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Site-specific modulation of morphine and swim-induced antinociception following thyrotropin-releasing hormone in the rat periaqueductal gray

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Site-Specific Modulation of Morphine and Swim-Induced Antinociception Following Thyrotropin-Releasing Hormone in the Rat Periaqueductal Gray

by

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Abstract

Site-Specific Modulation of Morphine and Swim-Induced Antinociception Following Thyrotropin-Releasing Hormone in the Rat Periaqueductal Gray

by

Judith A. Robertson

Advisor: Dr. Richard J. Bodnar

Thyrotropin-releasing hormone (TRH) appears to be a potent modulator of nonopioid forms of antinociception, but produces less consistent effects upon opioid-mediated antinociception. Previous studies have examined TRH effects in ventricular, cisternal or intrathecal locations, and thus could not specify the locus of action. The mesencephalic periaqueductal gray (PAG) was chosen as a potential site for the modulatory effects of TRH because: a) TRH-reactive fibers and receptors are localized in this area, b) it supports TRH antinociception, and c) it is a primary locus for supraspinal opiate analgesia. The present study examined intracerebral TRH effects upon continuous cold-water swim (CCWS) and morphine antinociception because of their clear dissociations and representations as nonopioid and opioid forms of antinociception. TRH effects upon basal nociception were also examined. Cannulated rats received intracerebral microinjections of TRH (0.1-10 ug) 20 min prior to CCWS (2°C for 3.5 min) or morphine (0.1-2.5 ug) administered into the PAG. Tail-flick latencies, jump thresholds and core body temperatures were assessed in that order following the basal, CCWS or morphine conditions.

TRH microinjections into the PAG significantly increased basal

tail-flick latencies and jump thresholds for up to 15 min following administration, confirming previous reports of a short-lived antinociception following supraspinal administration. Microinjections of TRH into the PAG failed to alter basal core body temperatures. TRH produced significant alterations in CCWS antinociception on the tail-flick and jump tests that varied as a function of the specific site of administration within the PAG. When TRH was microinjected into the anterior PAG, it significantly reduced CCWS antinociception on the tail-flick and jump tests in a dose-dependent fashion. In contrast, when TRH was microinjected into the posterior PAG, it significantly potentiated CCWS antinociception on the jump test. CCWS hypothermia was significantly reduced by TRH microinjections into both anterior and posterior PAG placements, suggesting a dissociation between antinociceptive and hypothermic effects.

In contrast, TRH microinjections into the both the anterior and posterior PAG significantly and dose-dependently potentiated morphine antinociception on the tail-flick and jump tests, as well as the hyperthermic effects of morphine. These results are discussed in terms of the role of the PAG in opioid and nonopioid forms of stress-induced antinociception as well as morphine antinociception, and the potential roles of TRH and the anterior PAG in a collateral inhibition model of antinociceptive responses.

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GLOSSARY

B-FNA	beta-funaltrexamine, mu opioid antagonist
CCWS	continuous cold-water swims, nonopioid antinociceptive stressor
DADL	D-Ala^2-D-Leu^5-enkephalin, delta opioid agonist
DALCE	[D-Ala^2, Leu^5, Cys^6]-enkephalin, delta$_1$ opioid antagonist
DAMGO	[D-Ala^2, NMe^4, Gly(ol)^5]-enkephalin, mu opioid agonist
DPDPE	[D-Pen$_2$, D-Pen^5]-enkephalin, delta$_1$ opioid agonist
DSLET	[D-Ser^2, Leu^5] enkephalin-Thr^6, delta/mu$_1$ opioid agonist
EKC	ethylketocyclazocine, kappa opiate agonist
ICWS	intermittent cold-water swims, opioid antinociceptive stressor
LC	locus coeruleus
NRGC	nucleus reticularis gigantocellularis
NRM	nucleus raphe magnus
OVLT	organum vasculosum lamina terminalis, circumventricular organ
PAG	periaqueductal gray
RVM	rostro-ventral medulla, includes NRM and NRGC
SIA	stress-induced antinociception
TRH	thyrotropin-releasing hormone
5HT	serotonin

INTRODUCTION

Pain-inhibition has been shown to be modulated by different intrinsic systems in the brain and spinal cord. One major pathway modulating centrifugal control of nociceptive stimuli originates in the mesencephalic periaqueductal gray (PAG) which projects to the medullary nuclei raphe magnus (NRM) and reticularis gigantocellularis (NRGC). In turn the NRM and NRGC send descending projections through the dorsolateral funiculus of the spinal cord into the dorsal horn where they synapse on nociceptive-sensitive neurons and inhibit their activity (see reviews: Basbaum and Fields, 1984; Fields and Basbaum, 1978). These mesencephalic, medullary and spinal structures are rich in opioid receptors (see review: Akil, Watson, Young, Lewis, Khachaturian, and Walker, 1984) which, if stimulated by morphine microinjections, activate an antinociceptive response (see reviews: Yaksh, 1981, 1984a,b; Yaksh and Rudy, 1978). However, several lines of evidence, including work in the area of stress-induced antinociception (see reviews: Bodnar, 1986, 1990; Bodnar, Kelly, Brutus, and Glusman, 1980b; Terman, Shavit, Lewis, and Cannon, 1984; Watkins and Mayer, 1982b), have indicated that both opioid and nonopioid forms of antinociception exist. Indeed, certain stressors like continuous cold-water swims have a nonopioid antinociceptive profile that is quite distinct from the mechanisms subserving morphine antinociception. Further, these two forms of antinociception display collateral inhibition in that simultaneous administration of morphine and continuous cold-water swims produces an antinociceptive response less than the

antinociception induced by either manipulation singly (Steinman, Faris, Mann, Olney, Komisaruk, Willis, and Bodnar, 1990).

A search for the neurochemical and neuroanatomical substrates mediating either nonopioid antinociception itself or its inhibitory interaction with opioid systems has included the pituitary-adrenal axis (Bodnar, Kelly, Mansour, and Glusman, 1979b; Bodnar, Sharpless, Kordower, Potegal, and Barr, 1982; Kelly, Silverman, Glusman, and Bodnar, 1993), the vasopressinergic system (Bodnar, Zimmerman, Nilaver, Mansour, Thomas, Kelly, and Glusman, 1980f; Bodnar, 1986; Kordower and Bodnar, 1984; Truesdell and Bodnar, 1987), catecholamine systems (Bodnar, Kelly, Brutus, Greenman, and Glusman, 1980c; Bodnar, Merrigan, and Sperber, 1983; Bodnar and Nicotera, 1982; Kepler and Bodnar, 1988) and histaminergic systems (Gogas and Hough, 1988; Gogas, Hough, Eberle, Lyon, Glick, Ward, Young, and Parsons, 1989; Robertson, Hough, and Bodnar, 1988). Another neuromodulatory substance implicated in opioid and nonopioid forms of antinociception is thyrotropin-releasing hormone (TRH). While failing to alter different forms of opioid antinociception (Holaday, Tseng, Loh, and Li, 1978; Holaday and Faden, 1983; Kasson and George, 1983; Osbahr, Nemeroff, Luttinger, Mason, and Prange, 1982), ventricular administration of TRH potentiates antinociception induced by nonopioid parameters of continuous cold-water swims and inescapable footshock (Butler and Bodnar, 1984, 1987).

Since TRH was administered into the lateral ventricles, the specific site of action upon nonopioid antinociception could not be

specified. Given the central role of the PAG in antinociceptive processes, the main aim of this dissertation was to determine whether the PAG is implicated in the modulation of central TRH effects upon either nonopioid antinociception as stimulated by continuous cold-water swims or opioid antinociception as stimulated by morphine. The following sections are designed to provide background information pertaining to: a) a description of intrinsic pain-inhibitory systems, b) the description of specific opioid and nonopioid pain-inhibitory systems, c) the afferent, efferent and intrinsic organization of the PAG, d) the anatomical distribution of TRH cells, fibers and receptors, e) TRH and nociceptive processes, and f) a rationale for the present studies.

A. Intrinsic Opioid Pain-Inhibitory System.

A pivotal issue contributing to the understanding of the modulation of pain perception was the discovery of endogenous pain inhibitory systems producing centrifugal inhibition of nociceptive input at both supraspinal and spinal levels of the neuraxis. The delineation of these systems was characterized by the central antinociceptive properties of morphine or electrical stimulation delivered to specific supraspinal sites (see reviews: Mayer and Price, 1976; Yaksh and Rudy, 1978) and the ability of opiates to produce antinociception following intrathecal administration into the spinal cord (see review: Yaksh, 1984a,b). The relationship of individual supraspinal loci with respect to other supraspinal and spinal loci implicated in pain inhibition has been the subject of neuroanatomical, neurophysiological and neurochemical analyses

(Basbaum and Fields, 1984; Fields and Basbaum, 1978; Fields, Heinricher and Mason, 1991; Gebhart, 1982). Microinjection mapping studies have identified mesencephalic, metencephalic and mylencephalic regions involved in the mediation of opiate antinociception, including the periaqueductal gray (PAG), locus coeruleus (LC), nucleus raphe magnus (NRM) and the nucleus reticularis gigantocellularis (NRGC) (Yaksh and Rudy, 1978; Jensen and Yaksh, 1986a,b; Fang, Fields, and Lee, 1986; Schmauss and Yaksh, 1984; Schmauss, Shimohigashi, Jensen, Rodbard, and Yaksh, 1985; Bodnar, Williams, Lee, and Pasternak, 1988).

Endogenous opiate pain inhibition has been proposed to originate in the mesencephalic PAG and adjacent dorsal raphe nucleus, project to the NRM and NRGC (collectively known as the rostro-ventral medulla: RVM), and send descending serotonergic (5HT) projections through the dorsolateral funiculus to the dorsal horn of the spinal cord (Basbaum and Fields, 1984; Bowker and Abbott, 1990). Electrophysiological studies have implicated activation of the NRM and NRGC in the mediation of opiate antinociception elicited from the PAG (Fields, Heinricher, and Mason, 1991; Fields and Basbaum, 1978; Gebhart, 1982). Indeed, a serotonergic synapse mediated by $5HT_2$ and $5HT_3$ receptors in the ventral medial medulla participates in the mediation of mesencephalic morphine antinociception (Kiefel, Cooper, and Bodnar, 1992a,b). Further, reciprocal connections between the LC and the medial medulla also exist (Moore and Bloom, 1979; Ennis and Aston-Jones, 1987; Clark and Proudfit, 1991) with the former contributing

spinal noradrenergic projections to the dorsal horn (Nygren and Olson, 1977; Nygren, Olson and Seiger, 1977; Olson and Fuxe, 1971, 1972).

B. Opioid and Nonopioid Forms of Pain-Inhibition.

Opioid Antinociception: Small amounts of morphine or endogenous opioids injected directly into various regions of the PAG of rats, cats, and monkeys produces strong antinociceptive effects (Pert and Yaksh, 1974; Tsou and Jang, 1964; Yaksh and Rudy, 1978; Yaksh, Yeung and Rudy, 1976). Moreover, opiate and opioid antinociception in the PAG was reversed by the opiate receptor antagonist naloxone. Subsequent mapping studies indicated that antinociception can also be elicited following microinjections of morphine into either the NRM, NRGC and nuclei reticularis paragigantocellularis and reticularis paragigantocellularis lateralis of the RVM (Takagi, Satoh, Akaike, Ahibata and Kuraisi, 1977; Akaike, Shibata, Satoh and Takagi, 1978; Azami, Llewelyn and Roberts, 1982; Dickenson, Oliveras and Besson, 1979; Levy and Proudfit, 1979).

Spinal Opioid Antinociception: Intrathecal administration of opiates and opioid analogues produces a dose-dependent antinociception which can be blocked by naloxone (Yaksh and Rudy, 1978; Yaksh, 1981). This suggests that the antinociception is mediated by the direct action of opiates on spinal cord opioid receptors. The modulation of spinal opioid antinociception has been delineated with the development of selective agonists and antagonists for specific opiate receptor subtypes. These studies

have found that intrathecal administration of mu selective agonists elicits antinociception which is blocked by beta-funaltrexamine, but not naloxonazine, indicating a mu_2 mechanism of action (Paul, Bodnar, Gistrak and Pasternak, 1989). Delta and kappa agonists produce predominantly spinal antinociception which is blocked by selective antagonists for these receptors (Yaksh, 1984a,b; Porreca, Heyman, Mosberg, Omnaas and Vaught, 1987; Porreca, Mosberg, Hurst, Hruby and Burks, 1984; Wuster, Schulz and Herz, 1980; Heyman, Mulvaney, Mosberg and Porreca, 1987; Heyman, Vaught, Raffa and Porreca, 1988). Thus, mu_2, delta and kappa receptors have been implicated in spinally mediated opioid antinociception.

Supraspinal Opioid Antinociception: Supraspinal opioid antinociception appears to be modulated by neurons which originate in the midbrain PAG, synapse in the medullary NRM, NRGC, and NRGC pars alpha, and project to the substantia gelatinosa of the spinal cord through the dorsolateral funiculus (Basbaum and Fields, 1984; Fields and Basbaum, 1978). This model of descending pain inhibition is supported by various lines of research. Lesions placed in either the NRM (Proudfit and Anderson, 1975) or the dorsolateral funiculus (Lebars, Menetrey, Conseiller and Besson, 1975; Murphin, Bennett and Mayer, 1976) attenuate antinociception elicited by either electrical stimulation or morphine microinjection into the PAG. Both of these forms of antinociception in the PAG excite RVM neurons (Pomeroy and Behbehani, 1979) and inhibit nociceptive-sensitive dorsal horn neurons (Mayer and Liebeskind, 1974).

Physiologically-distinct classes of neurons have been

identified in the RVM based upon the temporal correlation of changes in their firing with the execution of reflexes elicited by noxious stimulation (Fields, Barbaro and Heinricher, 1988). Cells of the first class, "on-cells," reliably show a sudden increase in firing just prior to the occurence of a response, which are activated to evoke a withdrawal reflex (Vanegas, Barbaro and Fields, 1984). "On-cells" are highly active just prior to and during the execution of the tail-flick test (D'Amour and Smith, 1941), which is one of the nociceptive measures that was used in the present study. This indicates that "on-cell" firing does not have a potent inhibitory action on nocifensive reflexes. Moreover, administration of systemic morphine or morphine microinjected into the PAG at doses sufficient to block the tail flick response supresses "on-cell" firing (Barbaro, Heinricher and Fields, 1986).

The second set of cells, "off-cells," show an abrupt pause in firing prior to the tail-flick response. "Off-cells" therefore, inhibit nociceptive transmission and these cells become continuously active following administration of morphine either systemically or by microinjection into the PAG (Fields, Vanegas, Hentall and Zorman, 1983). The opiate activation of "off-cells" is particularly significant because of the evidence that the modulatory output neurons in the RVM responsible for nocifensor reflex suppression is excited by opiates (Vanegas et al., 1984).

"On" and "off" cells play a central role in descending nociceptive modulation, and both cell classes are excited by electrical stimulation in the PAG (Vanegas et al., 1984). Thus,

any observed enhancement of nociception that is correlated with an increase in "on-cell" activity could be explained by the removal of descending inhibition exerted by "off cells." These two physiologically-distinct classes of RVM neurons project to the dorsal horn, where they are likely to exert opposing actions on nociceptive transmission (Fields et al., 1983). Moreover, "on" and "off" cells have also been described in the PAG and the nucleus cuneiformis (Heinricher, Cheng and Fields, 1987), sites which have major projections to the RVM. Therefore, several authors (Fields, et al., 1988; Vanegas et al., 1984; Mason and Fields, 1989) believe modulation of nociception by the RVM must be interpreted in terms of the interactions between these two populations of RVM cells and their termination in the dorsal horn.

With the recent development of selective agonists and antagonists for particular opioid receptor subtypes, the involvement of specific opioid receptors in the mediation of supraspinal opioid antinociception has been investigated. This research has demonstrated that the mu_1 receptor plays an important role in supraspinal opioid antinociception. Microinjections of morphine, DAMGO (mu) and DSLET (delta, mu_1) into the PAG, LC, NRM and NRGC elicits antinociception (e.g., Bodnar et al., 1988; Fang et al., 1986; Smith, Perotti, Crisp, Cabral, Long and Scalzitti, 1988) which suggests a primary role for the mu receptor in supraspinal responses in these mesencephalic and medullary structures. Furthermore, both morphine and DSLET antinociception elicited from the PAG, LC and NRM/NRGC can be blocked by

naloxonazine (Bodnar et al., 1988). Our laboratory (Rossi, Pasternak and Bodnar, 1993) has recently demonstrated multiplicative synergy between these structures. Sub-analgesic doses of morphine administered simultaneously into the PAG and into the NRM/NRGC elicit a strong and prolonged analgesic response which shifts morphine's dose-response curve for each site significantly to the left. Further, morphine analgesia elicited by simultaneous activation of the PAG and the NRM/NRGC is blocked by naloxonazine, again implicating the mu receptor in this response.

Finally, microinjections of either naltrexone, the mu-selective antagonist, B-FNA or the delta-selective antagonist, naltrindole into the NRM/NRGC significantly inhibited mesencephalic morphine analgesia (Kiefel, Rossi and Bodnar, 1993). The naltrexone and B-FNA antagonism is in keeping with the foregoing data implicating the mu receptor in opioid supraspinal analgesia. However, the ability of microinjections of the delta-selective antagonist, naltrindole into the RVM to also significantly reduce mesencephalic morphine analgesia appears to implicate the delta receptor in the medullary opioidergic synapse mediating opioid supraspinal analgesia. If this is the case, one would expect that delta receptor agonists should elicit analgesia from these medullary sites. Some investigations support a supraspinal role for delta receptors (Jensen and Yaksh, 1986a; Porreca et al., 1984, 1987). Recent work has indicated the existence of multiple delta (delta$_1$ and delta$_2$) receptors (Mattia, Farmer, Takemori, Sultana, Portoghese, Mosberg, Bowen and Porreca, 1992; Mattia, Vanderah,

Mosberg and Porreca, 1991; Negri, Potenza, Corsi and Melchiori, 1991). Whereas $delta_1$ agonists fail to elicit antinociception following microinjections into the PAG (Bodnar et al., 1988), $delta_2$ agonists produce a mild antinociceptive response. Naltrindole's actions at $delta_2$ sites are quite specific since it inhibits the antinociceptive effects of $delta_2$, but not $delta_1$ agonists. (Mattia et al., 1991; Jiang et al., 1990, 1991; Sofuoglu, Portoghese and Takemori, 1991). Finally, kappa receptors do not appear to participate in supraspinal antinociception following ventricular (Friedman, Jen, Chang, Lee and Loh, 1981; Chavkin, James and Goldstein, 1982) or intracerebral (Bodnar et al., 1991) administration.

