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NEUROMODULATORY ACTIONS OF THYROTROPIN-RELEASING HORMONE UPON STRESS-INDUCED ANALGESIA

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NEUROMODULATORY ACTIONS OF THYROTROPIN-RELEASING HORMONE UPON STRESS-INDUCED ANALGESIA

by

PAMELA D. BUTLER

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

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Abstract

NEUROMODULATORY ACTIONS OF THYROTROPIN-RELEASING HORMONE UPON STRESS-INDUCED ANALGESIA

by

Pamela D. Butler

Advisor: Professor Richard J. Bodnar

Thyrotropin-releasing hormone (TRH), a peptide with a wide central nervous system distribution, produces some centrally-mediated effects that are unrelated to its neuroendocrine actions. Although TRH itself elicits a transient analgesia, it antagonizes neurotensin analgesia and has mixed effects upon opioid analgesia. The purpose of this dissertation was to determine further the characteristics of the modulatory actions of TRH upon analgesic processes by examining its effects upon several forms of stress-induced analgesia (SIA) which have different pharmacological profiles. First, the results showed that while TRH itself only produced transient analgesia, it produced long-term potentiations of analgesia elicited by exposure to 20 or 80 footshocks delivered to all four paws, and analgesia elicited by brief shock delivered to the forepaws but not the hindpaws. Second, TRH potentiated analgesia elicited by acute exposure to swim temperatures ranging from 2 to 21 C. These effects appeared to be centrally-mediated

since TRH potentiated both swim and shock analgesia following intracerebroventricular, but not intravenous, administration. TRH's effects on swim analgesia may be due in part to its metabolism to histidyl-proline diketopiperazine (DKP) since this metabolite produced similar potentiations. However, the stable TRH analogue RX77368 also potentiated swim analgesia. Since the cholinergic system has been implicated in swim analgesia and TRH interacts with the cholinergic system, the observation that TRH potentiated analgesia induced by the muscarinic receptor agonist pilocarpine provides preliminary evidence for a possible cholinergic link in the TRH potentiation of various forms of SIA. Thus, while TRH itself has only transient analgesic properties, it is capable of producing long-term modulation of other forms of analgesia such as those produced by stress.

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I am particularly grateful to Dr. Richard Bodnar for being an excellent mentor and for giving so freely of his time, energy, and wisdom. I would like to thank Drs. Barry Komisaruk and Sandra Shapiro for being on my dissertation committee and for their valuable suggestions and comments. I would also like to thank Drs. Walter Essman and Thomas Frumkes for serving as outside readers.

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I would like to thank my family and friends for their encouragement and also for their patience with the demands of graduate school. My stepdaughter Allison was very understanding and even made friends with one of the rats whom she named Ricehead. Finally, I would like to thank my husband Jim whose love, supportiveness, and pleasure in my achievements have been invaluable to me.

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Introduction

The existence of multiple endogenous pain inhibitory systems (Basbaum & Fields, 1984) has stimulated attempts to define the neural substrates that either directly subserve or indirectly modulate these systems. Recent research suggests that the tripeptide thyrotropin-releasing hormone (TRH), classically described as a hypophyseal regulator (Guillemin & Burgus, 1972), may also act as a modulator of pain inhibition. The present series of studies examined the possible modulation by TRH of pain inhibition in rats, particularly in those situations in which the analgesic response is induced by acute exposure to a stressful stimulus. The analgesic stressors chosen for study (footshock, swims) were selected because of their different pharmacological and physiological profiles (see reviews: Bodnar, 1984; Watkins & Mayer, 1982b). The following sections will provide a background for the proposed studies including, a) the central nervous system (CNS) distribution of TRH and its receptors; b) the general behavioral effects of TRH; c) TRH modulation of analgesic and opioid/opiate responses; d) a description of endogenous pain inhibitory systems activated by footshock and swim stress; and e) a rationale for the proposed studies.

A. The CNS Distribution of TRH and Its Receptors

The distribution of TRH in the rat CNS is ubiquitous

(see Appendix A; Jackson & Reichlin, 1974; Johannson, Hokfelt, Jeffcoate, White, & Spindel, 1983) and includes areas involved in pain inhibition. Immunohistochemical (IHC) studies show that TRH-like immunoreactivity is located in cells in a number of hypothalamic nuclei, in the medullary nuclei raphe magnus, pallidus, and oralis, and in cells adjacent to and within the pyramidal tract (Hokfelt, Fuxe, Johannson, Jeffcoate, & White, 1975; Johannson & Hokfelt, 1980; Johannson, Hokfelt, Jeffcoate, White, & Sternberger, 1980; Johannson, Hokfelt, Pernow, Jeffcoate, White, Steinbusch, Verhofstad, Emson, & Spindel, 1981). Further, TRH coexists with serotonin (5-HT) and/or substance P (SP) in these medullary structures (Johannson et al., 1981). Cells containing TRH in mice, but not rats, are also found in the superficial laminae (II and III) of the dorsal horn of the spinal cord (Coffield, Zimmermann, Hoffent, Miletic, & Brooks, 1984).

TRH-containing fibers are located in the hypothalamic paraventricular nucleus (PVN) and medial basal hypothalamus (MBH) (Hokfelt et al., 1975; Johannson et al., 1980; Shioda & Nakai, 1983) as well as in such forebrain extrahypothalamic structures as the nucleus accumbens and septum (Hokfelt et al., 1975). TRH-containing fibers are also found caudally in the periaqueductal gray (PAG), the locus coeruleus, and the nucleus tractus solitarius, as well as in the medullary

raphe nuclei (Johannson et al., 1983). Although a few isolated fibers containing TRH are found within the nociceptive dorsal horn of the spinal cord, TRH fibers in the spinal cord are located in the ventral horn surrounding motor neurons, in the pericanalar gray, and in the sympathetic lateral column (Gilbert, Emson, Hunt, Bennett, Marsden, Sandberg, Steinbusch, & Verhofstad, 1982; Johannson et al., 1983; Johannson et al., 1981). The co-existence of TRH with SP and 5-HT is present in fibers in the ventral, but not dorsal horn (Bowker, Westlund, Sullivan, Wilber, & Coulter, 1982; Gilbert et al., 1982; Johannson et al., 1981).

