the pressor, but not the antidiuretic, effects of VP (Bankowski et al, 1978). Since hypertension itself elevates pain thresholds (Dworkin, Filewich, Miller, Craigmyle, and Pickering, 1979; Zamir and Segal, 1979; Zamir and Shuber, 1980; Zamir, Simantov, and Segal, 1980), VP-induced increases in tail-flick thresholds may be due

to the activation of baroreceptors (see review: Aisenbrey and Berl, 1982), which in turn elevate both blood pressure and nociceptive thresholds. However, both DDAVP and DGLVP elevate pain thresholds following central administration (Kordower et al, 1982) and possess minimal pressor effects (Cort and Schwartz, 1978; see: Kordower et al, 1982). Moreover, while VP, vasotocin, and phenylephrine, possess similar actions upon pressor responses, only VP produces analgesia. (Berkowitz and Sherman, 1982; Berson et al, 1983). These data argue strongly for the interpretation that VP exerts its effects upon pain thresholds independently of its pressor effects.

Although VP receptors have yet to be fully characterized, the present series of experiments suggest the existence of multiple VP receptor sub-types, which can be distinguished through the differential effects exerted by agonist and antagonist administration. Since both AVP and DDAVP elevate pain thresholds, and only AVP appreciably elevates blood pressure (Cort and Schwartz, 1978), it would appear that different receptor populations mediate the pressor and analgesic effects of these peptides. However, dPTyr(me)AVP decreases both the pressor and analgesic effects of AVP suggesting the existence of either a receptor sub-type which is differentially capable of mediating incoming pressor and nociceptive information, or one or more sub-types mediating each of these responses. An example of the

latter form of interaction is the noradrenergic receptor system. The alpha and beta noradrenergic sub-types possess differential effects upon central and peripheral nervous tissue and differentially bind endogenous ligands. The alpha-noradrenergic receptor displays preferential affinity and binding for norepinephrine, followed by epinephrine and then isoproterenol, and the beta noradrenergic receptors display the reverse pattern (see: Iverson and Iverson, 1975). However, alpha-noradrenergic neurons will bind isoproterenol, and beta adrenergic neurons will bind norepinephrine at high doses, suggesting an interaction , and not total independence, of the two receptor sub-types. Moreover, certain substrates (e.g. propranalol, timelol, atenalol) antagonize beta-noradrenergic receptors, while others (e.g. phentolamine, phenoxybenzamine) antagonize alpha-noradrenergic receptors, indicating conformational differences in receptor alignment. VP, then, like norepinephrine, may mediate multiple nervous system effects through a population of receptor sub-types that act independently under some conditions and interact under other conditions.

In addition to multiple receptor sub-types, the present data supports the idea of multiple pain-inhibitory systems. VP alters nociceptive thresholds independently of the endogenous opioids. However, if VP modulates pain thresholds through it efferents to the spinal cord, it would be sharing this dorsolateral funicul projection

with fibers involved in the expression of OA and SPA (see: review Fields and Basbaum, 1978). However, basal pain thresholds are not affected by dorsolateral funiculus transection (see: Mayer and Price, 1976) suggesting that both the VP and opioid systems are activational, rather than tonic. According to this view, these pain-inhibitory pathways are dormant until they are recruited, whereupon they are activated to decrease the sensation of noxious stimuli. This hypothesis was supported by the fact that central administration of a VP antagonist, at a dose sufficient to eliminate AVP and DDAVP analgesia, failed to alter basal tail-flick latencies. If the VP pain-inhibitory system is activational in nature, as the present results suggest, receptor blockade would not be expected to alter basal pain thresholds. If the VP pathway inhibiting painful stimulation was tonically active, it would be expected that administration of a VP antagonist would decrease the activity of that pathway, resulting in decreased basal pain perception. It should be pointed out, however, that there is data also supporting a tonic hypothesis. Both Brattleboro rats (Bodnar et al, 1980) and normal rats pretreated with an anti-serum raised against VP (Bodnar et al, 1982), display a slight hyperalgesia. Furthermore, in contrast to VP, dPTyr(me)AVP facilitates extinction (Koob et al, 1981) at a dose much higher than is needed for dPTyr(me)AVP to reverse VP's effects (LeMoal al, 1981; LeMoal et al,

1982). It is conceivable that much higher doses of the antagonist are necessary to elicit this small effect. In any case, the present result is similar to the findings reported with the endogenous opioid system. Naloxone produces either no effects or minimal decreases upon basal pain thresholds (see review: Sawtnok, Pinsky, and LaBella, 1979).