Nonopioid Pain Inhibition: Studies with stimulation-produced antinociception (SPA) (Cannon, Prieto, Lee, and Liebeskind, 1982) and stress-induced antinociception (SIA) provide evidence that both opioid-mediated and nonopioid-mediated pain inhibitory systems exist (Amir and Amit, 1978; Bodnar, Kelly, Spiaggia, Ehrenberg, and Glusman, 1978b; Lewis, Cannon and Liebeskind, 1980; Grau, Hyson, Maier, Madden and Barchas, 1981; Watkins and Mayer, 1982b), which have different pharmacological, hormonal, and neural profiles (see Bodnar, 1984, 1986, 1990, 1993 for reviews). Since the antinociception induced by continuous cold water swims (CCWS) is studied in this dissertation research, this section will therefore focus upon the nonopioid substrates of this stressor.

Antinociception can be produced for over 1 h by acute exposure to CCWS (3.5 min in a 2°C bath) across a range of swim temperatures

and across a range of nociceptive tests (e.g., jump, tail flick, tail-pinch and liminal escape tests: Bodnar, Kelly and Glusman, 1978a). The antinociceptive effects of CCWS do not appear to be a result of hypothermia since chronic daily exposure to CCWS over 14 days results in adaptation to the antinociceptive, but not hypothermic responses (Bodnar, Kelly, Spiaggia and Glusman, 1978c). CCWS antinociception fails to display cross-tolerance with morphine antinociception (Bodnar, Kelly, Steiner and Glusman, 1978d; Girardot and Holloway, 1984b), and is only partially decreased by a high dose of naloxone (Bodnar et al., 1978b; Bodnar and Sikorszky, 1983) or naltrexone (Girardot and Holloway, 1984a). These data provided the initial evidence that this form of antinociception was mediated through a nonopioid pain-inhibitory system. A number of other differences between opiate and CCWS antinociception subsequently emerged. First, administration of the putative enkephalinase inhibitor, D-phenylalanine, potentiated morphine antinociception (Alleva, Castellano and Oliverio, 1980), but reduced CCWS antinociception (Bodnar, Lattner and Wallace, 1980d). Second, naloxazone, a high- affinity mu_1 opioid receptor subtype antagonist (Pasternak, Childers and Snyder, 1980) reduced morphine antinociception, but potentiated CCWS antinociception (Kirchgessner, Bodnar and Pasternak, 1982). Third, chronic naloxone treatment significantly reduced morphine antinociception, but potentiated CCWS antinociception (Yoburn, Truesdell, Kest, Inturrisi and Bodnar, 1987). From these data, it was proposed that opioid (e.g., activated by morphine) and nonopioid (e.g., activated

by CCWS) pain-inhibitory systems interacted with each other through a mechanism of collateral inhibition (see review: Bodnar, 1986). Activation of the opioid pain-inhibitory system would produce an opioid-mediated antinociception that also inhibited the activation of the nonopioid pain-inhibitory system. Alternatively, activation of the nonopioid pain-inhibitory system would produce a nonopioid-mediated antinociception that also inhibited the activation of the opioid pain-inhibitory system. To evaluate this hypothesis directly, rats were administered either morphine alone, CCWS alone, or morphine and CCWS simultaneously. The simultaneous exposure to morphine and CCWS produced an antinociceptive response that was significantly less than that elicited by either morphine alone or CCWS alone (Steinman et al., 1990). These effects were temporally delimited and could also be observed for the interaction between morphine and other forms of nonopioid SIA (Grisel, Fleshner, Watkins and Maier, 1991; Grisel, Grahn, Sutton, Watkins and Maier, 1992).

This is not to say that all types of exposure to swims produce a nonopioid antinociceptive response. Increases in the swim temperature of CCWS to 15°C produces an antinociceptive response that is sensitive to naloxone antagonism (Bodnar and Sikorzsky, 1983). Further, acute exposure to intermittent cold-water swims (ICWS) produces an antinociceptive response which is both cross-tolerant with morphine antinociception and significantly reduced by naloxone and naltrexone pretreatment (Girardot and Holloway, 1984a,b, 1985). Like morphine antinociception, ICWS antinociception

is potentiated by endopeptidase 24.11 and 24.15 inhibition (Chipkin, Latranyi, Iorio, and Barnett, 1982; Greenberg and O'Keefe, 1982; Kest, Orlowski and Bodnar, 1991). It is interesting to note that the nonopioid antinociceptive response induced by CCWS displays reciprocal antinociceptive cross-tolerance with the opioid-mediated stressors, ICWS, cervical probing and 2-deoxy-D-glucose (Bodnar and Komisaruk, 1984; Pavlovic and Bodnar, 1993; Spiaggia, Bodnar, Kelly and Glusman, 1979).

CCWS antinociception has a hormonal component since it is decreased by hypophysectomy (Bodnar, Glusman, Brutus, Spiaggia and Kelly, 1979a). In contrast, CCWS antinociception is potentiated in adrenalectomized rats (Glusman, Bodnar, Mansour and Kelly, 1980) and in normal rats receiving corticosteroid synthesis inhibitors (Mousa, Miller and Couri, 1981a). The pituitary-adrenal axis appears to play a more prominent role in modulating CCWS antinociception than the sympatho-medullary axis for three reasons. First, administration of the synthetic glucocorticoid, dexamethasone decreases CCWS antinociception in normal rats (Mousa, Miller and Couri, 1981b) and in adrenalectomized rats (Marek, Ponocka and Hartmann, 1982). Second, the sympatho-medullary system does not seem to be involved in CCWS antinociception since adrenal demedullation and peripheral catecholamine depletion failed to affect CCWS antinociception following 2°C or 15°C swims (Bodnar et al., 1982). Third, removal of the intermediate and posterior lobes of the pituitary gland failed to alter the magnitude of CCWS antinociception (Kelly et al., 1993). The neural control of

pituitary-adrenal modulation of CCWS antinociception appears to involve the medial-basal hypothalamus. Neonatal treatment with monodium glutamate destroys the arcuate and medial-basal hypothalamus, and significantly reduced CCWS antinociception (Badillo-Martinez, Nicotera, Butler, Kirchgessner and Bodnar, 1984b; Bodnar, Abrams, Zimmerman, Krieger, Nicholson and Kizer, 1980a).

Information regarding the neural component of CCWS antinociception is derived mostly from neuropharmacological manipulations. For instance, noradrenergic systems have been implicated in CCWS antinociception since lesions placed in the locus coeruleus decrease CCWS antinociception (Bodnar, Wallace, Kordower, Kirchgessner, Simone, Merrigan, Scalisi and Lattner, 1980e). Further, desipramine, a noradrenergic reuptake blocker potentiates CCWS antinociception (Bodnar, Mann and Stone, 1985). However, both clonidine, an $alpha_2$ noradrenergic receptor agonist, and yohimbine, an $alpha_2$ noradrenergic receptor antagonist, potentiate CCWS antinociception (Bodnar et al., 1983; Kepler and Bodnar, 1988). Dopamine may play an antagonistic role in CCWS antinociception since dopamine receptor stimulation with apomorphine decreases CCWS antinociception (Bodnar et al., 1980c) and dopamine receptor blockade with either chlorpromazine or haloperidol potentiates CCWS antinociception (Bodnar and Nicotera, 1982). There is also cholinergic involvement in CCWS antinociception as both scopolamine and methylscopolamine selectively eliminated CCWS antinociception on the jump test

(Sperber, Kramer and Bodnar, 1986). A role for vasopressin in CCWS antinociception is supported by the observations that Brattleboro rats, genetically deficient in vasopressin, display significantly attenuated CCWS antinociception (Bodnar et al., 1980f). Further, lesions placed in the hypothalamic paraventricular nucleus significantly reduce both vasopressin antinociception and CCWS antinociception (Bodnar, Truesdell, Haldar, Aral, Kordower and Nilaver, 1986; Truesdell and Bodnar, 1987). Although CCWS antinociception is unaffected by serotonin depletion with parachlorophenylalanine (Bodnar, Kordower, Wallace and Tamir, 1981), the serotonin receptor antagonists, methysergide and pirenpirone significantly reduce this response (Kiefel, Paul and Bodnar, 1989). Pretreatment with the putative histamine$_2$ receptor antagonist, cimetidine, potentiated CCWS antinociception and hypothermia (Robertson et al., 1988). In contrast, neither agonists nor antagonists of GABA altered CCWS antinociception (Bodnar and Sperber, 1982).

C. The Intrinsic Organization of the PAG.

PAG Organization: The PAG, which surrounds the cerebral aqueduct throughout the midbrain, is a cytoarchitectonically complex region that consists of rather densely-packed, small cells difficult to subdivide anatomically (Gioia, Bianchi and Tredici, 1984). It is a heterogeneous region with functions including sensory integration, autonomic regulation and pain modulation based upon physiological stimulation studies (e.g., Mayer and Price, 1976). Recent anatomical tracing studies have compartmentalized the

PAG into functionally-distinct units (Beitz, 1985; Beitz, Shepard and Wells, 1983b; Conti, Barbaresi and Fabri, 1988; Ennis, Behbehani, Shipley, Van Bockstaele, and Aston-Jones, 1991; Van Bockstaele, Aston-Jones, Pieribone, Ennis and Shipley, 1991). The rostral ventromedial PAG dorsal to the oculomotor nucleus projects to the lateral retrofacial nucleus paragigantocellularis, and this projection is believed to subserve parasympathetic cardiac control and respiration. The caudal portion of the ventrolateral PAG elicits antinociception when electrically stimulated (Basbaum and Fields, 1984), and projects to the medial portion of the nucleus paragigantocellularis, an area that contributes to sympathetic blood pressure regulation. Lateral and ventrolateral juxtaqueductal regions of the PAG project heavily to the rostromedial pericoerulear area, especially Barrington's nucleus, and more sparsely to the locus coeruleus which is involved in antinociception (Bodnar et al., 1988, 1991; Proudfit, 1988). Chemical and electrical stimulation of Barrington's nucleus elicits increased urinary bladder pressure and evokes micturition (Sugaya, Matsuyama, Takakusaki, and Mori, 1987), and suggests that the PAG may be involved in control of micturition. Electrical stimulation of the dorsal PAG induces antinociception and strong aversive behavior (Fardin, Oliveras and Besson, 1984) or behavior characterizing a defense reaction (see review: Bandler, Carrive, and Zhang, 1992), and this area projects to the rostral juxtafacial nucleus paragigantocellularis and the NRM (Van Bockstaele et al., 1991).

The cytoarchitectural anatomical analysis of the PAG into lateral, dorso-lateral, dorsal, ventro-lateral and ventro-medial subdivisions (Beitz, 1985; Beitz and Shepard, 1985; Conti et al., 1988) appear important functionally especially in terms of defensive behavior (see review: Bandler et al., 1992). Whereas microinjections of excitatory amino acids into the anterior lateral PAG elicited backward defense in rats, the same injections into the posterior lateral PAG elicited forward avoidance behavior. Both of these sites also produced a rise in blood pressure and tachycardia. In contrast, glutamate injections into the posterior ventro-lateral PAG elicited immobility which was associated with a fall of blood pressure and bradycardia. PAG manipulations have differentially altered CCWS and other forms of stress-induced antinociception. Our laboratory (see review: Bodnar et al., 1980a) found that lesions placed in the anterior PAG rostral to the interpeduncular nucleus significantly reduced CCWS antinociception as measured by an operant liminal escape test, whereas lesions placed in the posterior PAG in the area of the dorsal raphe nucleus failed to affect CCWS antinociception on this measure. It should be noted however, that neither anterior nor posterior PAG lesions altered the magnitude of CCWS antinociception as measured by the tail-flick test. Despite its importance in the initiation of morphine antinociception (see reviews: Fields and Basbaum, 1978; Basbaum and Fields, 1984), lesions placed in the PAG have had variable effects upon other forms of stress-induced antinociception. Whereas antinociception induced by prolonged footshock was reduced by PAG

lesions, these lesions failed to affect antinociception induced by brief footshock or footshock delivered to the forepaws or hindpaws (Bragin, Vasilenko, and Durinjan, 1983; Watkins, Kinscheck, and Mayer, 1983). Interestingly, whereas classically-conditioned footshock antinociception was potentiated by anterior PAG lesions, it was attenuated by posterior PAG lesions (Kinscheck, Watkins, and Mayer, 1984). Further, the antinociception elicited by electrical stimulation of the anterior PAG is blocked by lesions placed in the posterior PAG (Rhodes and Liebeskind, 1978; Rhodes, 1979). Thus it appears that lesions placed in rostral and caudal PAG structures differentially alter various forms of antinociceptive responses, and therefore effects involving the PAG should be examined separately.

PAG Afferents and Efferents: The PAG has reciprocal connections with several regions of the brain. Anterograde tracing studies have revealed projections to numerous forebrain sites, the superior colliculus, the cuneiform nucleus, the NRM, the nucleus paragigantocellularis, the nucleus ambiguus, the nucleus of the solitary tract and the NRGC. In turn, brainstem inputs to the PAG include the nucleus cuneiformis, the pontine reticular formation, and the LC (Bandler and Tork, 1987; Beitz, 1982a; Mitchell, Dean and Redgrave, 1988; Van Bockstaele, Pieribone and Aston-Jones, 1989; Van Bockstaele et al., 1991). Taken together with the known direct spinal input to the PAG, the former two regions may provide a possible feedback relay regulating nociceptive input that activates PAG neurons in the dorsolateral, ventrolateral and

dorsomedial subregions (Beitz, Mullett and Weiner, 1983a; Shipley, McLean and Behbehani, 1987; Smith et al., 1988).

Opioid Peptides in the PAG: Antinociception appears to be effective following stimulation or opiate microinjection into the ventrolateral region of the PAG (Basbaum and Fields, 1984; Akil et al., 1984; Proudfit, 1988; Sharpe, Garnette and Cicero, 1974). The caudal and ventrolateral region of the PAG contains a dense innervation of enkephalin cells and terminals. This distribution shifts dorsally as one moves rostral in the midbrain (Beitz, 1982b). Dynorphin cells are concentrated just ventral to the aqueduct, along the rostro-caudal extent of the PAG (Burnett and Gebhart, 1991). Beta-endorphin-positive terminals are distributed within the ventral and ventro-lateral portions of the PAG, including the dorsal raphe nucleus (Khachaturian, Watson, Lewis, Coy, and Goldstein, 1982).

D. Distribution of TRH Cells, Fibers and Receptors:

TRH Perikarya and Fibers: Early radioimmunassay studies found that TRH was mostly present in the hypothalamus, with appreciable amounts in the septum, pre-optic area and brainstem, and negligible amounts in the cerebellum, and posterior cortex (Brownstein, Palkovits, Saavedra, Bassiri and Utiger, 1974; Kizer, Palkovits, and Brownstein, 1976; Oliver, Eskay, Ben-Jonathan and Porter, 1974; Winokur and Utiger, 1974). Immunocytochemical localization found that TRH cells were found in such hypothalamic areas as the preoptic, suprachiasmatic, periventricular, paraventricular, perifornical, dorsomedial, ventromedial and lateral hypothalamus.

TRH cells are also found in the nuclei raphe magnus and pallidus. TRH fibers were found in the median eminence, zona externa, and such hypothalamic areas as the the dorsomedial, perifornical, and paraventricular nucleus. TRH-positive fibers are also found in the OVLT, stria terminalis, lateral septal nucleus, oculomotor nucleus, trigeminal nucleus, periaqueductal gray, parabrachial nucleus, locus coeruleus, hypoglossal nucleus and superior olivary nucleus (Hokfelt, Fuxe, Johansson, Jeffcoate, and White, 1975a,b; Eskay, Long, and Palkovits, 1983; Johansson and Hokfelt, 1980; Johansson, Hokfelt, Pernow, Jeffcoate, White and Sternberger, 1980; Bowker, Westlund, Sullivan, Wilber and Coulter, 1982; Johansson, Hokfelt, Jeffcoate, White and Spindel, 1983; Kubek, Rea, Hodes and Aprison, 1983; Harkness and Brownfield, 1986). In colchicine-treated rats, the major cell groups containing TRH include the olfactory bulb, cortical and hippocampal areas, the caudate nucleus, hypothalamus, the periaqueductal gray, the pontine reticular formation, the medullary raphe nuclei, dorsal vagal complex, the area postrema and laminae II and III of the dorsal horn of the spinal cord (Tsuruo, Hokfelt and Visser, 1987). A 10-amino acid precursor for TRH has also been identified and found in hypothalamic and medullary raphe neurons that contain TRH (Jackson, Wu, and Lechan, 1985; Lechan, Wu, and Jackson, 1986a; Lechan, Wu, Jackson, Wolf, Cooperman, Mandel and Goodman, 1986b; Liao, Bulant, Nicolas, Vaudry and Pelletier, 1988).

Serotonin, substance P and TRH are each localized in the medullary nuclei raphe magnus, pallidus and obscuris with serotonin

about twice as prevalent as the others. These medullary perikarya can contain either serotonin and substance P, serotonin and TRH, or serotonin, TRH and substance P. Administration of the serotonin neurotoxins, 5,6- and 5,7-dihydroxytryptamine, eliminated immunoreactivity of serotonin, substance P and TRH in these medullary cells (Johansson et al., 1980). In contrast, lesions placed in the paraventricular nucleus of the hypothalamus reduce TRH immunoreactivity in the median eminence, but not in the spinal cord (Brownstein, Eskay and Palkovits, 1982; Lechan, Snapper, and Jackson, 1983). Using horseradish peroxidase retrograde tracing from the lumbo-sacral spinal cord, and orthograde immunocytochemistry in the medullary raphe, medullary raphe neurons containing TRH, serotonin, substance P and enkephalins project to the lumbo-sacral cord (Bowker et al., 1982). However, the descending raphe-spinal projection containing TRH and serotonin projects to the ventral horn (Johansson, Hokfelt, Pernow, Jeffcoate, White, Steinbusch, Verhofstead, Emson, and Spindel, 1981; Gilbert, Emson, Hunt, Bennett, Marsden, Sandberg, Steinbusch, and Verhofstad, 1982); TRH fibers in the dorsal horn appear to arise from a different population of TRH neurons (Harkness and Brownfield, 1986).

TRH Receptors: TRH-like receptors have been localized in the central nervous system using autoradiographic techniques (Manaker, Winokur, Rostene and Rainbow, 1985; Mantyh and Hunt, 1985; Pazos, Cortes and Palacios, 1985; Rostene, Morgat, Dussaillant, Rainbow, Sarrieau, Vial and Rosselin, 1984). Initial studies indicated that

TRH-like receptors are found to be predominantly in rhinencephalic structures, including the olfactory bulb, amygdala and hippocampus. Moderate amounts of TRH receptors were found throughout the rhombencephalon, including the mesencephalic central gray, medullary raphe nuclei and the nucleus reticularis gigantocellularis, as well as in the substantia gelatinosa of the dorsal horn of the spinal cord (Manaker et al., 1985; Manaker, Rainbow and Winokur, 1986; Sharif and Burt, 1986). It should be noted that it has been difficult to raise either specific antibodies against TRH which are not cross-reactive with other peptides, or selective agonists or antagonists to TRH. This has limited the ability to effectively map the brain for TRH receptors.