Specific, high-affinity binding sites for TRH have been found in several areas relevant to analgesia including the amygdala and septum (Pilotte, Sharif, & Burt, 1984; Taylor & Burt, 1982; Simasko & Horita, 1982), the PAG (Dean, Yamamura, Snowhill, & Wamsley, 1984), the periventricular gray of the fourth ventricle, the medulla (Taylor & Burt, 1982), and the dorsal and ventral horns of the spinal cord (Dean et al., 1984; Sharif, Pilotte, & Burt, 1983). The presence of TRH and its receptors in areas involved in pain perception and analgesia supports behavioral evidence for a modulatory role for TRH in pain inhibition.

B. Central Nervous System Effects of TRH

In addition to its well-characterized ability to

release thyrotropin stimulating hormone (TSH) from the anterior lobe of the pituitary gland, TRH also induces prolactin release (for review see: Prange, Nemeroff, Loosen, Bissette, Osbahr, Wilson, & Lipton, 1979). However, TRH also produces a number of effects which are dissociable from its hypophysiotropic actions including changes in behavioral activity, temperature, blood pressure, and glucose levels. TRH appears to have a neuromodulatory role in the CNS as seen by its interactions with sedative, hypothermic, and anti-depressant agents. Because many of the effects of TRH are CNS activating, it has been termed an ergotropic substance (Metcalf & Dettmar, 1981).

TRH increases locomotor activity as well as behaviors associated with arousal including 'wet-dog' shakes, paw tremor, tail elevation, piloerection, and grooming (Barlow, Cooper, Breese, Prange, & Lipton, 1975; Cowan & Vaught, 1983; Ervin, Schmitz, Nemeroff, & Prange, 1981; Holaday, Tseng, Loh, & Li, 1978; Martin, Dewey, Chan-Pham, & Prange, 1977; Masserano & King, 1976; Piva & Steiner, 1972; Schenkel-Hulliger, Koella, Hartmann, & Maitre, 1974; Segal & Mandel, 1974; Simon, Goujet, & Boissier, 1975; Vogel, Cooper, Barlow, Prange, Mueller, & Breese, 1978; Wei, Segal, Loh & Way, 1975). Since these effects are also observed following activation of the dopamine system (Manberg, Nemeroff, & Prange, 1979), the relationship

between TRH and the dopamine system were evaluated in this context. Although dopamine receptor antagonism suppresses TRH-induced hyperactivity (Miyamoto & Nagawa, 1975), the pattern and magnitude of effects associated with behavioral arousal were found to be different in amphetamine-treated and TRH-treated animals (Ervin et al., 1981). Further, anorexia elicited by TRH and amphetamine are mediated by different neurochemical substrates (for review see: Prange et al., 1979). Therefore, the behavioral arousal produced by TRH does not generally appear to be due to TRH interactions with the dopaminergic system. The role of TRH in the mediation of temperature regulation has been extensively studied. TRH produces hyperthermia in rats (Boschi & Rips, 1981; Brown, Rivier, & Vale, 1977; Cohn, Cohn, Krzysik, & Taylor, 1976; Goujet, Simon, Chermat, & Boissier, 1975; Prasad, Matsui, Williams, & Peterkofsky, 1978; but see Kasson & George, 1983) which appears to be mediated by activation of the medial preoptic nucleus (Boschi & Rips, 1981). TRH hyperthermia does not appear to be an epiphenomenon of hyperactivity (Boschi & Rips, 1981). Like many other peptides, transmitters, and pharmacological agents, TRH effects upon thermoregulation are species-specific since it increases core temperature in rabbits (Carino, Smith, Weick, & Horita, 1976; Horita & Carino, 1975) and decreases core temperature in cats (Metcalf, 1974; Myers,

Metcalf, & Rice, 1977). TRH increases mean arterial pressure (MAP) in rats through central and peripheral mechanisms of action (Beale, White, & Huang, 1977; Holaday, 1984; Holaday, D'Amato, & Faden, 1981). Finally, central TRH produces systemic hyperglycemia in rats (Brown, 1981) and systemic hypoglycemia in mice (Amir, Rivkind, & Harel, 1985).

TRH also acts as a CNS neuromodulator as seen by its modification of the behavioral effects of other agents. For instance, TRH antagonizes narcosis and hypothermia induced by pentobarbital, other barbiturates, and ethanol (Breese, Cott, Cooper, Prange, & Lipton, 1974; Breese, Cott, Cooper, Prange, Lipton, & Plotnikoff, 1975; Horita, Carino, & Chesnut, 1976; Prange, Breese, Cott, Martin, Cooper, Wilson, & Plotnikoff, 1974; Prange, Breese, Jahnke, Martin, Cooper, Cott, Wilson, Alltop, Lipton, Bissette, Nemeroff, & Loosen, 1975). This analeptic effect of TRH is not mediated through the pituitary-thyroid axis since it occurs in hypophysectomized animals (Breese et al., 1975). TRH also antagonizes the muscle-relaxant, sedative, and hypothermic effects of chlorpromazine (CPZ); this effect is potentiated in hypophysectomized and thyroidectomized animals (Kruse, 1975). Finally, TRH is active in pharmacological screens for anti-depressant drugs, potentiating the stimulatory effects of l-Dopa in pargyline-treated mice (Huidobro-Toro, Scotti De Carolis,

& Longo, 1974; Plotnikoff, Prange, Breese, Anderson, & Wilson, 1972; Plotnikoff, Breese, & Prange, 1975) and reversing reserpine-induced behavioral depresssion in mice (Morgan, Bower, Dettmar, Metcalf, Schafer, & Brown, 1979; Brewster, Dettmar, Lynn, Metcalf, Morgan, & Rance, 1980). Again, these TRH effects are not dependent on the pituitary-thyroid axis (Plotnikoff et al., 1972; Plotnikoff et al., 1975).