One question arising from the present data concerns the significance for an organism of possessing more than one pain-inhibitory system. Cannon (1939) suggested that the response to noxious or threatening stimuli is one of the basic adaptive mechanisms of animals and humans. It would be maladaptive for an organism to have only one pain-inhibitory system, which, if it failed, would leave the organisms defenseless against potentially injurious stimulation. Which pain system is activated may depend upon the quality or type of noxious stimulus, a point that was raised in the discussion of differential effects of VP upon PIFS and BCFS analgesia. It would appear that one role for VP in pain-inhibition might be to protect an organism from noxious thermal stimulation.

The fact that VP analgesia is unaffected by hypophysectomy (Berson et at, 1983) and possesses diffuse extrahypothalamic projections, suggests that VP may act in a neuromodulatory rather than a neuroendocrine fashion in producing analgesia. As described earlier, it has been

postulated that VP plays a neuromodulatory role in learning and memory processes. This suggestion was based upon VP's localization in limbic structures, including the hippocampus (Buijs et al, 1978; Kovacs et al, 1979), believed to be involved in learning and memory function (Scoville and Milner, 1957). et al, 1979). VP-induced effects upon learning and memory, however, may be due to its ability to alter perception of noxious stimuli (Bailey, 1982). Most of the studies reporting positive effects of VP upon memory employed aversive stimulation. Indeed, it has been postulated that the aversive properties of VP itself may account for its putative role in memory (Ettenberg et al, 1983). Rats developed both conditioned taste and place aversions following their respective pairing with AVP. While VP effects upon learning and memory function may be explained in terms of effects upon nociceptive processes, the converse does not appear to be true. If VP's effects upon pain thresholds were due to improved memory, and especially resistance to extinction, it would be expected that VP would decrease measures of pain thresholds by shortening the latency to escape from noxious stimuli. However, VP administration results in increased response latencies on measures in which the appropriate escape response results in the termination of the noxious stimulus (Berkowitz and Sherman, 1982; Berntson and Berson, 1980; Berson et al, 1983; Kordower et al, 1982). Therefore, it appears that

VP analgesia is due to specific interactions with nociceptive processes, rather than being an epiphenomenon of VP's neuromodulation of memory.

The present study employed a nociceptive measure using radiant heat as the noxious stimulus and confirmed previous reports of VP analgesia in response to heat stimuli, such as heat to the tail (Berntson and Berson, 1980; Berson et al, 1983; Kordower et al, 1982), heat to the feet, and intraperitoneal injections of acetic acid (Berkowitz and Sherman, 1982). Central administration of either VP or its analogue fails to alter pain thresholds on the jump test (Kordower et al, 1982), which measures reactivity to electric shock, suggesting that VP analgesia may be selective for noxious thermal stimulation. Anatomical considerations support this view. VP-containing neurons project from the PVN nucleus to the sensory nuclei of the fifth and tenth cranial nerves, brain regions that are involved in the perception of noxious input (Buijs, 1978, Swanson et al, 1980), VP-containing neurons also send a direct projection to the marginal zone of the spinal cord dorsal horn (Buijs, 1978; Swanson et al, 1980; Nilaver et al, 1980), a termination site important for the perception of both nociceptive and thermal stimuli. Together, the anatomical and behavioral evidence suggest that VP's effects upon noxious input may be mediated by this descending pathway, a hypothesis suggested by others (see review: Kozlowski et al, 1983).