E. TRH and Nociception:

TRH and Baseline Nociception: TRH produces a short-lasting (5-30 min) supraspinal antinociception when administered alone following intracerebroventricular administration (Boschi, Desiles, Reny, Rips and Wrigglesworth, 1983; Reny-Palasse, Monier and Rips, 1989; Rips, Reny and Desiles, 1983). Further, intracerebral administration of TRH also produces an antinociceptive response following microinjections into the PAG, the medullary NRM and NRGC, and the amygdala (Griffiths, Slater and Webster, 1981; Webster, Giffiths and Slater, 1983; Reny-Palasse et al., 1989). These effects are consistent with the presence of either TRH-immunoreactive cells and fibers in these structures, and/or the presence of known TRH receptors. In contrast, intrathecal administration of TRH failed to alter baseline nociceptive

thresholds, despite the presence of TRH-immunoreactive cells and fibers in the dorsal horn of the spinal cord (Watkins, Suberg, Thurston and Culhane, 1986). Both TRH and its metabolites, histidyl proline diketpiperazine and pyroglutamyl-histidyl-proline produce antinociception in mice as well (Kawamura, Sakurada, Sakurada, Kisara, Sasaki and Suzuki, 1985; Reny-Palasse and Rips, 1987). TRH antinociception appears to be modulated by the endogenous opioid system given that neither morphine-tolerant nor ethylketocyclazocine-tolerant mice display this antinociceptive effect (Reny-Palasse, Poncet and Rips, 1987). Further, TRH antinociception is blocked by either peripheral (Rips et al., 1983) or central (Reny-Palasse et al., 1989) naloxone administration.

TRH and Opioid Antinociception: TRH fails to alter beta-endorphin antinociception following either peripheral (Holaday et al., 1978) or intracisternal (Osbahr et al., 1982) administration. Further, TRH fails to affect morphine analgesia following either peripheral (Holaday and Faden, 1983) or intracerebroventricular (Kasson and George, 1983) administration. In contrast, the effects of intrathecal administration of TRH upon intrathecal morphine antinociception varied as a function of the TRH dose. Whereas low doses of intrathecal TRH potently attenuated intrathecal morphine antinociception, intermediate doses of intrathecal TRH had lesser inhibitory effects. Yet, high doses of intrathecal TRH actually potentiated intrathecal morphine antinociception (Watkins et al., 1986).

Despite its relative failure to alter morphine

antinociception, TRH reduces morphine tolerance, morphine dependence as measured by withdrawal signs, and reduces morphine hyperthermia (Bhargava, 1980, 1981; Holaday et al., 1978; Horita, Carino and Chestnut, 1976; Martin, Dewey, Chau-Pham and Prange, 1977). Despite the action of TRH as a physiological opioid antagonist in tolerance, withdrawal and thermoregulatory studies, it fails to alter ^{3}H-dihydromorphine or ^{3}H-naloxone binding in opioid receptor assays (Holaday et al., 1978; Martin et al., 1977; Tache, Lis and Collu, 1977). However, a TRH analogue has been found to decrease brain naloxone binding (Mori, Michimata, Ishihara, Yamada, Iriuchijima and Kobayashi, 1989), but it is not clear whether such actions work through TRH mechanisms. The effects of opioid compounds upon TRH binding have been examined using selective agonists or antagonists at the multiple opioid receptors (mu, kappa, delta: Martin, Eades, Thompson, Huppler and Gilbert, 1976; Lord, Waterfield, Hughes and Kosterlitz, 1977). Both delta-selective (ICI 154,129) and kappa-selective (ethylketocyclazocine) ligands interfered with brain TRH binding (Bhargava and Das, 1986). In contrast, mu-selective (morphine and naloxone) selective ligands failed to affect brain TRH binding.

TRH and Nonopioid Antinociception: Intracisternal TRH and its analogues inhibit neurotensin antinociception on the hot-plate, tail immersion and acetic acid writhing tests (Hernandez, Nemeroff, Valderama and Prange, 1984; Osbahr et al., 1982). Neurotensin antinociception is not antagonized by naloxone pretreatment (Clineschmidt, McGuffin and Bunting, 1979; Clineschmidt, Martin,

and Veber, 1982; Osbahr et al., 1982), but it is cross-tolerant with morphine antinociception (Luttinger, Burgess, Nemeroff and Prange, 1983), suggesting some interaction between opioids and neurotensin at some point beyond the opiate receptor. Butler and Bodnar (1984, 1987) investigated whether TRH participated in the modulation of nonopioid forms of stress-induced antinociception. Intracerebroventricular administration of TRH potentiated footshock antinociception on the tail-flick test induced by exposure to either 20 or 80 shocks. TRH also potentiated footshock antinociception on the writhing test induced by exposure to 80 shocks. Despite an earlier report (Grau et al., 1981), naloxone failed to alter footshock antinociception induced by 20, 40, 60 or 80 shocks. The potentiations in footshock antinociception by TRH were not due to long-duration increases in baseline nociceptive thresholds. Intraventricular administration of TRH also potentiated a nonopioid form of shock-induced antinociception elicited by shocks delivered to the forepaws (Butler and Bodnar, 1984). TRH effects upon nonopioid CCWS antinociception were then assessed, and intracerebroventricular administration of TRH significantly potentiated CCWS antinociception at bath temperatures of 2°, 8°, 15° and 21°C. This effect was not due to TRH-induced changes in the magnitude of CCWS hypothermia. This effect appeared to be centrally-mediated since intravenous administration of TRH failed to alter CCWS antinociception (Butler and Bodnar, 1987). Since CCWS antinociception was mediated in part by the cholinergic system (Sperber et al., 1986) and since TRH and acetylcholine displayed

functional interactions (see review: Yarbrough, 1983), intracerebroventricular TRH effects upon pilocarpine antinociception were evaluated. TRH potentiated pilocarpine antinociception on the tail-flick test (Butler and Bodnar, 1987).

F. Rationale

As indicated in the previous section, it appears that TRH is a potent modulator of nonopioid forms of antinociception, but produces less consistent effects upon opioid-mediated antinociception. All of the previous studies have examined TRH effects in ventricular, cisternal or intrathecal locations, and thus cannot specify the locus of action. Therefore, the present study evaluated the effects of intracerebral administration of TRH in the PAG. The PAG was chosen because: a) TRH-reactive fibers (Hokfelt et al., 1975a,b; Johansson et al., 1983; Tsuruo et al., 1987) and receptors (Manaker et al., 1985, 1986; Pazos et al., 1985) are localized in this area, b) it supports TRH antinociception (Griffiths et al., 1981; Webster et al., 1983; Reny-Palasse et al., 1989), and c) it is a primary locus for supraspinal opiate analgesia (see reviews: Fields and Basbaum, 1978; Basbaum and Fields, 1984). Finally, the effects of TRH in anterior and posterior PAG structures were examined separately because of differential effects observed in lesion studies evaluating changes in stress-induced antinociceptive responses (Bragin, Vasilenko, and Durinjan, 1983; Kinscheck, Watkins, and Mayer, 1984; Rhodes and Liebeskind, 1978; Rhodes, 1979; Watkins, Kinscheck, and Mayer, 1983).

The present study examined intracerebral TRH effects upon CCWS and morphine antinociception because of their clear dissociations and representations as nonopioid and opioid forms of antinociception. CCWS and morphine antinociception dissociate in studies investigating cross-tolerance (Bodnar et al., 1978d; Girardot and Holloway, 1984b), naloxone and naltrexone sensitivity (Bodnar et al., 1978b; Bodnar and Sikorszky, 1983; Girardot and Holloway, 1984a; Yoburn et al., 1987), endopeptidase inhibition (Alleva et al., 1980; Bodnar et al., 1980b), mu_1 antagonism (Kirchgessner et al., 1982) and collateral inhibition (Steinman et al., 1990). The present study evaluated only the effects of TRH pretreatment upon CCWS and morphine antinociception because a) this corresponded to the protocols used for previous antinociceptive responses (Butler and Bodnar, 1984, 1987; Holaday et al., 1978), and b) it avoided the administration of the peptide after CCWS in which pharmacokinetic effects such as hypothermia, altered drug absorption, and vasoconstriction might alter the distribution of TRH to pertinent sites.

The present study assessed intracerebral TRH effects upon opioid and nonopioid antinociception by using two nociceptive measures, the tail-flick (D'Amour and Smith, 1941) and jump (Evans, 1961) tests. Whereas the tail-flick test is a spinally-mediated reflex (Grossman, Basbaum and Fields, 1982) present in spinalized animals (Hayes, Bennett, Newlon and Mayer, 1978), the jump test is dependent upon supraspinal and suprasegmental mechanisms for its integrity. Given the role of TRH in modulating core body

temperature (e.g., Horita et al., 1976; Holaday et al., 1978; Bhargava, 1981; Hernandez et al., 1984; Butler and Bodnar, 1987), this measure was also used to assess TRH effects in the PAG under baseline conditions and following CCWS and morphine. This study was carried out in female rats since previous research observing positive TRH effects upon stress-induced antinociception used female rats (Butler and Bodnar, 1984, 1987). It should also be noted that neither CCWS nor central morphine antinociception in female rats is affected by estrous phase (Romero and Bodnar, 1986; Romero, Kepler, Cooper, Komisaruk and Bodnar, 1987; Romero, Cooper, Komisaruk and Bodnar, 1988; Kepler, Kest, Kiefel, Cooper and Bodnar, 1989).

MATERIALS AND METHODS

Subjects and Surgery: Adult female albino Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA, 80-120 days of age) were housed individually and maintained on a 12 h light: 12 h dark cycle with food and water available ad libitum. Following anesthesia with chlorpromazine (3 mg/kg, i.p.) and Ketamine HCl (100 mg/kg, i.m.), one stainless steel guide cannula (26 gauge, Plastic Products Co., Roanoke, VA) was placed stereotactically (Kopf Instruments) into the PAG at the following coordinates: incisor bar: -5 mm, 0.0-0.8 mm anterior to the lambda suture, 1.5-2.0 mm lateral to the sagittal suture, 6.5-7.0 mm from the top of the skull, and angled towards the sagittal suture at 12° (Bodnar et al., 1988, 1991). All animals were allowed one week to recover and clear anesthetic.

Nociceptive Tests: All animals were tested on the tail-flick and jump tests in that order to minimize carry-over effects between tests. On the tail-flick test, the beam (IITC Company) was mounted 8 cm above the dorsum and 3-9 cm proximal to the tip of the rat's tail with thermal intensity set to produce baseline tail-flick latencies between 2 and 3.5 s. Each session consisted of 3 latency determinations separated by 10-s intertrial intervals. To avoid tissue damage, a trial was automatically terminated if a response did not occur within 15 s. On the jump test, electric shock was delivered to the feet of the rat by a shock generator (BRS/LVE) and shock scrambler (Campden Instruments). The jump threshold was defined in mA as the lowest of two consecutive ascending

intensities in which the animal simultaneously removed both hindpaws from the grids. Each of six trials began with the animal receiving a 300-ms footshock at a current intensity of 0.10 mA with subsequent shocks increased in 0.05 mA steps at 10-s intervals until the jump threshold was determined. Baseline tail-flick latencies and jump thresholds were determined for at least 4 days before experimental testing began.

Protocol 1 - PAG TRH and Baseline Nociception: Twenty-eight cannulated rats received two microinjection conditions: a) vehicle and b) TRH (10 ug). TRH (Peninsula Laboratories, Belmont, CA) dissolved in normal saline was infused in a 0.5 ul volume over 1 min through a stainless steel internal cannula (33 gauge, Plastic Products, Roanoke, VA) using a Hamilton microsyringe and polyethylene tubing. Tail-flick latencies and jump thresholds were assessed 5, 15, 25, 50, 80, 110 and 140 min following microinjection. This time course was chosen for two reasons. The shorter intervals (5-25 min) were chosen because of previous reports of TRH-induced antinociception at these intervals (Griffiths et al., 1981; Boschi et al., 1983; Rips et al., 1983; Webster et al., 1983; Reny-Palasse et al., 1989). The longer intervals (50-140 min) were chosen because they matched the intervals between TRH administration and subsequent testing in the CCWS and morphine protocols.

Protocol 2 - PAG TRH and CCWS Antinociception: Twenty-three cannulated rats were exposed to three experimental conditions: a) no swim, b) CCWS, and c) TRH (10 ug, ic) administered 20 min prior

to CCWS. CCWS consisted of a 3.5 min swim in a 2°C bath that was deep enough to prevent the rat from standing. Following CCWS, each rat was returned to its home cage until testing commenced. One week elapsed between experimental conditions to minimize any possibility of adaptation effects (Bodnar et al., 1978b; Spiaggia et al., 1979; Butler and Bodnar, 1987). Tail-flick latencies, jump thresholds and core body temperatures were assessed in that order at 30, 60 and 90 min following the no swim or CCWS conditions. Core body temperature was ascertained by inserting a rectal probe of a digital thermometer (Sensortek, NJ) until a stable reading was achieved. To assess the dose-dependent effects of TRH, an additional nine rats were exposed to five experimental conditions: a) no swim, b) CCWS, and TRH administered 20 min prior to CCWS at doses of c) 0.1 ug, d) 1 ug and e) 10 ug. Latencies, thresholds and body temperature were assessed as described previously.

Protocol 3 - PAG TRH and PAG Morphine Antinociception: Cannulated rats received three microinjection conditions: a) vehicle (n=53), b) morphine at doses of 0.1 ug (n=20), 1 ug (n=17) or 2.5 ug (n=16), and c) TRH (10 ug) administered 20 min prior to morphine at doses of 0.1 ug (n=20), 1 ug (n=17) or 2.5 ug (n=16). Both TRH and morphine sulfate (Pennick Laboratories, West Lyndhurst, NJ) dissolved in normal saline, was infused in a 0.5 ul volume over 1 min through a stainless steel internal cannula (33 gauge, Plastic Products, Roanoke, VA) using a Hamilton microsyringe and polyethylene tubing. One week elapsed between microinjection conditions to minimize tolerance effects (Yaksh et al., 1976;

Bodnar et al., 1988, 1991). Tail-flick latencies and jump thresholds were assessed 30, 60, 90 and 120 min following the vehicle or morphine microinjections. To explore dose-dependent effects of TRH upon morphine antinociception, additional cannulated rats received three of the following microinjection conditions: a) vehicle (n=54), b) morphine at doses of 0.1 ug (n=19), 1 ug (n=22) or 2.5 ug (n=13), c) TRH (1 ug) administered 20 min prior to morphine at doses of 0.1 ug (n=19), 1 ug (n=11) or 2.5 ug (n=8), and d) TRH (0.1 ug) administered 20 min prior to morphine at doses of 1 ug (n=11) and 2.5 ug (n=5). One week elapsed between microinjection conditions, and tail-flick latencies and jump thresholds were assessed 30, 60, 90 and 120 min following the vehicle or morphine microinjections.

Histology: After testing, anesthetized (Euthanasia, H. Schein) rats received a transcardiac perfusion with 0.9% normal saline followed by 10% buffered formalin. Coronal (40-um) sections, stained with Cresyl violet were examined using light microscopy by an observer unfamiliar with the behavioral data to confirm the locus of each cannula placement within the PAG using the stereotaxic atlas of Paxinos and Watson (1986).

Statistical Analyses: Analyses of variance were performed for each dependent measure in each protocol with Dunnett comparisons used to ascertain significant differences relative to the control condition. Dunn comparisons were used to ascertain significant effects of TRH relative to the experimental condition. The potency of effects of TRH upon morphine antinociception were evaluated by

constructing log dose-response functions and performing linear regression analyses for peak (30 min) and total (differences in latencies or thresholds following drug relative to vehicle across the time course) antinociception for each nociceptive measure. The ED_{50} was calculated as a 50% increase in the maximal percentage effect (MPE = Experimental Score - Control Score / Cut-Off Score - Control Score).

RESULTS

Histological Placements: Figures 1, 2 and 3 illustrate the mesencephalic cannula placements which are divided into two different groups according to rostral-caudal dimensions. The dorsal raphe nucleus served as the defining characteristic (Figure 2, Panel D) between anterior PAG placements which were found rostral to this area, and posterior PAG placements which were at the level of this nucleus. Panels A-C of Figure 1 display anterior PAG placements which were found to be within the periaqueductal gray area and immediately adjacent tegmentum at the level of or rostral to the III cranial nerve complex. Anterior PAG placements were also found in the nucleus linearis. Panels D-F of Figure 2 and Panels G-I of Figure 3 display posterior PAG placements which were found to be within the periaqueductal gray area and immediately adjacent tegmentum at the level of the dorsal raphe nucleus. Posterior PAG placements were also found in the dorsal raphe nucleus, the nucleus raphe medianus and the central tegmental tract. The remainder of the Results section is organized to separately describe the effects of TRH at A) anterior PAG placements and B) posterior PAG placements upon: i) baseline nociceptive thresholds, ii) CCWS antinociception and hypothermia, and iii) morphine antinociception and hyperthermia.

Figure 1. Histological representations of anterior mesencephalic cannulae placements for animals tested with TRH paired with either continuous cold-water swims (squares) or morphine (circles). The representations are adapted from the rat brain atlas of Paxinos and Watson (1986). Anterior PAG placements were found both within and immediately adjacent to the periaqueductal gray area at the level of or rostal to the III cranial nerve complex and nucleus linearis. These placements are depicted in Panels A, B and C which are representations of Plates 39, 43 and 45 of Paxinos and Watson. Please note that an individual symbol may represent placements in more than one animal.