Thus, TRH produces behavioral effects itself and by modulating other systems. These effects are frequently independent of its hypophysiotropic role. The following evidence shows that TRH also plays a neuromodulatory role in opiate and analgesic responses.

C. TRH Modulation of Analgesic and Opiate Responses

Although most studies failed to observe changes in pain thresholds following either central or peripheral administration of TRH (Holaday et al., 1978; Kasson & George, 1983; Martin et al., 1977; Nemeroff, Osbahr, Manberg, Ervin, & Prange, 1979; Osbahr, Nemeroff, Luttinger, Mason, & Prange, 1981), short-acting (5-15 min) effects were observed in mice on visceral and pressure pain tests, but not on thermal pain tests (Boschi, Desiles, Reny, Rips, & Wrigglesworth, 1983). Firther, central injection of TRH into the PAG or spinal cord increased tail-flick latencies for up to 15 min (Griffiths, Slater, & Webster, 1981; Watkins, Suberg,

Thurston, & Culhane, 1986; Webster, Griffiths, & Slater, 1983; but see Sullivan & Pert, 1981).

Although TRH does not bind to opiate receptors (Holaday et al., 1978; Martin et al., 1977; Tache, Lis, & Collu, 1977), it reverses a number of opiate effects. Holaday and co-workers have termed it a physiologic opiate antagonist because of its naloxone-like effects in improving cardiovascular functioning following hemorrhagic and endotoxic shock (Holaday et al., 1981). TRH also reverses such opioid actions as morphine hypothermia, catalepsy, and respiratory depression, and beta-endorphin hypothermia, catalepsy, hypoactivity, and increases in growth hormone secretion (Holaday et al., 1978; Horita et al., 1976; Kasson & George, 1983; Tache et al., 1977). "Wet dog shakes", which occur during opiate withdrawal, can be elicited by central injection of either TRH or naloxone into the same brain areas (Wei et al., 1975). This effect is blocked by pretreatment with morphine, CPZ, apomorphine, and delta-9-tetrahydrocannabinol (Martin et al., 1977). However,TRH fails to elicit other morphine withdrawal symptoms like jumping behavior and weight loss in mice (Martin et al., 1977).

Although TRH appears to act generally as a physiological opiate antagonist, its effects upon opioid analgesia have ranged from potentiation (Holaday & Faden, 1983; Watkins et al., 1986), to antagonism (Watkins et

al., 1986), to a failure to observe changes (Holaday et al., 1978; Kasson & George, 1983; Martin et al., 1977; Osbahr et al., 1981). Further, while TRH, like naloxone, inhibits tolerance to the analgesic effects of morphine (Bhargava, 1981), TRH antagonizes naloxone hyperalgesia (Rips, Reny, & Desiles, 1983). Finally, TRH blocks neurotensin analgesia in mice on the hot plate, tail immersion, and acetic acid writhing tests (Osbahr et al., 1981). Although neurotensin analgesia is not decreased by naloxone (Clineschmidt, Martin, & Veber, 1982; Clineschmidt, McGuffin, & Bunting, 1979; Osbahr et al., 1981; but see Yaksh, Schmauss, Micevych, Abay, & Go, 1982) it is cross tolerant with morphine (Luttinger, Burgess, Nemeroff & Prange, 1983), indicating that the interaction between opiates and neurotensin occurs at some point beyond the opiate receptor.

In conclusion, TRH appears to be a weak analgesic when given alone. While TRH has been termed a physiologic opiate antagonist because it reverses a number of opiate responses (Holaday et al., 1981), it produces inconsistent effects upon opiate analgesia. It does, however, appear to antagonize neurotensin analgesia. Thus, TRH has some neuromodulatory effects on pain inhibitory systems, the nature of which are unclear at present.

D. Endogenous Pain Inhibitory Systems

The initial gate control model of centrifugal pain

inhibition (Melzack and Wall, 1965) was supported by subsequent findings that analgesia could be elicited by electrical brain stimulation (see Mayer and Price, 1976, for review) or morphine microinjection (Yaksh and Rudy, 1978). Further support for opioid-mediated pain inhibition includes observations that central administration of either B-endorphin (Jacquet & Marks, 1976; Bloom & Segal, 1976) or the enkephalins (Belluzi, Grant, Garsky, Sarantakis, Wise, & Stein, 1976; Pert, Pert, Chang, & Fong, 1976) produced analgesia which was eliminated by pretreatment with the opiate antagonist naloxone (Belluzi et al., 1976; Jacquet & Marks, 1976). Basbaum and Fields (1978; 1984) have proposed models of an endogenous opioid-mediated pain inhibitory system in which the PAG, rostral and ventral medullary (RVM) nuclei, and the dorsal horn of the spinal cord play integral roles. Several lines of evidence strongly implicate these areas in pain inhibition. Both electrical stimulation or morphine microinjection into the PAG or the RVM produce profound analgesia as does opiate microinjection directly into the subarachnoid space of the spinal cord (Akaike, Shibata, Satoh, & Takagi, 1978; Basbaum, Marley, O'Keefe, & Clanton, 1977; Dickenson, Oliveras, & Besson, 1979; Lewis & Gebhart, 1977; Mayer, Wolfe, Akil, Carder, & Liebeskind, 1971; Murfin, Bennett, & Mayer, 1976; Oleson & Liebeskind, 1975; Proudfit & Anderson, 1975; Reynolds, 1969; Yaksh,