VP plays a role in the expression of stress-induced analgesia with the direction of the effect dependent upon which pain-inhibitory system is activated. dPTyr(me)AVP decreases PIFS analgesia, yet potentiates BCFS analgesia. Additionally, the Brattleboro rat displays an impaired analgesic response to cold water swim stress (Bodnar et al, 1980). An increase in pain thresholds is only one of the many coping responses an animal makes upon exposure to a stressful environment. Other adjustments include changes in heart rate, muscle vasodilation, pupillary dilation, glucose and fat mobilization, constriction of capillary beds in the skin, and altered respiration, as well as a complex but integrated neuroendocrine response, involving pituitary-adrenal-cortical and sympatho-medullary activation (Selye, 1953). Of these responses, VP is capable of altering blood pressure (see review: Aisenbrey and Berl, 1982) heart rate (Gardiner and Bennett, 1982), neuroendocrine secretion (e.g. ACTH ; see: McCann, 1980), and pituitary-adrenal-cortical function (McCann, Antunes-Rodriquez, Naller, Valtin, 1966). It is possible that VP modulates stress-induced analgesia via either of two mechanisms: a) direct interactions with pain-inhibitory pathways that are exposed to stress; b) alterations of the organism's perception of the stressful consequences of the stimulus. In this regard, neonatal administration of monosodioum glutamate attenuates cold water swim analgesia (Bodnar, Abrams, Zimmerman, Kreiger,

Nicholson, and Kizer, 1980), and potentiates 2-DG analgesia (Badillo-Martinez, Nicotera, Butler, Kirchgessner, Sperber, and Bodnar, 1982) in the adult rat. However, these animals also display profound alterations in thermoregulatory and hyperphagic responsivity to these stressors, suggesting that MSG induced damage produces an abnormal perception of the stressful properties of these manipulations. At the present time, it is not known whether VP alters the analgesic response to stress through activation of pain-inhibitory pathways or through altering the perceptual consequences of stress. Certainly, there is evidence for VP subserving an intrinsic pain-inhibitory system. However, VP sends projections to the zona externa of the median eminence where it interacts with CRF (Gilles, Clinton and Lowry, 1979; Gilles and Lowry, 1982), and to the thoracic regions of the spinal cord (Swanson and McKeller, 1979), the source of sympathetic outflow from the central nervous system. These pathways suggest that VP may play a role in a more complex and integrated response to stress. Future research should begin to clarify the multidimensional aspects of this facinating and multifunctioned peptide.

# Appendix A

## Ontogeny of VP and OXY Neurons

Ontogenetically, VP, OXY, and their neurophysins can be detected in the brain by immunocytochemical and radioimmunoassay techniques as early as fetal day 16-19 in the rat (Buijs, Velis, and Swabb, 1982) and by mid-trimester in the human (Burford and Robinson, 1982). VP appears in the PVN and pituitary gland by fetal day 18 in the rat (Buijs et al, 1982) with levels of VP increasing dramatically until term in both rats and humans (Buijs et al, 1982; Burford and Robinson, 1982). OXY levels decrease between fetal days 16-18 and then increase on fetal days 18-22 in the rat, however the level of OXY is about five percent of that for VP (Buijs et al, 1982). In contrast, VP first appears in the SCN on the second post-natal day (deVries, Buijs, and Swabb, 1981).

## Historical Perspective of the Magnocellular Neurosecretory System

The magnocellular neurosecretory system was the first peptidergic system to be characterized in detail. In 1894, Raymon y Cajal described neural projections from the hypothalamus to the posterior lobe of the pituitary gland (see: Anderson and Haymaker, 1974). However it was not until the 1940's that it was postulated that these hypothalamic neurons synthsized neurosecretory material, and transported these substances to the neurohypophysis

for subsequent release (Scharrer and Scharrer, 1940). Scharrer and Scharrer called this neurosecretory material "colloidal" substance and postulated that the neurons of the paraventricular nucleus (PVN) and supraoptic nucleus (SON) of the hypothalamus could produce physiological effects at a locus distal to the nerve cell via this substance (Scharrer and Scharrer, 1954). This was later confirmed neuroanatomically using the staining techniques of Gomori (1941; 1950). At the same time, the peptides VP and OXY were being characterized, sequenced (du Vigneaud, 1956), and synthesized (du Vigneaud, Gish, and Katsoyannis, 1954; du Vigneaus, Ressler, Swan, Roberts and Katsoyannis, 1954; du Vigneaud, Ressler, Swan, Roberts, Katsoyannis, and Gordon, 1953) in both the central and peripheral nervous tissue. In addition, their protein carrier molecules, the neurophysins were discovered (van Dyke, Chow, Greep, and Rothen, 1941), although aside from their designations as 'carrier molecules', little is known about their function. It is known that both VP and OXY have neurochemically distinct neurophysins (Robinson and Franz, 1973) and that both the peptides and their carrier molecules are probably derived from the to be fully elucidated (Brownstein et al, 1980; Marshall,