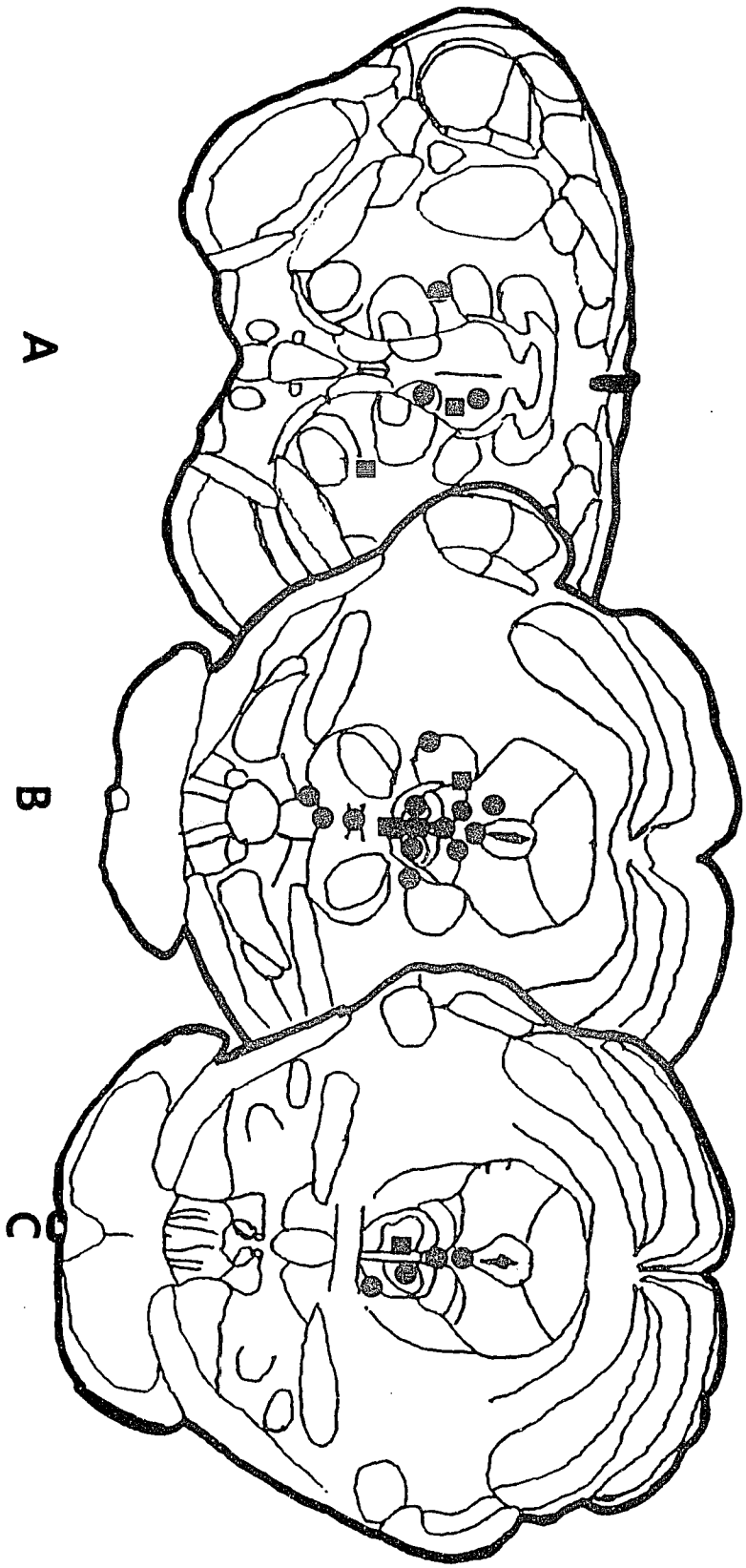

A
B
C

Figure 2. Histological representations of posterior mesencephalic cannulae placements for animals tested with TRH paired with either continuous cold-water swims (squares) or morphine (circles). The representations are adapted from the rat brain atlas of Paxinos and Watson (1986). Posterior PAG placements were found both within and immediately adjacent to the periaqueductal gray area at the level of the dorsal raphe nucleus, the nucleus medianus and the central tegmental tract. These placements are depicted in Panels D, E and F which are representations of Plates 46, 47 and 48 of Paxinos and Watson. Please note that an individual symbol may represent placements in more than one animal.

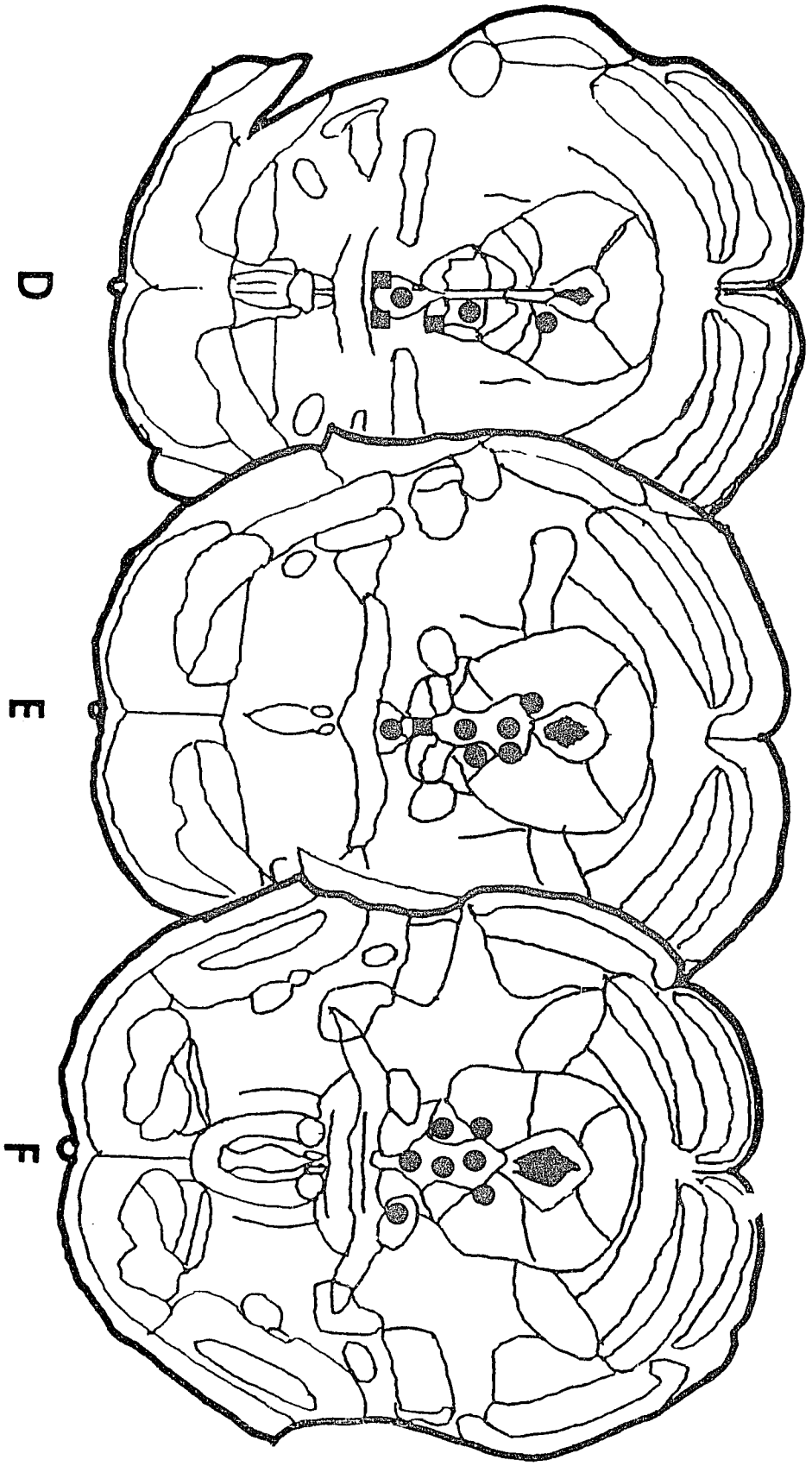
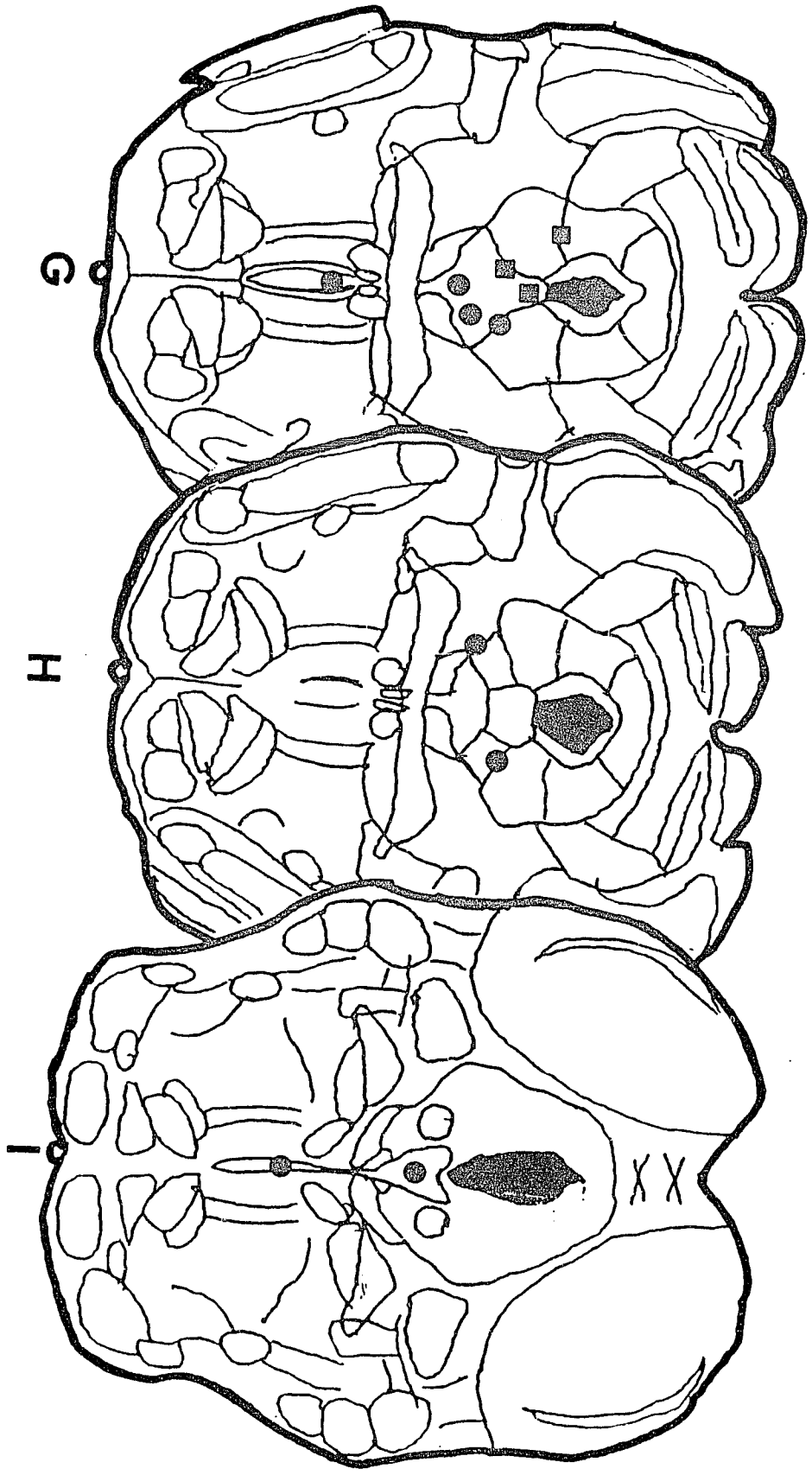

D
E
F

Figure 3. Histological representations of posterior mesencephalic cannulae placements for animals tested with TRH paired with either continuous cold-water swims (squares) or morphine (circles). The representations are adapted from the rat brain atlas of Paxinos and Watson (1986). Posterior PAG placements were found both within and immediately adjacent to the periaqueductal gray area at the level of the dorsal raphe nucleus, the nucleus medianus and the central tegmental tract. These placements are depicted in Panels G, H and I which are representations of Plates 49, 50 and 51 of Paxinos and Watson. Please note that an individual symbol may represent placements in more than one animal.

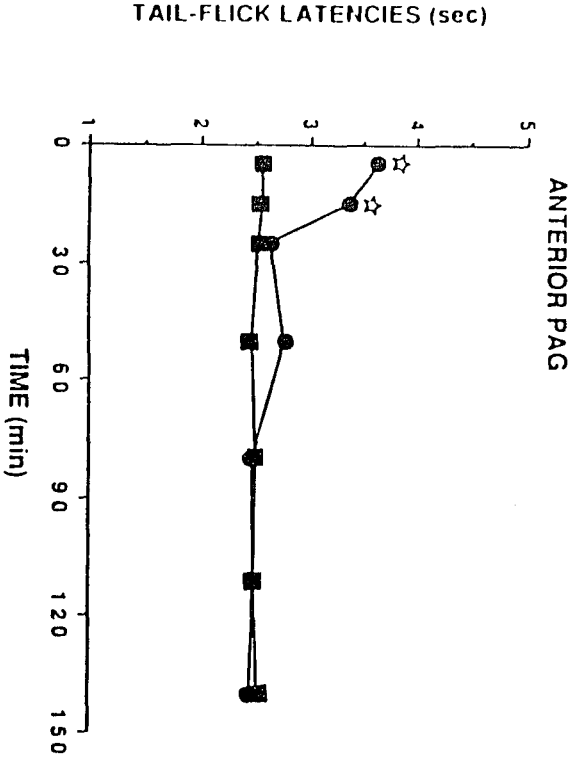

G
H
I
X X

A. TRH and Anterior PAG Placements:

i. Baseline Nociceptive Thresholds: Figure 4 illustrates the significant increases in tail-flick latencies (41%) and jump thresholds (10%) following TRH in rats with anterior PAG placements. These effects persisted for up to 15 min after TRH treatment, but failed to alter either measure thereafter. In contrast, Table 1 indicates that rats with anterior PAG placements failed to display significant alterations in core temperatures following TRH.

ii. CCWS Antinociception and Hypothermia: Figure 5 illustrates changes in CCWS antinociception on the tail-flick (upper panel) and jump (middle panel) tests as well as CCWS hypothermia (lower panel) following TRH in the anterior PAG. CCWS significantly increased latencies (132%) for up to 90 min and thresholds (46%) for up to 60 min in rats with anterior PAG placements. TRH significantly decreased the magnitude of CCWS antinociception for up to 60 min on the tail-flick test by 37% and on the jump test by 63%. The significant decreases (30-60 min) in core body temperature (13%) following CCWS were significantly reduced (47%) by TRH. All rats tested for dose-dependent effects of TRH upon CCWS antinociception had placements in the anterior PAG. CCWS significantly increased latencies (99%) for up to 90 min and thresholds (39%) for up to 60 min in rats with anterior PAG placements. Table 2 indicates that the magnitude of CCWS antinociception was significantly decreased across the dose range of TRH on the tail-flick test (30-90 min; 0.1 ug (46%), 1 ug (51%),

Figure 4. Alterations in tail-flick latencies (left panels) and jump thresholds (right panels) following pretreatment with vehicle (closed squares) or thyrotropin releasing hormone (TRH: 10 ug, closed circles) administered into the anterior periaqueductal gray. Significant differences in latencies and thresholds were observed between conditions (tail-flick: $F(1,16)= 3.88$, $p<.066$; jump: $F= 33.26$, $p<.001$), across the time course (tail-flick: $F(6,96)= 10.21$, $p<.001$; jump: $F= 8.11$, $p<.001$) and for the interaction between conditions and times (tail-flick: $F(6,96)= 7.18$, $p<.001$; jump: $F= 3.31$, $p<.005$). The open stars (Dunnett comparisons, $p<.05$) indicate significant increases in latencies or thresholds following TRH relative to vehicle treatment.


TAIL-FLICK LATENCIES (sec)
1
2
3
4
5
0
30
60
90
120
150
TIME (min)
ANTERIOR PAG

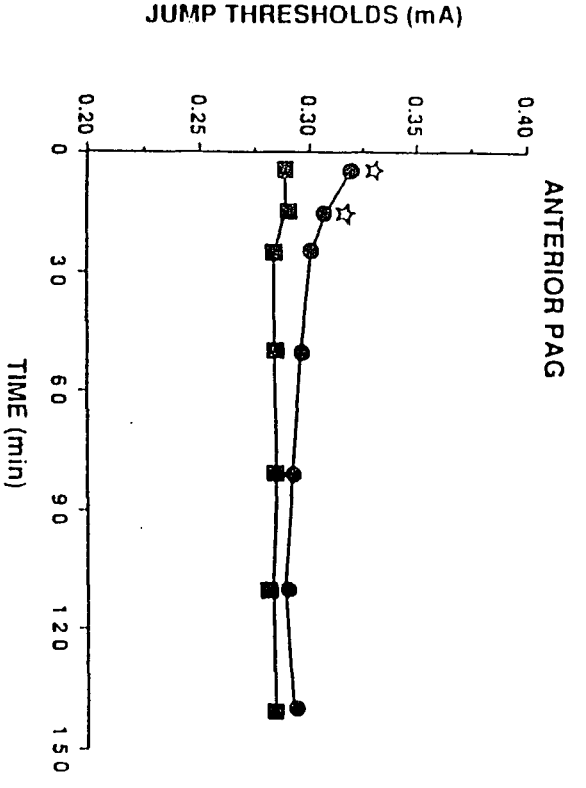
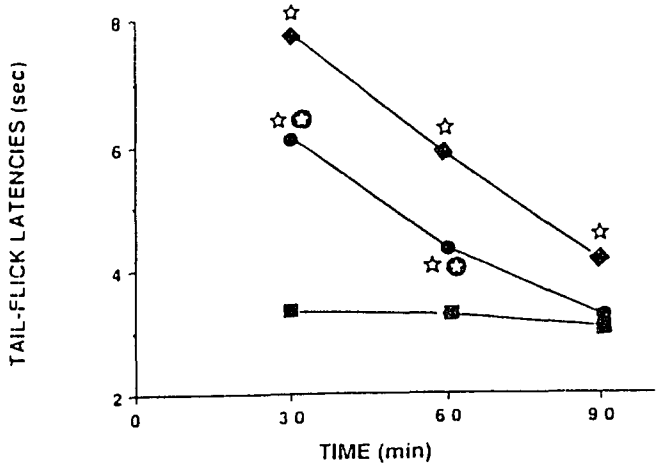

JUMP THRESHOLDS (mA)
0.20
0.25
0.30
0.35
0.40
0
30
60
90
120
150
TIME (min)
ANTERIOR PAG

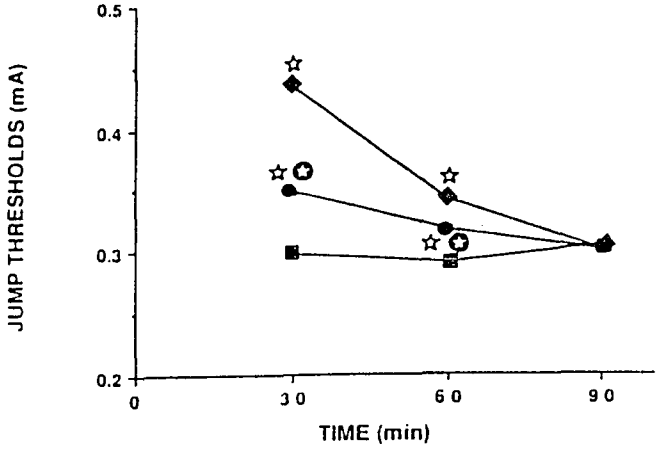
TABLE 1. Effects of thyrotropin-releasing hormone 10 ug administered into the anterior periaqueductal gray upon baseline core body temperature (°C).

POST-INJECTION (min)	CONDITION	
	Vehicle	TRH 10 ug
5	37.9	37.9
15	38.3	38.5
25	38.4	38.8
50	38.3	38.6
80	38.2	38.5
110	38.2	38.5
140	38.2	38.5

Note: Core body temperatures failed to change either between conditions ($F(1,7)= 2.64$) or for the interaction between conditions and times ($F(6,42)= 1.76$), but showed significant effects across the post-injection time course ($F(6,42)= 7.00$, $p<.001$).