1978). Both opioid peptides and opioid receptors are localized in the PAG (see Basbaum & Fields, 1984, for review), the RVM (Beitz, 1982a), and in lamina I, II, and IV of the dorsal horn of the spinal cord (see Basbaum & Fields, 1984, for review). Since the PAG and RVM each receive ascending nociceptive information, Basbaum & Fields (1984) postulated that an opioid inhibitory interneuron (Nicoll, Alger, & Nicoll, 1980) in the PAG may disinhibit tonic PAG efferents. Since both electrical stimulation of and opiate microinjection into the PAG excite NRM neurons (Oleson & Liebeskind, 1975; Oleson, Twombly, & Liebeskind, 1978) and inhibit the firing of dorsal horn neurons (LeBars, Menetrey, Conseiller, & Besson, 1974), these PAG output cells would produce analgesia by exciting the RVM which in turn in turn would inhibit neurons in the dorsal horn of the spinal cord (Basbaum & Fields, 1984). Indeed, the RVM receives PAG projections (see Basbaum & Fields, 1984, for review; Beitz, 1982b) and in turn projects to the dorsal horn of the spinal cord through the dorsolateral funiculus (DLF) (Basbaum & Fields, 1984). Though their original model (Fields & Basbaum, 1978) concentrated on the role of medullary 5-HT-containing projections exerting control over spinal nociceptive neurons, other peptides, including TRH have recently been localized in cells in the RVM. These cells are either interneurons or project to the

spinal cord (Bowker, Steinbusch, & Coulter, 1981; Hokfelt, Terenius, Kuypers, & Dann, 1979). Thus, the heterogenous population of neurons in the RVM (especially those 5-HT and SP-containing neurons that project to the dorsal horn) appear to provide descending control of pain inhibition. However, since RVM neurons containing TRH project to the ventral horn (Bowker et al., 1982; Gilbert et al., 1982; Johannson et al., 1981), it would appear that any invovlement of TRH in opioid analgesia would not act through descending TRH-containing neurons.

Studies with stimulation produced analgesia (SPA) (Cannon, Prieto, Lee, & Liebeskind, 1982) and stress induced analgesia (SIA) provide evidence that both opioid-mediated and non-opioid-mediated pain inhibitory systems exist (Amir & Amit, 1978; Bodnar, Kelly, Spiaggia, Ehrenberg, & Glusman, 1978b; Lewis, Cannon, & Liebeskind, 1980; Grau, Hyson, Maier, Madden, & Barchas, 1981; Watkins & Mayer, 1982b), which have different pharmacological, hormonal, and neural profiles (see Bodnar, 1984, for review). Analgesia induced by two forms of footshock and cold water swims will be reviewed in this section.

1. Analgesia Induced by 20 and 80 Footshocks: Variation in the number of inescapable shocks appears to activate opioid or non-opioid forms of analgesia. Rats receiving 5-sec inescapable tailshocks delivered on a variable interval 1 min (VI-1) schedule displayed

naltrexone reversible analgesia on the tail-flick test after 60 or 80 shocks, but not after 20 shocks (Grau et al., 1981; Hyson, Ashcraft, Drugan, Grau, & Maier, 1982). Lesions placed in the DLF of the spinal cord, but not decerebration, eliminated both forms of tailshock analgesia, indicating that the substrate of these effects originates in the caudal brain stem and is dependent on the DLF for expression (Watkins, Drugan, Hyson, Moye, Ryan, Mayer, & Maier, 1984a).

2. Forepaw and Hindpaw Shock Analgesia: Variation in the region of the body that is shocked also differentially activates opioid or nonopioid forms of analgesia which do not have a hormonal component. Rats receiving shock (1.6 mA for 90 sec) displayed naloxone-reversible analgesia on the tail-flick test when the shock was delivered to the forepaws, but not the hindpaws (Watkins, Cobelli, Faris, Aceto, & Mayer, 1982a). Further, analgesia induced by forepaw, but not hindpaw shock developed cross-tolerance with morphine analgesia (Watkins et al., 1982a). Neither forepaw nor hindpaw shock analgesia is dependent upon hormonal factors since they are not significantly decreased by either hypophysectomy, adrenalectomy, dexamethasone pretreatment, or inhibition of sympathic-medullary activity (Watkins, Cobelli, Newsome, & Mayer, 1982d). The importance of spinal opioids is observed

by the ability of intrathecal naloxone to reduce forepaw, but not hindpaw shock analgesia (Watkins & Mayer, 1982a). Both analgesic responses are dependent upon some spinal influences since either DLF lesions or spinal transections reduce forepaw and hindpaw shock analgesia (Watkins, Cobelli, & Mayer, 1982c). From these data it was hypothesized that hindpaw analgesia is also dependent upon an intraspinal component (Watkins et al., 1982c). Lesions placed in the medulla indicated that the primary locus of forepaw shock analgesia is the nucleus raphe magnus and palladus (Watkins, Young, Kinscheck, & Mayer, 1983b). In contrast, hindpaw shock analgesia is only partially mediated by medullary raphe nuclei and involves some other, as yet unknown brainstem site (Watkins et al., 1983b). Neither manipulation is affected by rostral or caudal PAG lesions or by decerebration, indicating that midbrain and forebrain areas appear not to be necessary for these analgesic effects (Watkins, Kinscheck, & Mayer, 1983a). Thus, forepaw shock analgesia is dependent on pathways descending in the DLF from the medullary raphe nuclei and is mediated by an opioid synapse in the spinal cord. Hindpaw shock analgesia is mediated by descending pathways in the DLF which originate only partly in the medullary raphe nuceli, and by intraspinal pathways and is not dependent upon an opioid synapse in the spinal cord.