Neurophysiolgy of the Magnocellular Neurosecretory System-

While Cross and Green (1959) initially demonstrated that neither the shape of action potentials nor the

patterns of electrical activity were significantly different in the magnocellular hypothalamus as compared to any other part of the central nervous system, these experiments were hindered by the inability to identify the magnocellular neurons under study. In the sole report of electrical activity following intracellular recording from confirmed magnocellular hypothalamic neurons (Koizumi and Yamashita, 1972), the resting potential (-40 mV) and spike amplitude (50-80 mV) did not differ from non-neurosecretory nerve cells. However the action potentials were of long duration (5 msec) and displayed a graded potential, probably due to the numerous afferent imputs onto these neurons (see review: Kozlowski, Nilaver, and Zimmerman, 1983). The application of antidromic stimulation techniques to magnocellular neurosecretory neurons (Yagi, Azuma, Matsuda, 1966) constituted a significant methodological advance since nerve terminals can easily be stimulated in the neurohypophysis through transaural (Dyball, 1969), dorsal (Lincoln and Wakerley, 1974; Negoro and Holland, 1972) and ventral (Dreifuss and Ruf, 1972; Sundsten, Novin, and Cross, 1970) approaches. Extracellular antidromic recording reveals three patterns of background firing of magnocellular neurons: 1- a slow irregular pattern characterized by a very low mean firing rate (<3 spike/sec); 2- a fast continuous pattern characterized by a mean firing rate > 3 spikes/sec; 3- and a phasic pattern characterized by successive periods of

electrical activity and electrical silence occurring in a more or less regular manner.

Exogenous stimuli will selectivly elicit the electrical discharge and subsequent release of VP or OXY. Upon suckling, OXY is released, eliciting the milk ejection reflex in both awake and anesthetized rats (Lincoln and Wakerley, 1974; Wakerley and Lincoln, 1973) allowing extracellular recording for up to 4-5h. During most of the suckling period, OXY neurons in the PVN or SON are unresponsive to the suckling efforts of the pups (Lincoln and Wakerley 1974; Wakerley and Lincoln, 1973) and are characterized by synchronous slow irregular or fast continuous, but not phasic activity. However, just prior to (15-20 sec) the periodic milk ejection these neurons fire rapidly and synchronously with 70-80 spikes occuring within 2-4 sec, followed by a brief period of quiescence. Greater activity is noted for OXY neurons in the PVN. In contrast to OXY neurons, many VP neurons display phasic activity. In unanesthetized monkeys,deprived of water for five days, the percentage of SON cells displaying a phasic pattern rose from 10% on day 1 to 50-60% on day 5 (Arnauld, Dufy, and Vincent, 1975). In the anesthetized rat, 6 h of water deprivation resulted in phasic activity in 94% of VP (Wakerley et al, 1978).

The functional significance of the firing patterns of these neurons remains in question. In the rat, the brief

activation of OXY fibers induced by pup suckling corresponds to the pulsatile nature of OXY release at milk ejection (Poulain and Wakerley, 1982). However, the sudden and brief excitation of OXY neurons cannot be entirely explained by the continuous stimulation by the pups. From anatomical evidence (Findlay, 1966), it would seem that the suckling of the pups would provide a large amount of input to the spinal cord of the dam. Even if the pathway from the spinal cord to the hypothalamus is multisynaptic, the impulse would reach the hypothalamus within milliseconds, and presumably would reach both the SON and PVN simultaneously to account for the synchonous firing of these two nuclei (Poulain and Wakerley, 1982). Therefore, the actual stimulus producing these electrophysiological effets is unkown. Additionally, the functions of VP and OXY in regulating neuroendocrine function appear to be dependent upon other neurotransmitter and/or neuropeptide systems in that many systems that synapse upon or co-exist within the PVN and SON (see review: Kozlowski et al, 1983), and pharmacological or anatomical manipulations of these systems can alter the firing patterns of VP and OXY neurons (Poulain and Wakerley, 1982).

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