Figure 5. Alterations in tail-flick latencies (upper panel), jump thresholds (middle panel) and core body temperature (lower panel) following a no-swim control condition (closed squares), and continuous cold-water swims (CCWS: 2°C for 3.5 min) paired with either vehicle (closed diamonds) or TRH (10 ug, closed circles) administered into the anterior periaqueductal gray. Significant differences in antinociception and hypothermia were observed among conditions (tail-flick: $F(2,24)= 14.47$, $p<.0001$; jump: $F= 11.73$, $p<.0003$; temperature: $F= 32.77$, $p<.0001$), across the time course (tail-flick: $F(2,24)= 19.54$, $p<.0001$; jump: $F= 24.49$, $p<.0001$; temperature: $F= 154.45$, $p<.0001$) and for the interaction between conditions and times (tail-flick: $F(4,48)= 8.64$, $p<.0001$; jump: $F= 22.50$, $p<.0001$; temperature: $F= 65.51$, $p<.0001$). The open stars (Dunnett comparisons, $p<.05$) indicate significant alterations in latencies, thresholds or temperatures relative to the no-swim condition. The enclosed stars (Dunn comparisons, $p<.05$) indicate the significant alterations induced by TRH in latencies, thresholds or temperatures following CCWS relative to vehicle treatment.


TAIL-FLICK LATENCIES (sec)
8
6
4
2
0
30
60
90
TIME (min)


JUMP THRESHOLDS (mA)
0.5
0.4
0.3
0.2
0
30
60
90
TIME (min)

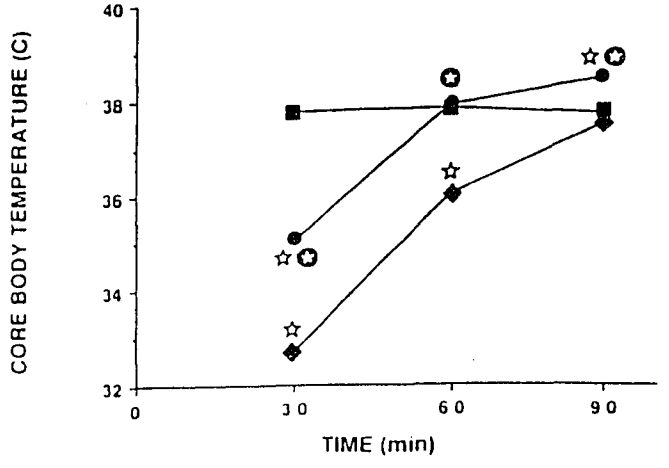

CORE BODY TEMPERATURE (C)
40
38
36
34
32
0
30
60
90
TIME (min)

TABLE 2. Dose-dependent effects of thyrotropin-releasing hormone administered into the anterior periaqueductal gray upon continuous cold-water swim (CCWS) analgesia and hypothermia.

CONDITION	POST-SWIM (min)		
	30	60	90
A. Tail-Flick Latencies (sec):			
No-Swim Control	3.43	3.37	3.30
TRH 0/CCWS	6.81*	5.33*	4.30*
TRH 0.1 ug/CCWS	5.25*+	4.00*+	3.18*+
TRH 1.0 ug/CCWS	5.07*+	4.04+	3.48+
TRH 10 ug/CCWS	4.90*+	3.82+	3.52+
B. Jump Thresholds (mA):			
No-Swim Control	.300	.291	.306
TRH 0/CCWS	.417*	.342*	.299
TRH 0.1 ug/CCWS	.332*+	.299+	.293
TRH 1.0 ug/CCWS	.323+	.307+	.301
TRH 10 ug/CCWS	.319+	.292+	.290
C. Core Body Temperatures (°C):			
No-Swim Control	37.8	37.7	37.7
TRH 0/CCWS	33.0*	35.8*	37.2
TRH 0.1 ug/CCWS	35.2*+	37.9+	38.4*+
TRH 1.0 ug/CCWS	35.7*+	37.6+	37.9+
TRH 10 ug/CCWS	36.2*+	37.7+	38.1+

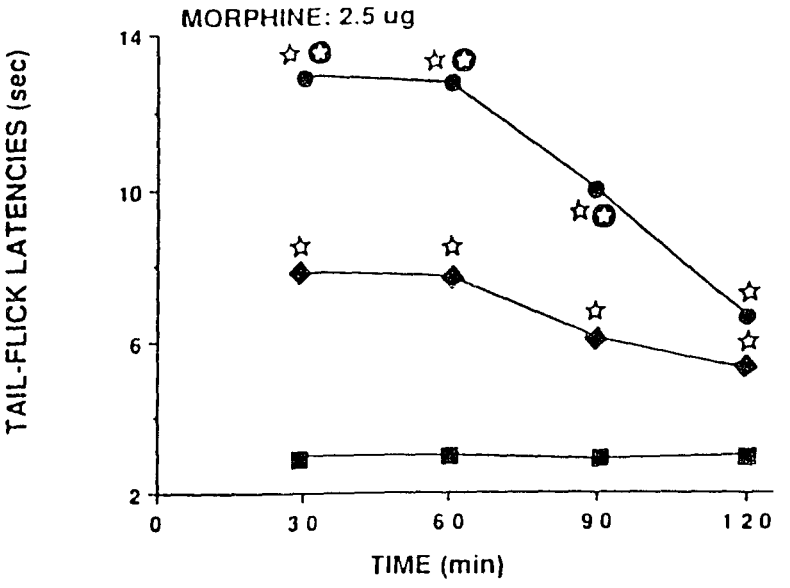
Note: Significant differences in CCWS antinociception and CCWS hypothermia were observed among conditions (tail-flick: $F(4,32)= 7.01$, $p<.0004$; jump: $F= 7.53$, $p<.0002$; temperature: $F= 16.57$, $p<.0001$), across the time course (tail-flick: $F(2,16)= 100.35$, $p<.0001$; jump: $F= 22.75$, $p<.0001$; temperature: $F= 66.61$, $p<.0001$) and for the interaction between conditions and times (tail-flick: $F(8,64)= 6.13$, $p<.0001$; jump: $F= 10.28$, $p<.0001$; temperature: $F= 23.87$, $p<.0001$). The asterisks denote a significant alteration relative to the no-swim control condition (Dunnett comparisons, $p<.05$). The crosses denote a significant alteration in the CCWS effect by TRH (Dunn comparison, $p<.05$).

10 ug (57%)) and jump test (30-60 min; 0.1 ug (73%), 1 ug (80%), 10 ug (83%)). The significant decreases (30-60 min) in core body temperature (13%) following CCWS were significantly reduced by the TRH doses of 0.1 (46%), 1 (56%) and 10 (65%) ug (Table 2).

iii. Morphine Antinociception and Hyperthermia: The effects of TRH at a dose of 10 ug upon different morphine doses were assessed first. Morphine microinjected into the anterior PAG significantly and dose-dependently increased latencies following the 1 (30 min) and 2.5 (30-120 min) ug doses, and increased thresholds following the 0.1 (30-60 min), 1 (30-60 min) and 2.5 (30-120 min) ug doses. Figure 6 illustrates the significant increases in the magnitude of morphine (2.5 ug) antinociception induced by TRH (10 ug) on the tail-flick test for up to 90 min (Figure 6, upper panel), and on the jump test for up to 120 min (Figure 6, lower panel). An identical pattern of facilitatory effects by TRH was observed on the tail-flick (Figure 7, upper panel) and jump (Figure 7, lower panel) tests following the 1 ug morphine dose, and on the tail-flick (Figure 8, upper panel) and jump (Figure 8, lower panel) tests following the 0.1 ug morphine dose.

Regression analyses revealed significant differences in the slopes and intercepts of the peak dose-response curves of morphine antinociception for anterior PAG placements on the tail-flick ($F(2,58)= 7.74$, $p<.001$) and jump ($F= 11.67$; $p<.0001$) tests. The ED_{50} on the tail-flick test for morphine alone (1.85 ug) was shifted 6-fold to the left in anterior PAG placements when a 10 ug

Figure 6. Alterations in tail-flick latencies (upper panel) and jump thresholds (lower panel) following a no-injection control condition (closed squares), and morphine 2.5 ug paired with either vehicle (closed diamonds) or TRH (10 ug, closed circles) administered into the anterior periaqueductal gray. Significant differences were observed among conditions (tail-flick: $F(2,16)= 18.64$, $p<.0001$; jump: $F= 16.03$, $p<.0002$), across the time course (tail-flick: $F(3,24)= 9.41$, $p<.0003$; jump: $F= 31.84$, $p<.0001$), and for the interaction between conditions and times (tail-flick: $F(6,48)= 7.44$, $p<.0001$; jump: $F= 7.62$, $p<.0001$). The open stars (Dunnett comparisons, $p<.05$) indicate significant alterations in latencies and thresholds relative to the no-injection condition. The enclosed stars (Dunn comparisons, $p<.05$) indicate the significant alterations induced by TRH in latencies and thresholds following morphine relative to vehicle treatment.


MORPHINE: 2.5 ug
TAIL-FLICK LATENCIES (sec)
14
10
6
2
0
30
60
90
120
TIME (min)

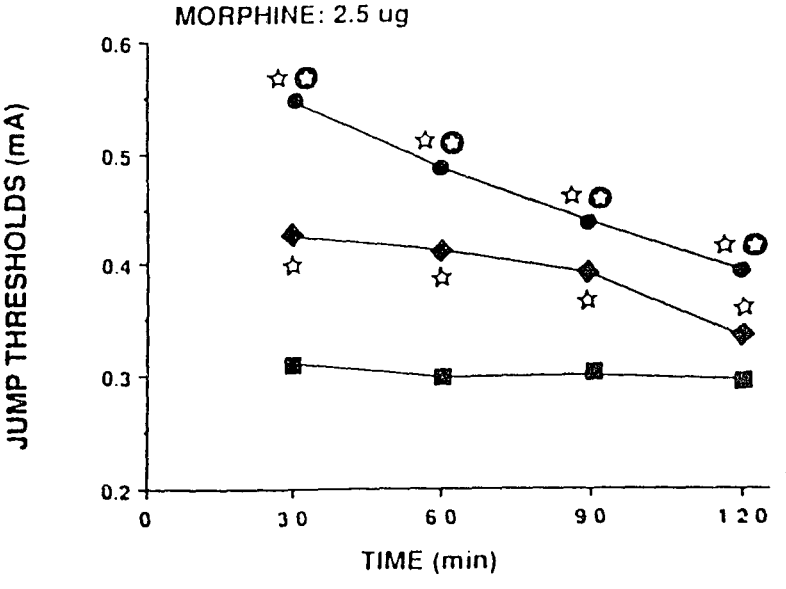
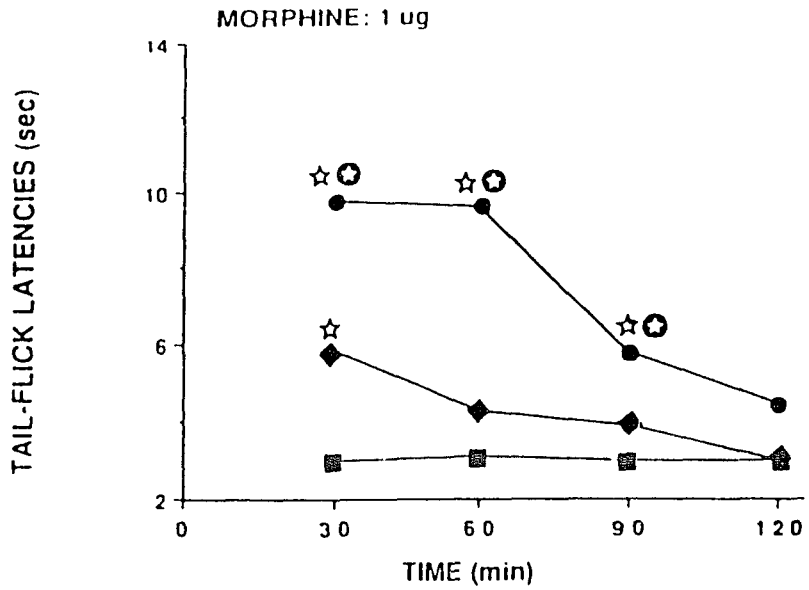

MORPHINE: 2.5 ug
JUMP THRESHOLDS (mA)
0.6
0.5
0.4
0.3
0.2
0
30
60
90
120
TIME (min)

Figure 7. Alterations in tail-flick latencies (upper panel) and jump thresholds (lower panel) following a no-injection control condition (closed squares), and morphine 1 ug paired with either vehicle (closed diamonds) or TRH (10 ug, closed circles) administered into the anterior periaqueductal gray. Significant differences were observed among conditions (tail-flick: $F(2,16)= 13.13$, $p<.0004$; jump: $F= 22.09$, $p<.0001$), across the time course (tail-flick: $F(3,24)= 9.27$, $p<.0003$; jump: $F= 18.18$, $p<.0001$), and for the interaction between conditions and times (tail-flick: $F(6,48)= 5.89$, $p<.0001$; jump: $F= 6.53$, $p<.0001$). The open stars (Dunnett comparisons, $p<.05$) indicate significant alterations in latencies and thresholds relative to the no-injection condition. The enclosed stars (Dunn comparisons, $p<.05$) indicate the significant alterations induced by TRH in latencies and thresholds following morphine relative to vehicle treatment.


MORPHINE: 1 ug
TAIL-FLICK LATENCIES (sec)
14
10
6
2
0
30
60
90
120
TIME (min)

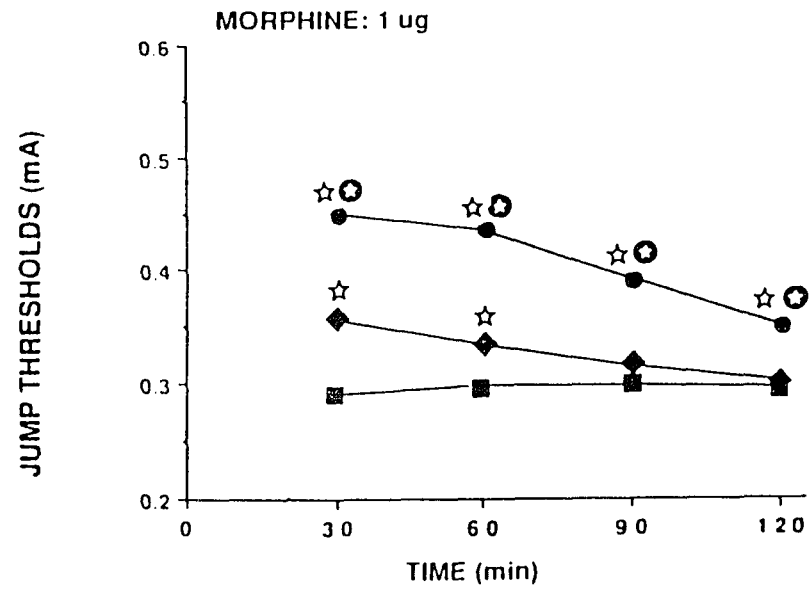
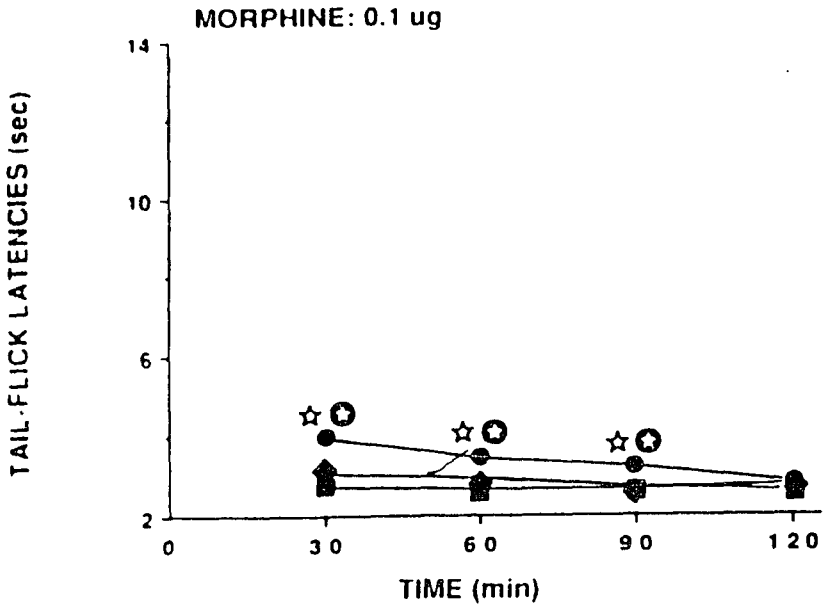
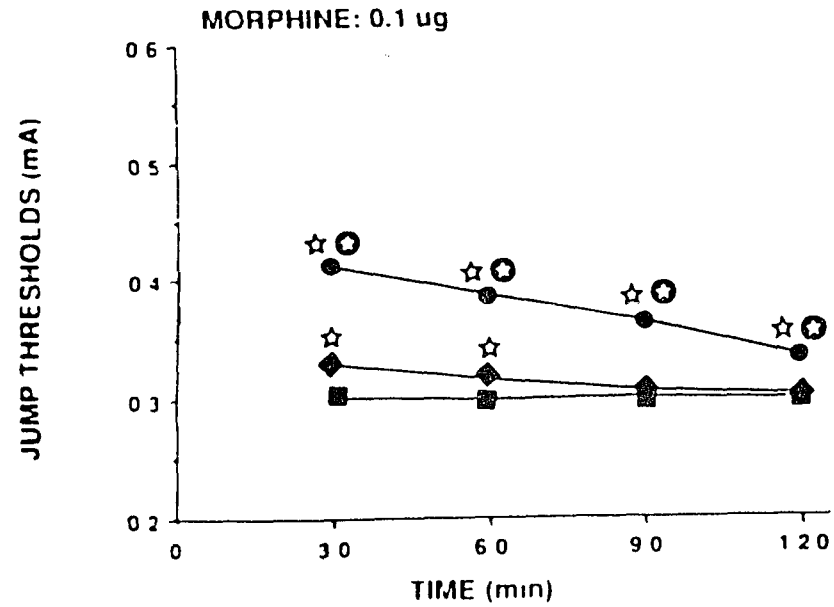

MORPHINE: 1 ug
JUMP THRESHOLDS (mA)
0.6
0.5
0.4
0.3
0.2
0
30
60
90
120
TIME (min)

Figure 8. Alterations in tail-flick latencies (upper panel) and jump thresholds (lower panel) following a no-injection control condition (closed squares), and morphine 0.1 ug paired with either vehicle (closed diamonds) or TRH (10 ug, closed circles) administered into the anterior periaqueductal gray. Significant differences in antinociception were observed among conditions (tail-flick: $F(2,24)= 11.42$, $p<.0003$; jump: $F= 13.13$, $p<.0001$), across the time course (tail-flick: $F(3,36)= 6.16$, $p<.002$; jump: $F= 18.38$, $p<.0001$), and for the interaction between conditions and times (tail-flick: $F(6,72)= 4.58$, $p<.0005$; jump: $F= 15.25$, $p<.0001$). The open stars (Dunnett comparisons, $p<.05$) indicate significant alterations in latencies and thresholds relative to the no-injection condition. The enclosed stars (Dunn comparisons, $p<.05$) indicate the significant alterations induced by TRH in latencies and thresholds following morphine relative to vehicle treatment.