The muscarinic receptor antagonist scopolamine,

but not its quartenary derivative methylscopomaine decreases hindpaw, but not forepaw, shock analgesia (Watkins, Katayama, Kinscheck, Mayer, & Hayes, 1984b). Further, the site of action appears to be supraspinal since intrathecal scopolamine failed to decrease hindpaw shock analgesia (Watkins et al., 1984b).

3. Cold Water Swim (CWS) Analgesia: Analgesia can also be produced by cold water swims across a range of swim temperatures. Further, the pharmacological profile of CWS analgesia differs from footshock analgesia. A 3.5 min swim in 2°C water produced analgesia in rats on the flinch-jump, tail flick, and tail-pinch tests for at least 1 hour (Bodnar, Kelly, & Glusman, 1978a). The analgesic effects of CWS do not appear to be a result of CWS hypothermia since chronic CWS results in adaptation of the analgesic, but not the hypothermic, effects of CWS (Bodnar, Kelly, Spiaggia, and Glusman, 1978c). Continuous CWS analgesia is not cross-tolerant with morphine (Bodnar, Kelly, Steiner, & Glusman, 1978d; Girardot & Holloway, 1984b) and is only partially decreased by a high dose of naloxone (Bodnar et al., 1978b) or naltrexone (Girardot & Holloway, 1984a), suggesting that this analgesia is mediated through a non opioid pain-inhibitory system. There are a number of other differences between opiate and CWS analgesia including the finding that the putative anti-enkephalinase D-phenylalanine potentiates morphine

analgesia (Alleva, Castellano, & Oliverio, 1980) but reduces CWS analgesia (Bodnar, Lattner, & Wallace, 1980b). Further, naloxazone, a high affinity opiate antagonist (Pasternak, Childers, & Snyder, 1980) reduces morphine analgesia but potentiates CWS analgesia (Kirchgessner, Bodnar, & Pasternak, 1982). The opiate nature of CWS analgesia can be changed by increasing the swim temperature. While continuous swims in 2°C or 8°C baths produces non opiate analgesia, naloxone can eliminate swim analgesia at a bath temperature of 15°C (Bodnar & Sikorzsky, 1983).

CWS analgesia has a hormonal component since it is decreased by hypophysectomy (Bodnar, Glusman, Brutus, Spiaggia, & Kelly, 1979a). Bodnar and co-workers (Bodnar, Sharpless, Kordower, Potegal, & Barr, 1982) suggest that glucocorticoids may inhibit CWS analgesia since adrenalectomy (Glusman, Bodnar, Mansour, & Kelly, 1980) and corticosteroid synthesis inhibtion (Mousa, Miller, & Couri, 1981a) potentiate CWS analgesia and the synthetic glucocorticoid dexamethasone decreases CWS analgesia (Mousa, Miller, & Couri, 1981b). Further, Bodnar et al. (1982) suggest that the anterior pituitary may be the site of glucocorticoid inhibition since dexamethasone still decreases CWS analgesia in adrenalectomized rats (Marek, Ponocka, & Hartmann, 1982) and removal of the intermediate and posterior lobes of the pituitary does not decrease CWS

analgesia (Glusman, Bodnar, Kelly, Sirio, Stern, & Zimmerman, 1979). The sympathomedullary system does not seem to be involved in CWS analgesia as adrenal demedullation did not affect CWS analgesia following 2°C or 15°C swims (Bodnar et al., 1982).

Information regarding the neural component of CWS analgesia is derived mostly from neuropharmacological manipulations. For instance, noradrenergic systems have been implicated in CWS analgesia as locus coeruleus lesions decrease CWS analgesia (Bodnar, Wallace, Kordower, Kirchgessner, Simone, Merrigan, Scalisi, and Lattner, 1980c) and clonidine, an alpha noradrenergic receptor agonist, potentiates CWS analgesia (Bodnar, Merrigan, & Sperber, 1983a). Further, acute desipramine, which blocks the reuptake of NE, also potentiates CWS analgesia (Bodnar, Mann, & Stone, 1985). Dopamine may play an antagonistic role in CWS analgesia since dopamine receptor stimulation with apomorphine decreases CWS analgesia (Bodnar, Kelly, Brutus, Greenman, & Glusman, 1980a) and dopamine receptor blockade with CPZ potentiates CWS analgesia (Bodnar & Nicotera, 1982). There is also cholinergic involvement in CWS analgesia as both scopolamine and methylscopolamine eliminated CWS analgesia on the flinch-jump test, though not on the tail-flick test (Sperber, Kramer, & Bodnar, in press). Finally, Brattleboro rats genetically deficient in vasopressin have

attenuated CWS analgesia (Bodnar, Zimmerman, Nilaver, Mansour, Thomas, Kelly, & Glusman, 1980d). In contrast, GABA and 5-HT do not appear to be involved in CWS analgesia (Bodnar & Sperber, 1982; Bodnar, Kordower, Wallace, & Tamir, 1981).

E. Rationale

The purpose of this dissertation was to examine whether TRH exerts modulatory actions upon different forms of stress-induced analgesia, and if so, to determine some mechanisms of action. To this end, five studies were performed. First, the dose-dependent effects of central administration of TRH upon analgesia induced by 20 and 80 footshocks were examined on two pain tests, the tail-flick test and the hypertonic saline writhing test. Second, the dose-dependent effects of TRH upon forepaw and hindpaw shock analgesia were examined on the tail-flick test following intracerebroventricular (ICV) and intravenous (IV) administration in order to assess the central versus peripheral actions of TRH upon these forms footshock analgesia. Third, the dose-dependent effects of TRH on CWS analgesia across a range of swim temperatures were evaluated following ICV and IV routes of administration on two pain tests, the tail-flick test and the jump test. Core temperatures were also monitored to determine whether any TRH effects upon analgesia or hypothermia are related or unrelated.