MORPHINE: 0.1 ug
TAIL-FLICK LATENCIES (sec)
14
10
6
2
0
30
60
90
120
TIME (min)


MORPHINE: 0.1 ug
JUMP THRESHOLDS (mA)
0.6
0.5
0.4
0.3
0.2
0
30
60
90
120
TIME (min)

dose of TRH was paired with morphine (0.30 ug). The ED_{50} on the jump test for morphine alone (4.11 ug) was shifted 2.8-fold to the left in anterior PAG placements when a 10 ug dose of TRH was paired with morphine (1.45 ug).

The effects of other TRH doses upon morphine antinociception from anterior PAG placements were then analyzed. Table 3 indicates that a 1 ug dose of TRH significantly increased the magnitude of antinociception following the 0.1 ug morphine dose on the tail-flick (77%, 30 min) and jump (136%, 30-120 min) tests, the 1 ug morphine dose on the jump test (47%, 30-60 min) and the 2.5 ug morphine dose on the jump test (89%, 30 min). Table 4 indicates that a 0.1 ug dose of TRH significantly increased the magnitude of antinociception following the 2.5 ug morphine dose on the tail-flick 91%, 30-60 min) and jump (174%, 30-90 min) tests.

Morphine Hyperthermia: Both doses of morphine microinjected into the anterior PAG significantly increased core body temperature across the time course. TRH (10 ug) significantly increased the magnitude of morphine hyperthermia in rats with anterior PAG placements at both morphine doses across the time course (Table 5).

TABLE 3. Effects of thyrotropin-releasing hormone 1.0 ug administered into the anterior periaqueductal gray upon morphine analgesia.

CONDITION	POST-INJECTION (min) 30	60	90	120
A. Tail-Flick Latencies (sec):				
Control	2.94	2.86	2.82	2.95
TRH 0/Morphine 0.1 ug	3.25*	3.07*	2.91	2.84
TRH 1.0 ug/Morphine 0.1 ug	3.49*+	3.15*	3.05*	2.87
Control	2.56	2.57	2.66	2.62
TRH 0/Morphine 1.0 ug	7.72*	7.11*	5.89*	4.57*
TRH 1.0 ug/Morphine 1.0 ug	6.05*+	6.94*	5.07*	3.71
Control	2.74	2.63	2.66	2.66
TRH 0/Morphine 2.5 ug	5.65*	5.03*	4.55*	3.09
TRH 1.0 ug/Morphine 2.5 ug	7.29*	6.56*	4.99*	3.59
B. Jump Thresholds (mA):				
Control	.309	.308	.306	.306
TRH 0/Morphine 0.1 ug	.353*	.334*	.318	.312
TRH 1.0 ug/Morphine 0.1 ug	.413*+	.377*+	.358*+	.337*+
Control	.358	.365	.362	.361
TRH 0/Morphine 1.0 ug	.478*	.443*	.412*	.390
TRH 1.0 ug/Morphine 1.0 ug	.547*+	.521*+	.448*	.424*
Control	.285	.284	.284	.284
TRH 0/Morphine 2.5 ug	.360*	.345*	.317	.285
TRH 1.0 ug/Morphine 2.5 ug	.427*+	.372*	.341*	.315

TRH (1 ug) and morphine (2.5 ug) produced significant differences among conditions (jump: $F= 7.92$, $p<.005$), across the time course (tail-flick: $F(3,21)= 7.33$, $p<.002$; jump: $F= 25.34$, $p<.0001$) and for the interaction between conditions and times (jump: $F= 4.53$, $p<.001$). TRH (1 ug) and morphine (1 ug) produced significant differences among conditions (jump: $F= 7.89$, $p<.009$), across the time course (tail-flick: $F(3,15)= 4.20$, $p<.03$; jump: $F= 16.01$, $p<.0001$) and for the interaction between conditions and times (tail-flick: $F(6,30)= 3.33$, $p<.013$; jump: $F= 479$, $p<.001$). TRH (1 ug) and morphine (0.1 ug) produced significant differences among conditions (jump: $F= 6.40$, $p<.008$), across the time course (tail-flick: $F(3,27)= 5.46$, $p<.005$; jump: $F= 29.66$, $p<.0001$) and for the interaction between conditions and times (tail-flick: $F(6,54)= 3.59$, $p<.005$; jump: $F= 6.49$, $p<.001$). Significant alterations are denoted relative to control (asterisks) and morphine (crosses) conditions.

TABLE 4. Effects of thyrotropin-releasing hormone 0.1 ug administered into the anterior periaqueductal gray upon morphine analgesia.

CONDITION	POST-INJECTION (min) 30	60	90	120
A. Tail-Flick Latencies (sec):				
Control	2.56	2.57	2.66	2.62
TRH 0/Morphine 1.0 ug	7.72*	7.11*	5.89*	4.57*
TRH 0.1 ug/Morphine 1.0 ug	5.77*+	5.14*+	3.76+	3.21
Control	2.88	2.73	2.79	2.77
TRH 0/Morphine 2.5 ug	4.88*	3.36	2.89	2.83
TRH 0.1 ug/Morphine 2.5 ug	6.69*+	5.27*+	4.31	3.15
B. Jump Thresholds (mA):				
Control	.358	.365	.362	.361
TRH 0/Morphine 1.0 ug	.478*	.443*	.412*	.390
TRH 0.1 ug/Morphine 1.0 ug	.472*	.471*	.422*	.408*
Control	.288	.288	.285	.288
TRH 0/Morphine 2.5 ug	.361*	.332*	.309	.292
TRH 0.1 ug/Morphine 2.5 ug	.415*+	.367*+	.335*+	.308

TRH (0.1 ug) and morphine (2.5 ug) produced significant differences among conditions (jump: $F= 5.36$, $p<.035$), across the time course (tail-flick: $F(3,12)= 4.37$, $p<.03$; jump: $F= 10.26$, $p<.002$) and for the interaction between conditions and times (jump: $F= 8.46$, $p<.0001$). TRH (0.1 ug) and morphine (1.0 ug) produced significant differences among conditions (jump: $F= 4.18$, $p<.05$), across the time course (tail-flick: $F(3,15)= 4.92$, $p<.015$; jump: $F= 7.06$, $p<.004$) and for the interaction between conditions and times (tail-flick: $F(6,30)= 3.09$, $p<.02$; jump: $F= 5.60$, $p<.0005$). The asterisks and crosses respectively denote significant alterations relative to control and morphine conditions.

TABLE 5. Effects of thyrotropin-releasing hormone 10 ug administered into the anterior periaqueductal gray upon morphine hyperthermia (°C).

CONDITION	POST-INJECTION (min)			
	30	60	90	120
Control	37.9	37.9	37.9	37.7
TRH 0/Morphine 1 ug	38.2*	38.3*	38.4*	38.3*
TRH 10 ug/Morphine 1 ug	38.8*+	39.0*+	38.9*+	38.9*+
Control	37.5	37.6	37.7	37.5
TRH 0/Morphine 2.5 ug	37.9*	38.3*	38.3*	38.6*
TRH 10 ug/Morphine 2.5 ug	38.9*+	39.4*+	39.4*+	39.5*+

Significant differences in core body temperatures were observed among injection conditions (morphine dose of 1 ug: $F(2,14)= 14.34$, $p<.0004$; 2.5 ug: $F(2,14)= 33.68$, $p<.0001$), across the time course (1 ug: $F(3,21)= 1.12$, ns; 2.5 ug: $F(3,21)= 8.47$, $p<.0007$), but not for the interaction between conditions and times. The asterisks denote a significant alteration relative to the control condition (Dunnett comparisons, $p<.05$). The crosses denote a significant alteration in morphine hyperthermia by TRH (Dunn comparison, $p<.05$).

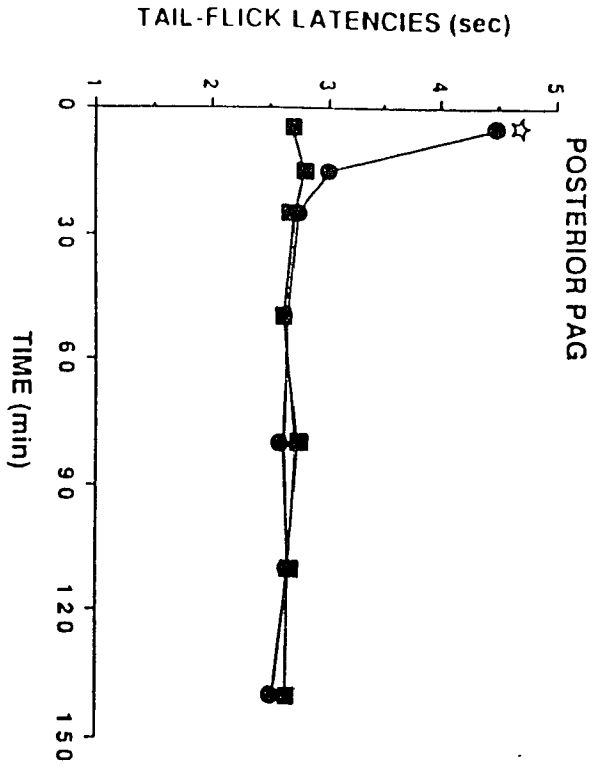
B. TRH and Posterior PAG Placements:

i) Baseline Nociceptive Thresholds: TRH significantly increased latencies (65%) and thresholds (11%) at only 5 min after TRH treatment, but failed to alter either measure thereafter (Figure 9). Rats with posterior PAG placements failed to display significant alterations in core body temperatures either between conditions or for the interaction between conditions and times but showed significant effects across the post-injection time course. Table 6 indicates the non-significant changes in core body temperature following TRH in the posterior PAG.

ii) CCWS Antinociception and Hypothermia: Significant differences in CCWS antinociception and CCWS hypothermia were also observed in rats with posterior PAG placements among conditions, across the time course, and for the interaction between conditions and times. CCWS significantly increased latencies (120%) and thresholds (26%) for up to 60 min in rats with posterior PAG placements. However, TRH significantly increased the magnitude of CCWS antinociception on the jump test (57%) after 30 min (Figure 10, middle panel), but failed to alter CCWS antinociception on the tail-flick test (13% increase) (Figure 10, upper panel). The significant decreases (30-60 min) in core body temperature (13%) following CCWS were significantly reduced (30%) by TRH (Figure 10, lower panel).

iii) Morphine Antinociception and Hyperthermia: The effects of TRH at a dose of 10 ug upon different morphine doses were assessed first. Morphine microinjected into the posterior PAG

Figure 9. Alterations in tail-flick latencies (left panels) and jump thresholds (right panels) following pretreatment with vehicle (closed squares) or thyrotropin releasing hormone (TRH: 10 ug, closed circles) administered into the posterior periaqueductal gray. Significant differences in tail-flick latencies and jump thresholds were observed between conditions (tail-flick: $F(1,10)= 1.73$, ns; jump: $F= 10.59$, $p<.009$), across the time course (tail-flick: $F(6,60)= 4.21$, $p<.001$; jump: $F= 3.98$, $p<.002$) and for the interaction between conditions and times (tail-flick: $F(6,60)= 4.22$, $p<.0013$; jump: $F= 1.63$, ns). The open stars (Dunnett comparisons, $p<.05$) indicate significant increases in latencies or thresholds following TRH relative to vehicle treatment.


TAIL-FLICK LATENCIES (sec)
1
2
3
4
5
0
30
60
90
120
150
TIME (min)
POSTERIOR PAG

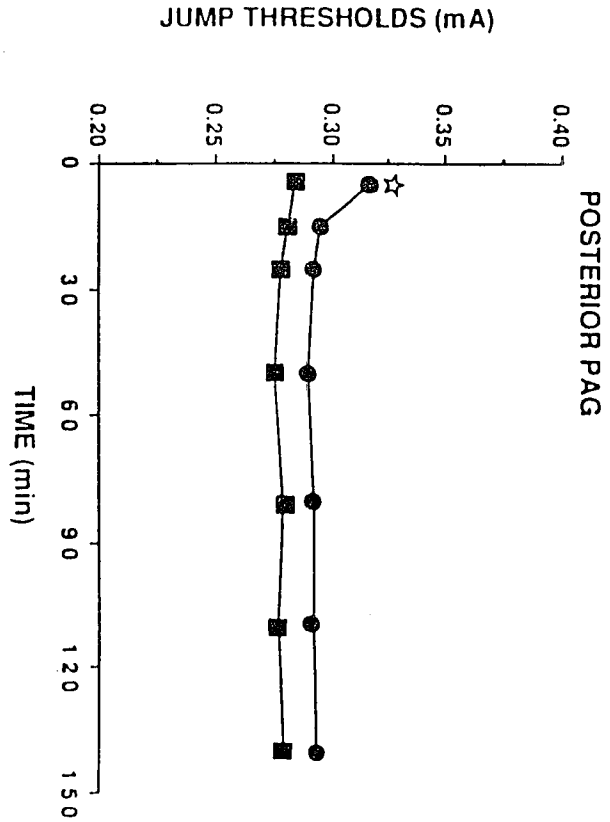
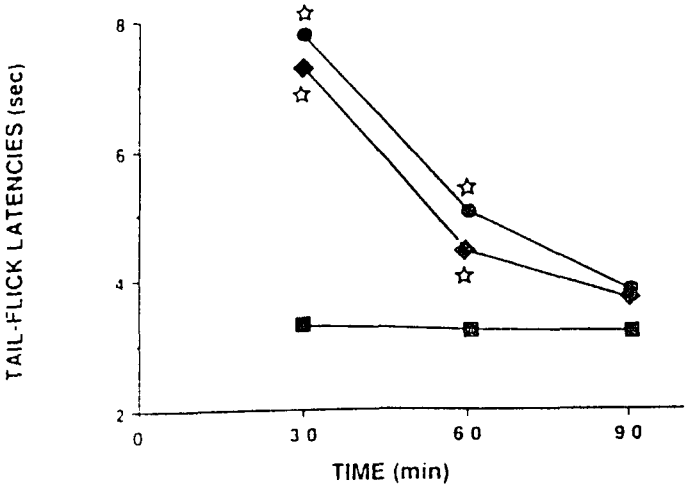

JUMP THRESHOLDS (mA)
0.20
0.25
0.30
0.35
0.40
0
30
60
90
120
150
TIME (min)
POSTERIOR PAG

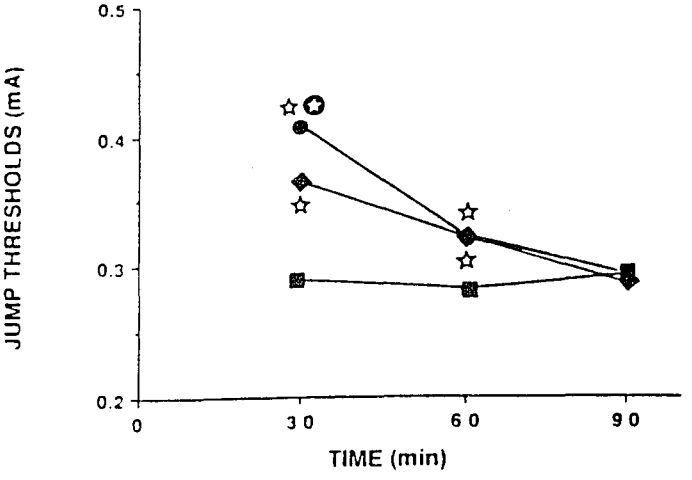
TABLE 6. Effects of thyrotropin-releasing hormone 10 ug administered into the posterior periaqueductal gray upon baseline core body temperature (°C).

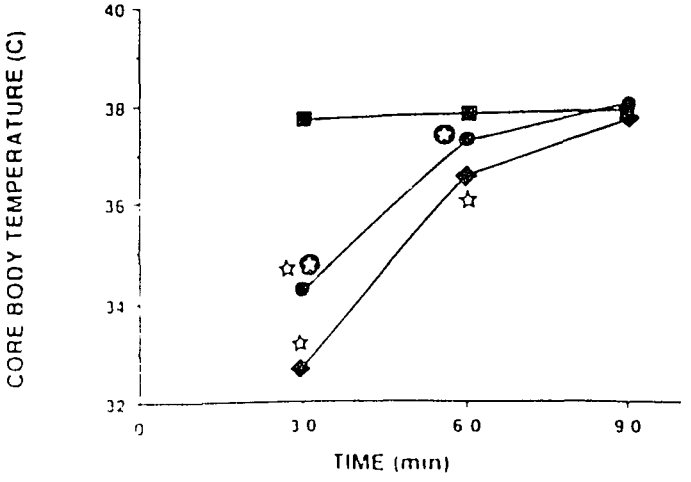
POST-INJECTION (min)	CONDITION	
	Vehicle	TRH 10 ug
5	37.7	37.8
15	38.3	38.1
25	38.3	38.6
50	38.1	38.4
80	38.1	38.4
110	38.0	38.4
140	38.0	38.6

Note: Core body temperatures failed to change either between conditions ($F(1,3)= 4.88$) or for the interaction between conditions and times ($F(6,18)= 2.50$), but showed significant effects across the post-injection time course ($F(6,18)= 3.79$, $p<.01$).

Figure 10. Alterations in tail-flick latencies (upper panel), jump thresholds (middle panel) and core body temperature (lower panel) following a no-swim control condition (closed squares), and continuous cold-water swims (CCWS: 2°C for 3.5 min) paired with either vehicle (closed diamonds) or TRH (10 ug, closed circles) administered into the posterior periaqueductal gray. Significant differences in antinociception and hypothermia were observed among conditions (tail-flick: $F(2,18)= 14.97$, $p<.0001$; jump: $F= 6.21$, $p<.009$; temperature: $F= 18.29$, $p<.0001$), across the time course (tail-flick: $F(3,27)= 85.06$, $p<.0001$; jump: $F= 36.15$, $p<.0001$; temperature: $F= 51.74$, $p<.0001$), and for the interaction between conditions and times (tail-flick: $F(6,54)= 19.93$, $p<.0001$; jump: $F= 14.56$, $p<.0001$; temperature: $F= 34.89$, $p<.0001$). The open stars (Dunnett comparisons, $p<.05$) indicate significant alterations in latencies, thresholds or temperatures relative to the no-swim condition. The enclosed stars (Dunn comparisons, $p<.05$) indicate the significant alterations induced by TRH in thresholds and temperatures following CCWS relative to vehicle treatment.


TAIL-FLICK LATENCIES (sec)
8
6
4
2
0
30
60
90
TIME (min)


JUMP THRESHOLDS (mA)
0.5
0.4
0.3
0.2
0
30
60
90
TIME (min)

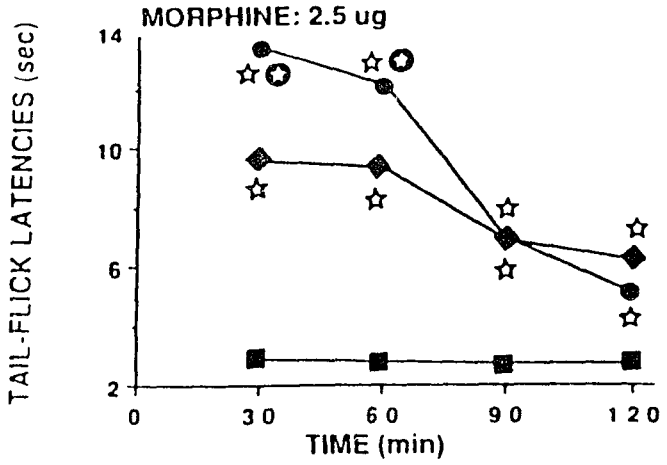

CORE BODY TEMPERATURE (C)
40
38
36
34
32
0
30
60
90
TIME (min)

significantly and dose-dependently increased latencies and thresholds following the 1 (30-90 min) and 2.5 (30-120 min) ug doses, but not following the 0.1 ug dose (Figures 11-13). Figure 11 illustrates the significant increases in the magnitude of morphine (2.5 ug) antinociception induced by TRH (10 ug) on the tail-flick test for up to 60 min (Figure 11, upper panel), and on the jump test for up to 60 min (Figure 11, lower panel). An identical pattern of facilitatory effects by TRH was observed on the tail-flick (Figure 12, upper panel) and jump (Figure 12, lower panel) tests following the 1 ug morphine dose, but not on the tail-flick (Figure 13, upper panel) or jump (Figure 13, lower panel) tests following the 0.1 ug morphine dose.

Regression analyses revealed significant differences in the slopes and intercepts of the peak dose-response curves of morphine antinociception of posterior PAG placements on the tail-flick ($F(2,40)= 3.16$, $p<.05$) and jump ($F= 5.97$, $p<.005$) tests. The ED_{50} on the tail-flick test for morphine alone (1.27 ug) was shifted 3.3-fold to the left in posterior PAG placements when a 10 ug dose of TRH was paired with morphine (0.38 ug). The ED_{50} on the jump test for morphine alone (5.94 ug) was shifted 1.8-fold to the left in posterior PAG placements when a 10 ug dose of TRH was paired with morphine (3.34 ug). Pairing either 0.1 or 1 ug doses of TRH with morphine in rats with posterior PAG placements failed to significantly alter morphine antinociception at any dose and on any test (data not shown).

Both doses of morphine microinjected into the posterior PAG

Figure 11. Alterations in tail-flick latencies (upper panel) and jump thresholds (lower panel) following a no-injection control condition (closed squares), and morphine 2.5 ug paired with either vehicle (closed diamonds) or TRH (10 ug, closed circles) administered into the posterior periaqueductal gray. Significant differences were observed among conditions (tail-flick: $F(2,12)= 13.31$, $p<.0001$); jump: $F= 12.76$, $p<.001$), across the time course (tail-flick: $F(3,18)= 13.59$, $p<.0001$; jump: $F= 24.15$, $p<.0001$), and for the interaction between conditions and times (tail-flick: $F(6,36)= 9.30$, $p<.0001$; jump: $F= 7.37$, $p<.0001$). The open stars (Dunnett comparisons, $p<.05$) indicate significant alterations in latencies and thresholds relative to the no-injection condition. The enclosed stars (Dunn comparisons, $p<.05$) indicate the significant alterations induced by TRH in latencies and thresholds following morphine relative to vehicle treatment.


MORPHINE: 2.5 ug
TAIL-FLICK LATENCIES (sec)
14
10
6
2
0
30
60
90
120
TIME (min)

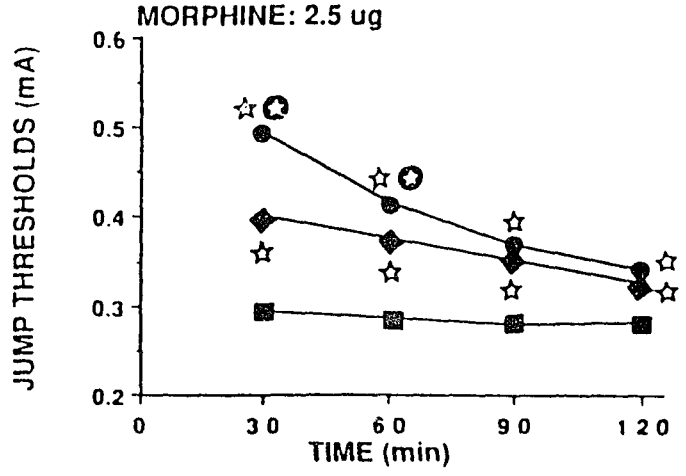
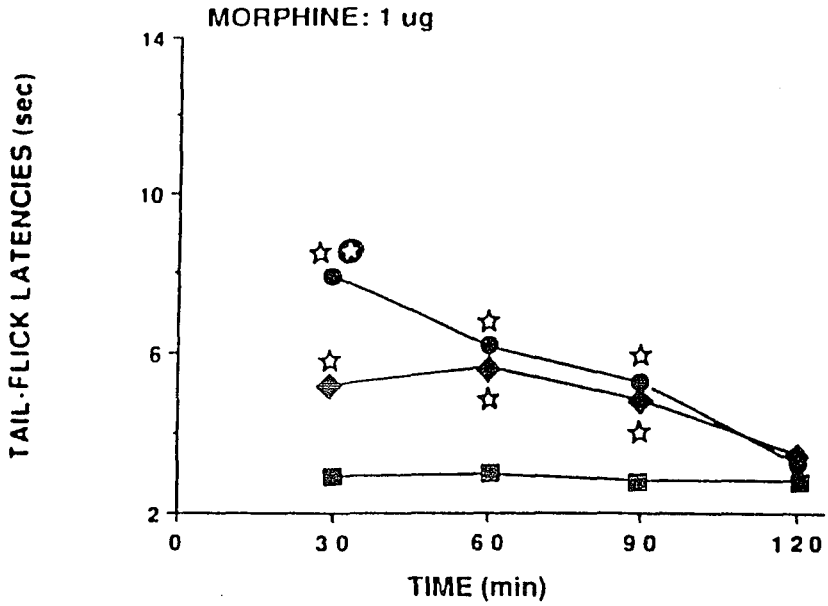

MORPHINE: 2.5 ug
JUMP THRESHOLDS (mA)
0.6
0.5
0.4
0.3
0.2
0
30
60
90
120
TIME (min)

Figure 12. Alterations in tail-flick latencies (upper panel) and jump thresholds (lower panel) following a no-injection control condition (closed squares), and morphine 1 ug paired with either vehicle (closed diamonds) or TRH (10 ug, closed circles) administered into the posterior periaqueductal gray. Significant differences were observed among conditions (tail-flick: $F(2,14)= 4.46$, $p<.03$; jump: $F= 9.23$, $p<.003$), across the time course (tail-flick: $F(3,21)= 5.05$, $p<.01$; jump: $F= 12.94$, $p<.0001$), and for the interaction between conditions and times (tail-flick: $F(6,42)= 4.54$, $p<.001$; jump: $F= 8.40$, $p<.0001$). The open stars (Dunnett comparisons, $p<.05$) indicate significant alterations in latencies and thresholds relative to the no-injection condition. The enclosed stars (Dunn comparisons, $p<.05$) indicate the significant alterations induced by TRH in latencies and thresholds following morphine relative to vehicle treatment.


MORPHINE: 1 ug
TAIL-FLICK LATENCIES (sec)
14
10
6
2
0
30
60
90
120
TIME (min)

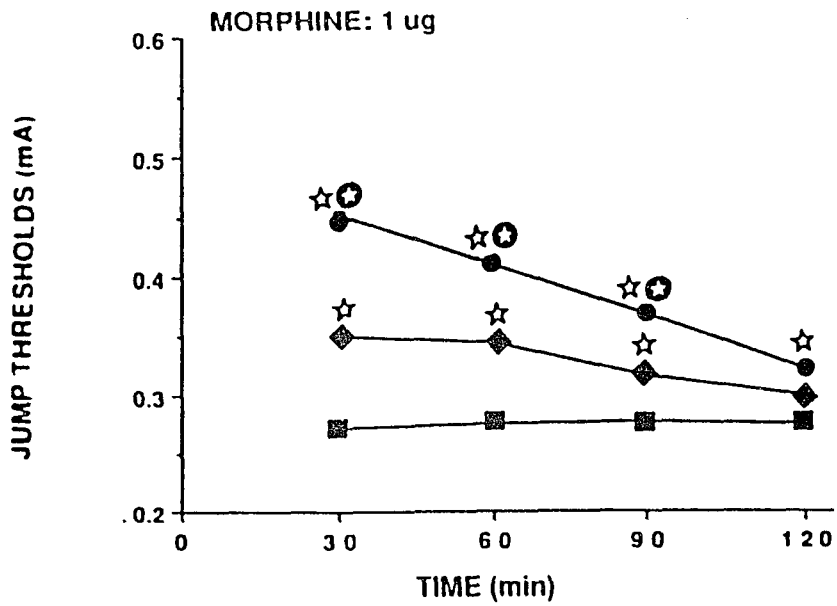
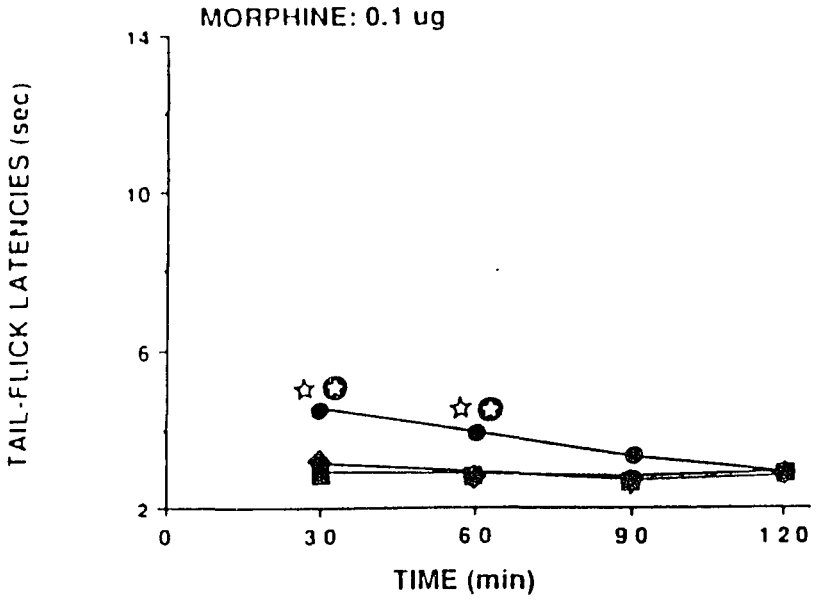
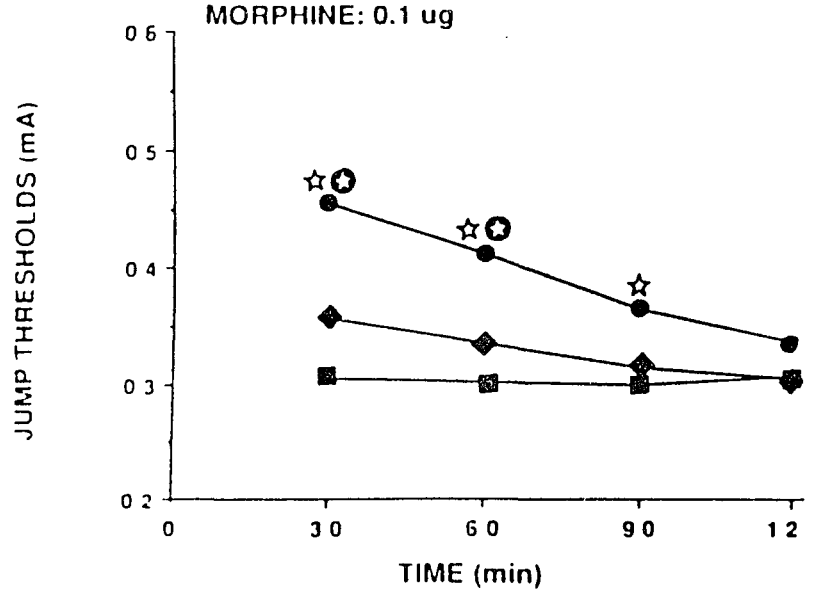

MORPHINE: 1 ug
JUMP THRESHOLDS (mA)
0.6
0.5
0.4
0.3
.0.2
0
30
60
90
120
TIME (min)

Figure 13. Alterations in tail-flick latencies (upper panel) and jump thresholds (lower panel) following a no-injection control condition (closed squares), and morphine 0.1 ug paired with either vehicle (closed diamonds) or TRH (10 ug, closed circles) administered into the posterior periaqueductal gray. Significant differences were observed among conditions (tail-flick: $F(2,12)= 11.40$, $p<.002$; jump: $F= 24.83$, $p<.0001$), across the time course (tail-flick: $F(3,18)= 20.41$, $p<.0001$; jump: $F= 18.46$, $p<.0001$), and for the interaction between conditions and times (tail-flick: $F(6,36)= 10.56$, $p<.0001$; jump: $F= 13.71$, $p<.0001$). The open stars (Dunnett comparisons, $p<.05$) indicate significant alterations in latencies and thresholds relative to the no-injection condition. The enclosed stars (Dunn comparisons, $p<.05$) indicate the significant alterations induced by TRH in latencies and thresholds following morphine relative to vehicle treatment.


MORPHINE: 0.1 ug
TAIL-FLICK LATENCIES (sec)
14
10
6
2
0
30
60
90
120
TIME (min)


MORPHINE: 0.1 ug
JUMP THRESHOLDS (mA)
0 6
0 5
0 4
0 3
0 2
0
30
60
90
12
TIME (min)

significantly increased core body temperature across the time course (Table 7). TRH (10 ug) significantly increased the magnitude of morphine hyperthermia in rats with posterior PAG placements following the 1 (60-120 min) and 2.5 (30-90 min) ug doses of morphine (Table 7).

TABLE 7. Effects of thyrotropin-releasing hormone 10 ug administered into the posterior periaqueductal gray upon morphine hyperthermia (°C).

CONDITION	POST-INJECTION (min)			
	30	60	90	120
Control	37.9	37.9	37.8	37.9
TRH 0/Morphine 1 ug	38.4*	38.6*	38.6*	38.6*
TRH 10 ug/Morphine 1 ug	38.6*	38.9*+	39.0*+	39.1*+
Control	37.6	38.1	37.8	38.0
TRH 0/Morphine 2.5 ug	38.4*	38.6*	39.1*	39.2*
TRH 10 ug/Morphine 2.5 ug	39.4*+	39.7*+	39.6*+	39.5*

Significant differences in core body temperatures were observed among injection conditions (morphine doses of 1 ug $F(2,12)= 8.52$, $p<.005$; 2.5 ug: $F(2,10)= 27.83$, $p<.0001$), across the time course (1 ug: $F(3,18)= 1.86$, ns; 2.5 ug: $F(3,15)= 6.11$, $p<.006$), and for the interaction between conditions and times (1 ug: $F(6,36)= 1.22$, ns; 2.5 ug: $F(6,30)= 2.58$, $p<.039$). The asterisks denote a significant alteration relative to the control condition (Dunnett comparisons, $p<.05$). The crosses denote a significant alteration in morphine hyperthermia by TRH (Dunn comparison, $p<.05$).

DISCUSSION

The present study evaluated the effects of intracerebral microinjections of TRH into the PAG upon the antinociceptive responses following CCWS and morphine as measured by the tail-flick and jump tests in rats. First, TRH microinjections into the PAG significantly increased tail-flick latencies and jump thresholds for up to 15 min following administration, confirming previous reports of a short-lived antinociception following supraspinal administration (Griffiths et al., 1981; Boschi et al., 1983; Rips et al., 1983; Webster et al., 1983; Reny-Palasse et al., 1989). TRH failed to alter either latencies or thresholds at those time intervals when it was combined with either CCWS or morphine. Microinjections of TRH into the PAG also failed to alter core body temperatures. Female rats were utilized in this study because they were used in previous positive TRH effects upon stress-induced antinociception (Butler and Bodnar, 1984, 1987). Although neither CCWS nor central morphine antinociception in female rats is affected by estrous phase (Romero and Bodnar, 1986; Kepler et al., 1989), it is not known whether TRH effects upon either baseline nociception, CCWS antinociception or central morphine antinociception were sensitive to estrous differences. Since evaluation of estrous phase was not examined in the present study, this variable deserves some consideration in the interpretation of results, and should be systematically studied in future research.

Second, TRH microinjections into the PAG produced significant alterations in CCWS antinociception on the tail-flick and jump

tests that varied as a function of its location in the PAG. When TRH was microinjected into the anterior PAG, it significantly reduced CCWS antinociception on the tail-flick and jump tests in a dose-dependent fashion. In contrast, when TRH was microinjected into the posterior PAG, it significantly potentiated CCWS antinociception on the jump test. This latter effect was similar to the increased magnitude in CCWS antinociception following intracerebroventricular administration of TRH (Butler and Bodnar, 1987). The differential effects of TRH upon CCWS antinociception as a function of cannula placement were specific to antinociceptive responses since CCWS hypothermia was significantly reduced by TRH microinjections into both anterior and posterior PAG placements.

Third, TRH microinjections into the both the anterior and posterior PAG significantly and dose-dependently potentiated the magnitude of morphine antinociception on the tail-flick and jump tests, and shifted morphine's dose-response curve to the left. This effect is in striking contrast to the inability of either peripheral or intracerebroventricular TRH to alter opioid antinociception induced by either morphine (Holaday and Faden, 1983; Kasson and George, 1983) or beta-endorphin (Holaday et al., 1978; Osbahr et al., 1982). The consistent potentiation of the magnitude of morphine antinociception and its increased sensitivity in the presence of PAG TRH are also in contrast to the attenuation of morphine antinociception at low doses of intrathecal TRH and enhancement of morphine antinociception at high doses of intrathecal TRH (Watkins et al., 1986). TRH also significantly

potentiated the hyperthermic effects of morphine following microinjections into anterior and posterior PAG placements. Thus, the effects of PAG TRH upon PAG morphine antinociception constitutes both an upward shift in terms of magnitude of effect, and a leftward shift in terms of effective morphine dose to elicit a criterion antinociceptive response.