The fourth experiment compared the effects of the TRH metabolite histidyl-proline diketopiperazine (DKP) and the potent TRH analogue RX77368 to the effects of TRH on swim analgesia. This experiment was undertaken because of the suggestion that some of the effects of TRH may be due to its metabolism to DKP (Peterkofsky & Battaini, 1980; Prasad, Matsui, & Peterkofsky, 1977; Webster & Griffiths, 1983). For instance, DKP is a more effective antagonist of ethanol-induced sleep than TRH (Prasad et al., 1977; Breese et al., 1974). DKP and TRH each antagonize delta-9-tetrahydrocannabinol hypothermia (Bhargava, 1980) and cholesterol synthesis (Gebhard, Morley, Prigge, Goodman, & Prasad, 1981), as well as produce anorexia (Morley, Levine, & Prasad, 1981) and stereotypy following injection into the nucleus accumbens (Webster & Griffiths, 1983). However, TRH, but not DKP, produces hyperthermia in rabbits, reverses reserpine hypothermia in mice (Brewster et al., 1980), produces wet dog shakes in rats (Webster & Griffiths, 1983), and antagonizes pentobarbitol narcosis (Prasad et al., 1977). It was of interest to determine whether the TRH analogue RX77368 would produce more potent effects than TRH since it has a longer half-life in brain (190 versus 9 min) and plasma (390 versus 22 min) than TRH (Brewster, 1983). Metcalf (1983) found that RX77368 was 10-200 times more potent than TRH in reversing reserpine and clonidine hypothermia in mice, in reversing

barbiturate and ethanol sleeping time in mice, and in producing learned immobility and forepaw tremor in mice, and hyperthermia in rabbits. Like TRH, RX77368 antagonizes neurotensin hypothermia and analgesia in mice (Burgess, Luttinger, Hernandez, Kalivas, Nemeroff, & Prange, 1983). Further, while some actions of TRH may be due to its metabolism to DKP, any effects of RX77368 are probably due to TRH mimicry since RX77368 is not metabolized to DKP (Brewster, 1983; Brewster, Humphrey, & Wareing, 1981; Griffiths, McDermott, & Visser, 1983).

The fifth experiment examined the effects of TRH on analgesia induced by the muscarinic cholinergic agonist pilocarpine for the following reasons. Of the many neurotransmitters and peptides with which TRH interacts, recent research has emphasized interactions between TRH and cholinergic systems (see Yarbrough, 1983, for review). For instance, TRH analepsis is antagonized by muscarinic receptor antagonists (Breese et al., 1975; Horita et al., 1976; Nagai, Narumi, Nagawa, Sakurada, Ueno, & Ishii, 1980; but see Santori, Schmidt, Kalivas, & Horita, 1981). The septum appears to mediate TRH antagonism of pentobarbital narcosis in the rat (Kalivas & Horita, 1980), an effect which is eliminated by either the muscarinic receptor antagonist atropine or by lesions of septohippocampal fibers (Kalivas & Horita, 1983). Cholinomimetics in general and pilocarpine in particular

increase pain thresholds, effects which are blocked by the muscarinic receptor antagonist scopolamine (Houser, 1976; Houser & VanHart, 1974). Finally, the muscarinic receptor appears integral in several forms of SIA, including hindpaw shock analgesia and CWS analgesia, as seen by scopolamine antagonism of these forms of analgesia (Sperber et al., in press; Watkins et al., 1984b). Thus, this experiment will examine whether TRH interactions with the cholinergic system can be extended to TRH modulation of cholinergic analgesia. This experiment will also give a preliminary, indirect indication as to whether a TRH interaction with the cholinergic system may underlie TRH effects upon SIA.

GENERAL METHOD

Subjects: Female Sprague Dawley rats (230-330 g) were individually housed and were maintained on a 12 h light: 12h dark cycle with food and water available ad libitum.

Intracerebroventricular (ICV) Surgery: Rats in Experiments 1 through 5 received an intraperitoneal (IP) injection of chlorpromazine HCl (3mg/ml normal saline/kg body weight) followed 15 min later by an intramuscular (IM) injection of Ketamine HCl (95mg/ml sterile water/kg body weight). A stainless steel 22 gauge guide cannula (Plastic Products) was stereotaxically (Kopf) implanted so that its tip was positioned 0.3 mm above the left lateral

ventricle. With the incisor bar set at +5 mm, coordinates were 0.5 mm anterior to the bregma suture, 1.3 mm lateral to the sagittal suture and 3.6 mm from the top of the skull. The cannula was secured to three stainless steel screws with dental acrylic. All animals were allowed 10 days to recover from surgery before behavioral testing began.

Intravenous (IV) Surgery: Rats in Experiments 2 and 3 were anesthetized with Ketamine HCl (95 mg/ml sterile water/kg body weight, IM), which was supplemented with ether to maintain sufficient anesthesia. An incision was made in the right ventrolateral throat and the muscles were moved to expose the jugular vein. Then polyethylene tubing filled with saline was threaded into the right jugular vein as far as the right atrium and secured in place. The vein above the catheter was tied off to prevent blood loss. The other end of the tubing was threaded subcutaneously and pulled out through a small incision made in the back of the neck. Heparin was injected into the tubing to keep the blood from coagulating and the tubing was then tied off. All animals were allowed to recover for 48 hours before behavioral testing began.

Tail-Flick Latencies: The tail flick test, a somatic spinal pain test (Grossman, Basbaum, & Fields, 1982) was used in all experiments. Rats were tested for their

responsiveness to radiant heat in a modification of the procedure of D'Amour and Smith (1941). A radiant heat source (IITC Analgesia Meter) was mounted 8 cm above the dorsum and 4 cm proximal to the tip of the tail of a lightly restrained animal. A timer (0.01 sec) started and stopped respectively when the radiant heat was applied and when the rat flicked its tail to activate a photocell. Thus, the tail-flick latency is defined as the duration of the heat stimulus, in seconds. The intensity of the thermal stimulus was set so as to produce stable baseline tail-flick latencies between 2.5 and 3.5 sec. If a tail-flick response did not occur within 10 sec, the trial was automatically terminated to avoid tissue damage. Baseline tail flick latencies were determined over three trials spaced at 30 sec intervals.

Jump Thresholds: The jump test was used in Experiments 3, 4, and 5. Jump thresholds were determined using a modification of the procedure described by Evans (1961). The shock was delivered by a 60 Hz constant current shock generator (BRS/LVE) and grid scrambler (Campden Instruments) to a 30 cm by 24 cm floor composed of 16 grids. Using an ascending method of limits of successively more intense shocks, the jump threshold was defined in mA as the lowest of two consecutive intensities that elicited simultaneous withdrawal of both hindpaws from the grids. Each trial began with the animal receiving a 300 msec foot

shock at a current intensity of 0.1 mA. Subsequent shocks occurred at 10 sec intervals and were increased in equal 0.05 mA steps until the nociceptive threshold was determined. After each trial, the current intensity was reset to 0.1 mA for the next trial until 6 trials were completed. Jump thresholds were computed as the mean of these six trials and four days of stable baseline thresholds were determined for each animal.

Writhing Test: The writhing test was used in Experiment 1. The writhing response, which is a measure of visceral pain (Giesler & Liebeskind, 1976; Levine, Wilcox, Grace & Morley, 1982), was defined as an elongation and contraction of the abdominal wall following a hypertonic saline injection (1.5 ml 4% saline/kg body weight, IP). The latency to the first writhe and total number of writhes were determined over a 2 min period in a clear plastic cage. In addition, writhing duration was categorized as lasting for either less than or more than 4 sec.

Core Body Temperature: Rectal temperatures were determined in Experiments 3 and 4 by inserting a probe which was held in place until a stable reading (0.1^{o} C) was obtained from a digital thermometer (Bailey Instruments).

ICV and IV Administration: All doses of TRH (Peninsula Laboratories) were dissolved in 10 ul normal

saline for ICV injections and in 1 ml normal saline for IV injections. ICV injections were infused at a rate of 1 ul every 15 sec into the lateral ventricle using a microsyringe (Hamilton Company) which was connected to a stainless steel 28 guage internal cannula by polyethylene tubing (PE50, Fisher). The internal cannula extended 0.5 mm ventral to the guide cannula. IV TRH was injected into the tubing protruding from the back of the neck. The TRH injections were preceeded and followed by heparin injections to clear the tubing and to ensure that the drug reached the heart, respectively.

Histology: Following experimental testing, all animals with cannulae were anesthetized with sodium pentobarbital (100 mg/2ml normal saline/kg body weight, IP). Randomly selected animals were perfused through transcardiac puncture with 0.9% saline followed by 10% buffered formalin. Each brain was blocked, sliced in 40 um coronal sections through the lateral ventrical and stained with cresyl violet for cell body visualization. Sections were analyzed under the light microscope for cannula placement. The remainder had their brains removed and gross dissection through the cannula track was performed. Only those animals with cannula tips located in the lateral ventricle were included in the data analysis. Animals that had undergone IV catheter implantations were anesthetized following behavioral

testing with sodium pentobarbitol (100 mg/2ml normal saline/kg body weight, IP) and their incisions were opened to verify that the catheter had remained in the vein throughout the experiment.

Statistical Analysis: Split plot analyses of variance (ANOVA) were used to determine significant main effects and interactions. The Dunnett test was employed to compare experimental to control conditions. While post-hoc comparisons are frequently done when only one of the main effects or interactions are significant, the present comparisons were done in a more conservative manner since there is no previous data on the effects of TRH on stress-induced analgesia. Thus, comparisons between baseline and experimental conditions were done if time was significant, but comparisons between vehicle and drug groups were done only if the main effect between drug groups or the interaction between time and drug was significant. The motor effects were analyzed with the Chi Square (χ^2) test since this test is appropriate when there is no variability in some of the groups, which was the case for the vehicle-treated rats. For each animal, the presence or absence of a particular motor behavior during any of the test times was noted and used for the χ^2 analysis.

Experiment 1A: Effects of Central TRH Upon 20 and 80 Footshock Analgesia: Tail Flick Latencies.

While analgesia induced by 20 inescapable tail shocks is not affected by naltrexone, analgesia induced by exposure to 60 or 80 inescapable tail shocks appears naltrexone-sensitive (Grau et al., 1981). Since TRH alters both opioid-mediated and nonopioid-mediated responses (Holaday et al., 1978; Horita et al., 1976; Osbahr et al., 1981; Tache et al., 1977), the present experiment examined whether TRH would alter either of these forms of shock analgesia on the tail-flick test.

Method

Six groups of eight rats each were matched on the basis of baseline tail flick latencies. The first three groups received an ICV injection of either 0, 10, or 50 ug of TRH respectively. Immediately thereafter, each rat received 20 inescapable foot shocks (1 mA, 5 sec/min) delivered according to a variable interval (VI-1 min) schedule. Following the last foot shock, each rat was removed from the chamber and tail-flick latencies were assessed at 1-min intervals for 15 min and once again at 20 min after shock. The second three groups also received an ICV injection of either 0, 10, or 50 ug of TRH respectively. However, these three groups received 60 inescapable foot shocks (1 mA, 5 sec/min, VI-1 min) prior to injection and 20 inescapable foot shocks after the injection. Again, tail-flick latencies were assessed at 1-min intervals for 15 min and again at 20 min after the

last shock.