Before discussing specific implications of the present results, an evaluation of the specificity of the results must be examined, particularly the site of effect. Since all microinjections were made in and immediately around the PAG, one must consider the possibility that TRH effects might occur distally from this structure due to diffusion of the peptide into the cerebral aqueduct. One obvious control would be to place direct microinjections of TRH into the aqueduct, and observe whether similar effects occurred. Especially for anterior PAG placements, this would be an inadequate control because the cannula would occlude the aqueduct itself. Further, it does not control for the possibility that the peptide would immediately diffuse into the PAG and exert effects. Evidence against a diffusion hypothesis can be found in comparisons of intracerebroventricular and intracerebral effects. Intracerebroventricular administration of TRH at a dose of 50 ug significantly potentiated CCWS antinociception, while intracerebral administration of TRH into anterior PAG placements reduced CCWS antinociception at doses as low as 0.01 ug. A diffusion hypothesis would argur for an identical pattern of effects along the medial neuraxis. Further, whereas higher doses of

TRH administered intracerebroventricularly failed to alter morphine antinociception, very low (0.1-10 ug) of TRH significantly and dose-dependently potentiated morphine antinociception, and significantly shifted its dose-response curve to the left. A diffusion hypothesis cannot explain such differential effects. The remainder of the discussion will evaluate: a) possible mechanisms of action modulating differential PAG TRH effects upon CCWS antinociception, b) possible mechanisms of action modulating PAG TRH effects upon morphine antinociception, and c) a possible modulatory role for PAG TRH in collateral inhibition mechanisms (see Kirchgessner et al., 1982; Bodnar, 1990; Steinman et al., 1990).

A. PAG TRH and CCWS Antinociception: Our laboratory (Butler and Bodnar, 1987) has previously found that intracerebroventricular administration of TRH (10-50 ug) significantly potentiated CCWS antinociception at bath temperatures of 21°C, 15°C and 2°C on the tail-flick test in rats. Intracerebroventricular TRH significantly reduced CCWS hypothermia at a bath temperature of 21°C, and significantly potentiated CCWS hypothermia at a bath temperature of 2°C. The present study was designed to determine a central locus of action through which TRH exerted its effects upon CCWS antinociception and hypothermia, and the findings that the rostro-caudal extent of mesencephalic cannula placements were a critical variable in determining whether TRH potentiated or reduced CCWS antinociception was quite unexpected. Administration of TRH into the anterior PAG significantly reduced CCWS antinociception on both

nociceptive tests in a dose-dependent manner, while administration of TRH into the posterior PAG significantly potentiated CCWS antinociception on the jump test alone. These differential effects upon CCWS antinociception were not due to site-specific differences of TRH effects upon either baseline nociceptive thresholds or CCWS hypothermia. Both anterior and posterior PAG placements were capable of eliciting short-term antinociceptive responses on both tests following TRH administration, and these effects dissipated over time such that baseline thresholds were back to baseline levels at the time of CCWS antinociception determinations. Further, TRH administration into both anterior and posterior PAG placements significantly reduced the magnitude and duration of CCWS hypothermia. Manipulations altering CCWS antinociception and CCWS hypothermia have been shown to dissociate in some instances, but not others (see review: Bodnar, 1993). Dissociations between CCWS antinociception and CCWS hypothermia occur following repeated exposure to CCWS, hypophysectomy, D-phenylalanine treatment and antagonism of muscarinic and noradrenergic receptors as well as in aging and gender differences. Changes in CCWS antinociception and CCWS hypothermia have been associated with each other following neonatal monosodium glutamate treatment as well as following noradrenergic agonists and histaminergic antagonists. That different mesencephalic sites differentially alter TRH-induced effects upon CCWS antinociception, but uniformly reduce CCWS hypothermia suggests that the antinociceptive and hypothermic alterations are not related to each other.

The differentiation of TRH effects upon CCWS antinociception as a function of anterior and posterior PAG placements should be examined in terms of previous effects of PAG manipulations upon CCWS antinociception and other forms of stress-induced antinociception. Our laboratory (see review: Bodnar et al., 1980a) found that lesions placed in the anterior PAG rostral to the interpeduncular nucleus significantly reduced CCWS antinociception as measured by an operant liminal escape test, whereas lesions placed in the posterior PAG in the area of the dorsal raphe nucleus failed to affect CCWS antinociception on this measure. It should be noted however, that neither anterior nor posterior PAG lesions altered the magnitude of CCWS antinociception as measured by the tail-flick test. Despite its importance in the initiation of morphine antinociception (see reviews: Fields and Basbaum, 1978; Basbaum and Fields, 1984), lesions placed in the PAG have had variable effects upon other forms of stress-induced antinociception. Whereas antinociception induced by prolonged footshock was reduced by PAG lesions, these lesions failed to affect antinociception induced by brief footshock or footshock delivered to the forepaws or hindpaws (Bragin, Vasilenko, and Durinjan, 1983; Watkins, Kinscheck, and Mayer, 1983). Interestingly, whereas classically-conditioned footshock antinociception was potentiated by rostral PAG lesions, it was attenuated by caudal PAG lesions (Kinscheck, Watkins, and Mayer, 1984). Further, the antinociception elicited by electrical stimulation of the rostral PAG is blocked by lesions placed in the

caudal PAG (Rhodes and Liebeskind, 1978; Rhodes, 1979). Thus it appears that lesions placed in rostral and caudal PAG structures differentially alter various forms of antinociceptive responses. It is not clear on the basis of TRH-like immunocytochemistry and TRH receptor autoradiography how distribution of the peptide or its receptors in the PAG can account for the differential effects since most of these studies regarded the PAG as a homogeneous area.

Cytoarchitectural anatomical analysis of the PAG has indicated its heterogeneity into lateral, dorso-lateral, dorsal, ventro-lateral and ventro-medial subdivisions (Beitz, 1985; Beitz and Shepard, 1985; Conti et al., 1988). Functional subdivisions of the PAG have been proposed as well especially in terms of defensive behavior (see review: Bandler et al., 1992). Defensive behavior has been subcategorized into the two aspects of responsivity to aggressive encounters, historically labelled "fight" or "flight". Such agonistic behavior has been explained in terms of cognitive decision-making, and the elicitation of the aggressive encounter would trigger the action. Recent data however argue that separate pathways within the PAG exist that separately activate each response. The "fight" response is typically measured by the "backward defense" posture in rats in which the animal rears to free its forepaws for aggressive acts, pins back its ears, and makes both audible and inaudible vocalizations. The "flight" response is typically measured by the "forward avoidance" movement in rats in which the animal darts away from the intruder. Whereas microinjections of excitatory amino acids into the <u>anterior</u> lateral

PAG elicited backward defense in rats, the same injections into the posterior lateral PAG elicited forward avoidance behavior. Both of these sites also produced a rise in blood pressure and tachycardia. In contrast, glutamate injections into the posterior ventro-lateral PAG elicited immobility which was associated with a fall of blood pressure and bradycardia. These data taken together with the present data suggests that the magnitude of antinociception elicited by the stressful consequences of CCWS is differentially determined at different levels of the PAG, and that TRH may be one of the bioactive substances participating in this differential response. Indeed, there may be a relationship between an animal's antinociceptive response to stress, and the type of defensive behavior that is employed by the animal. In the anterior PAG, TRH reduces the magnitude of nonopioid antinociception, and increases backward defensive behavior that is associated with the "fight" aspect of the "fight-flight" reaction. This reduction in antinociceptive potency by TRH may then allow the animal to assess the extent of its wounds during the "fight", and thereby withdraw if the wounds are very serious. In the posterior PAG, TRH increases the magnitude of nonopioid antinociception, and increases forward avoidance behavior that is associated with the "flight" aspect of the "fight-flight" reaction. This increase in antinociceptive potency by TRH may then allow the animal to withdraw in an efficacious manner during "flight", and would prevent interruption of this "flight" behavior by attention to injury. It would be interesting to note whether changes in the strategy of "fight-

flight" behavior is associated with differential TRH activity in the anterior and posterior PAG, and whether the efficacy of nonopioid antinociception is altered as well.

A second interesting hypothesis associated with behavioral specificity in the PAG is the relationship between blood pressure and pain sensitivity. Injections of excitatory amino acids into the anterior and posterior lateral PAG elicit rises in blood pressure and tachycardia, while glutamate injections in the posterior PAG elicited falls in blood pressure and bradycardia. Randich and Maixner (1984a) proposed that systems controlling cardiovascular function are coupled to systems modulating antinociception such that increases in blood pressure decrease pain sensitivty. Activation of the sinoaortic baroreceptor reflex arc produces antinociception, and this activation appears to explain some of the antinociceptive actions of endogenous and exogenous opioids (Randich and Hartunian, 1983; Randich and Maixner, 1984). Further, the cardiovascular alterations observed in spontaneously hypertensive rats correspond with alterations in their nociceptive responsivity (Randich, 1982). Finally, cervical vagal stimulation produces an antinociceptive response related to activity of "on" and "off" cells in the rostro-ventral medulla, and there is a coupling of these physiological responses to effects upon nociceptive and cardiovascular processes (Thurston and Randich, 1992). TRH has other neuromodulatory roles in the central nervous system, including control of blood pressure (see review: Metcalf and Dettmer, 1981) such that blood pressure increases following TRH

administration (Holaday et al., 1981). Since hypertension produces antinociception (Randich, 1982; Zamir and Segal, 1979; Zamir and Shuber, 1980), this would suggest that TRH produces its increases in antinociceptive responses through its hypertensive properties. However, TRH produces its most potent hypertensive responses following peripheral administration (Holaday et al., 1981), yet this route of administration fails to alter different forms of opioid or nonopioid antinociception (Butler and Bodnar, 1984, 1987; Holaday et al., 1983). Further, the antinociceptive responses following vasopressin have been dissociated from its more potent pressor responses (Kordower and Bodnar, 1984), indicating that these two physiological responses can respond independently to the same bioactive substance. To investigate this hypothesis further, it will be important to assess the time course and pattern of PAG TRH effects upon blood pressure and antinocieption.

B. PAG TRH and Morphine Antinociception: The antinociceptive responses to morphine as measured by the tail-flick and jump tests were significantly potentiated by intracerebral administration of TRH into either the anterior or posterior PAG. TRH-induced mediation of morphine antinociception in the PAG is quite specific since it failed to alter beta-endorphin (Holaday et al., 1978; Osbahr et al., 1982) and morphine (Holaday and Faden, 1983; Kasson and George, 1983) antinociception following peripheral or ventricular administration. Further, intrathecal TRH attenuated morphine antinociception at low doses, yet enhanced morphine antinociception at high doses (Watkins et al., 1986). Again, the

ability of TRH to potentiate morphine antinociception in PAG placements was independent of its effects upon baseline nociceptive thresholds since these effects were observed after baseline latencies and thresholds were recovered. It should be noted however that the potentiation of morphine antinociception by TRH was accompanied by a significant increase in the magnitude of morphine hyperthermia following injection into both anterior and posterior PAG placements, suggesting that the alterations in nociceptive thresholds may be related to alterations in core body temperature.

Other transmitter systems also appear to participate locally in morphine antinociception in the PAG. Intracerebral pretreatment with the serotonin receptor antagonist, methysergide significantly reduces mesencephalic morphine antinociception (Schul and Frenk, 1991). Further, whereas the GABA agonist THIP microinjected into the PAG decreased mesencephalic morphine antinociception, the GABA antagonist, picrotoxin microinjected into the PAG potentiated mesencephalic morphine antinociception (Depaulis, Morgan, and Liebeskind, 1987). Administration of the partial mu_1 agonist, ethylketocyclazocine into the PAG inhibits mesencephalic morphine antinociception (Bodnar et al., 1991). Finally, NMDA antagonists microinjected into the PAG also modulate mesencephalic morphine antinociception (Jacquet, 1988). The ability of mesencephalic TRH to potentiate mesencephalic morphine antinociception was quite potent in that TRH doses as low as 0.1 ug potentiated morphine antinociception, and a TRH dose of 10 ug paired with a morphine dose of 0.1 ug resulted in significant antinociception on both

nociceptive tests. In addition to potentiating the magnitude of mesencephalic morphine antinociception, TRH produced significant leftward shifts in morphine's dose-response curve on both the tail-flick and jump tests.

C. PAG TRH and Collateral Inhibition: As indicated in the Introduction, the antinociceptive responses following morphine and CCWS clearly dissociate from each other. Whereas morphine antinociception is potentiated by either putative endopeptidase inhibition with D-phenylalanine or hypophysectomy, CCWS antinociception is reduced by such treatments (Bodnar et al., 1979a,b, 1980b). Further, whereas CCWS antinociception is potentiated by either chronic naloxone treatment (Yoburn et al., 1987) or pretreatment with the mu_1 antagonist, naloxazone (Kirchgessner et al., 1982), morphine antinociception is reduced by such treatments. On the basis of these data, our laboratory (Kirchgessner et al., 1982; Bodnar, 1986, 1990) proposed that selective activation of one antinociceptive system should actively inhibit other antinociceptive systems in addition to producing its pain-inhibitory effects. In directly testing this hypothesis, our laboratory (Steinman et al., 1990) found that whereas morphine and CCWS produce potent antinociception themselves, simultaneous treatment of morphine and CCWS significantly reduces each other's antinociceptive responses. This finding has been replicated using other forms of opioid-mediated and nonopioid-mediated antinociception (Grisel et al., 1991, 1992). Since the pituitary gland has been implicated in the maintainance, and not the

initiation of CCWS antinociception (Bodnar et al., 1979a,b; Bhargava, 1981; Kelly et al., 1993), it has not been clear where the central locus (loci) of collateral inhibition between these different antinociceptive systems may occur. The ability of TRH to both potentiate mesencephalic morphine antinociception and reduce CCWS antinociception following administration into anterior PAG placements suggests that this site and this peptide may be candidates for the modulatory effects of collateral inhibition. If TRH is acting as a natural modulator of different forms of antinociception in the anterior PAG, it is not clear whether extrinsic or intrinsic TRH neurons are responsible for this action. The ability of the anterior PAG to differentially modulate these different forms of antinociception is another instance of this region's importance in mediating integrated responses to external stimuli that are relevant to an organism's survival and which activate autonomic and other adaptive responses (see review: Bandler et al., 1992).

FUTURE DIRECTIONS AND CONCLUSIONS

Microinjection of TRH into the anterior and posterior PAG altered the level of opioid and nonopioid antinociception as well as the hyperthermic and hypothermic effects associated with these forms of antinociception. Future studies should include exploration of other sites where TRH might play a neuromodulatory role, particularly since earlier studies found that hyperthermic effects upon CCWS were not consistent when TRH was microinjected into the lateral ventricles (Butler and Bodnar, 1987), although the effects of PAG TRH were consistent in the present study. Potential sites include the RVM and locus coeruleus which are interconnected with the PAG both functionally (Kiefel et al., 1992a,b; 1993) and synergistically (Rossi et al., 1993) as described in the anatomical section.

Since the lateral and ventrolateral portions of the PAG are heavily implicated in opioid antinociception, this study focused on these areas. As indicated in the studies of Beitz (1985; Beitz Shepard and Wells, 1983), there are other subdivisions in the PAG where TRH is found. A mapping study should explore the effects of TRH microinjections in other portions of the PAG, possibly in conjunction with an examination of the relationship between antinociception and defensive behavior.

In the present study PAG TRH differentially altered a prototypical form of opioid antinociception induced by morphine, but the specific opioid receptor subtypes involved are not clear, nor whether TRH would have similar effects upon other forms of

opioid antinociception. To more clearly delineate the specific opioid receptor subtypes involved, the effects of TRH upon antinociception of selective receptor subtype agonists should be examined, as well as whether opioid receptor subtype antagonists block morphine antinociception in the PAG and its potentiation by TRH. Other forms of nonopioid antinociception should be explored to extend and support the present findings. These might include inescapable footshock, glucoprivation, and immobilization.

Given the relatively brief half-life of TRH within the brain, a study including microinjection of its metabolites and a synthetic analogue into the PAG would determine whether antinociceptive effects are due to TRH itself, a metabolite, or both, and where such activity might occur. The results of intraventricular administration indicated that both TRH and its metabolite DKP seemed to be involved in the potentiation of CCWS antinociception (Butler and Bodnar, 1987), however the specific sites of action were not clear, and it is quite possible that they are different for the two substances.

Since TRH is known to co-exist with serotonin and substance P in medullary cells, manipulations which include the others would help to determine whether various types of antinociceptive neuromodulation in the PAG can be attributed solely to TRH, or how TRH might interact with other neurotransmitters or peptides. Recent work has shown that a serotonergic synapse mediated by $5HT_2$ and $5HT_3$ receptors in the ventral medial medulla participates in the mediation of mesencephalic morphine antinociception (Kiefel,

Cooper, and Bodnar, 1992a,b). Given the recent development of substance P antagonists, the participation and interaction of this peptide in antinociceptive processes can be explored as well.

Since previous studies evaluating supraspinal TRH effects used female rats (Butler and Bodnar, 1984, 1987), the present one did so as well. As noted previously, alterations in estrous phase fails to alter the magnitude of either CCWS or central morphine antinociception (Romero and Bodnar, 1986; Kepler et al., 1989). However, both gender and gonadectomy differences have been observed for both CCWS and morphine antinociception (Romero and Bodnar, 1986; Romero et al., 1987, 1988; Kepler et al., 1989; Kavaliers and Innes, 1987). The role of TRH in nociceptive processes has not been evaluated in terms of gender, gonadal or estrous differences, although the short-term antinociceptive actions of TRH have been observed in both male and female rodents (Butler and Bodnar, 1987; Boschi et al., 1983; Reny-Palasse and Rips, 1983; Rips et al., 1983). Although estrous phase does not affect morphine and CCWS antinociception itself, it is conceivable that it could alter the interaction between TRH and these other manipulations. It is also conceivable that since there are gender and gonadectomy differences for both forms of antinociception, TRH interactions could be differentially sensitive as functions of these organismic variables. This is especially relevant since the locus of effects (the PAG) is both critical for antinociception and a target for gonadal steroid hormones. Therefore, it would be interesting to examine whether TRH in the PAG might be a candidate peptide that

modulates observed gonadectomy and gender differences, and might be sensitive to estrous effects.

A future physiological study might serve to clarify the nature of TRH's modulatory effects upon antinociception, such as whether enhanced activation or disinhibition are involved. Fields and coworkers (Barbaro et al., 1986; Fields et al., 1983; Vanegas et al., 1984) have identified distinct cell populations in the RVM that modulate opioid antinociception elicited in the PAG. Since TRH reliably potentiates mesencephalic morphine antinociception, it would be of interest to examine whether the firing characteristics of "on" and "off" cells change as a function of mesencephalic morphine in the presence and absence of TRH.

In conclusion, the following three findings emerged from this dissertation. First, TRH microinjections into the PAG significantly increased tail-flick latencies and jump thresholds for up to 15 min following administration, confirming previous reports of a short-lived antinociception following supraspinal administration. TRH failed to alter either latencies or thresholds at those time intervals when it was combined with either CCWS or morphine. Microinjections of TRH into the PAG also failed to alter core body temperatures. Second, TRH microinjections into the PAG produced significant alterations in CCWS antinociception on the tail-flick and jump tests that varied as a function of its location in the PAG. When TRH was microinjected into the <u>anterior</u> PAG, it significantly <u>reduced</u> CCWS antinociception on the tail-flick and jump tests in a dose-dependent fashion. In contrast, when TRH was

microinjected into the posterior PAG, it significantly potentiated CCWS antinociception on the jump test. Third, TRH microinjections into the both the anterior and posterior PAG significantly and dose-dependently potentiated morphine antinociception on the tail-flick and jump tests. TRH also significantly potentiated the hyperthermic effects of morphine following microinjections into anterior and posterior PAG placements.

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