Results

20 Footshock Condition: Significant effects were observed across the time course ($F(18,756)=12.86$, $p<0.001$) among doses ($F(2,42)=4.77$, $p<0.014$) and for the interaction between test time and dose ($F(36,756)=1.74$, $p<0.005$) but not between the 20 and 80 foot shock conditions ($F(1,42)=1.98$). Following 20 shocks, tail-flick latencies were significantly elevated relative to pre-injection levels in vehicle-treated rats (1 and 2 min) and in rats receiving the 10 ug (1-4, 7, 8, and 10 min) and 50 ug (1-11) min doses of TRH (Dunnett comparison, $p<.05$). Figure 1 shows that significant potentiations in analgesia following 20 inescapable footshocks occurred in animals pretreated with the 10 ug (1 and 10 min) and 50 ug (1, 6, and 8-11 min) doses of TRH relative to vehicle-treated rats.

80 Footshock Condition: Tail-flick latencies were significantly increased relative to pre-injection levels following exposure to 80 inescapable shocks in vehicle-treated rats and in rats receiving the 10 ug (1 and 6 min) and 50 ug (1, and 3-20 min) doses of TRH. Figure 2 shows the significant potentiations in analgesia elicited by 80 foot shocks in rats receiving the 10 ug (3 and 6 min) and the 50 ug (1, and 3-14 min) doses of TRH relative to vehicle-treated rats.

Figure 1. Centrally administered TRH significantly potentiated analgesia induced by 20 footshocks on the tail-flick test as compared to vehicle treatment.
* Dunnett comparison, $p<0.05$.

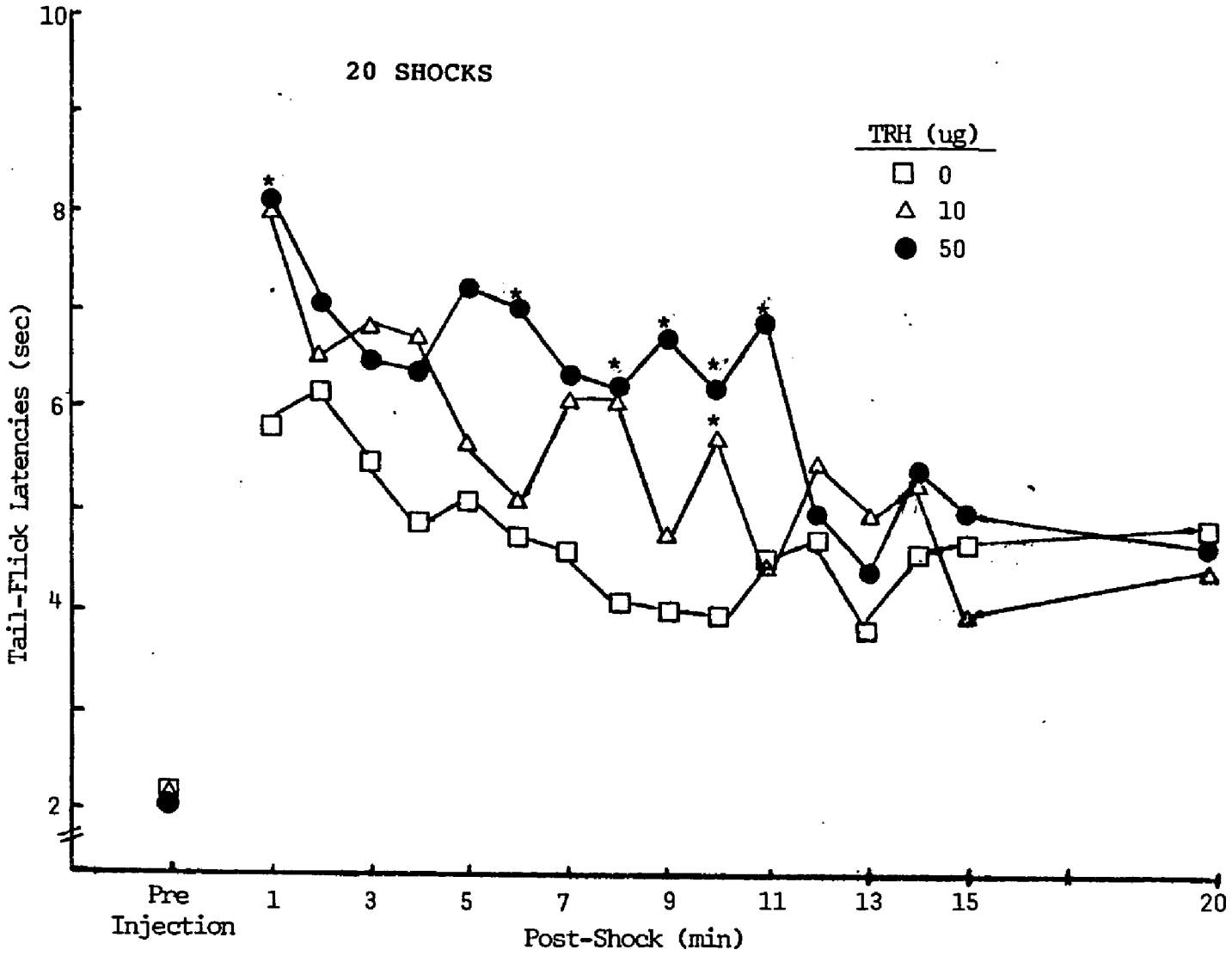
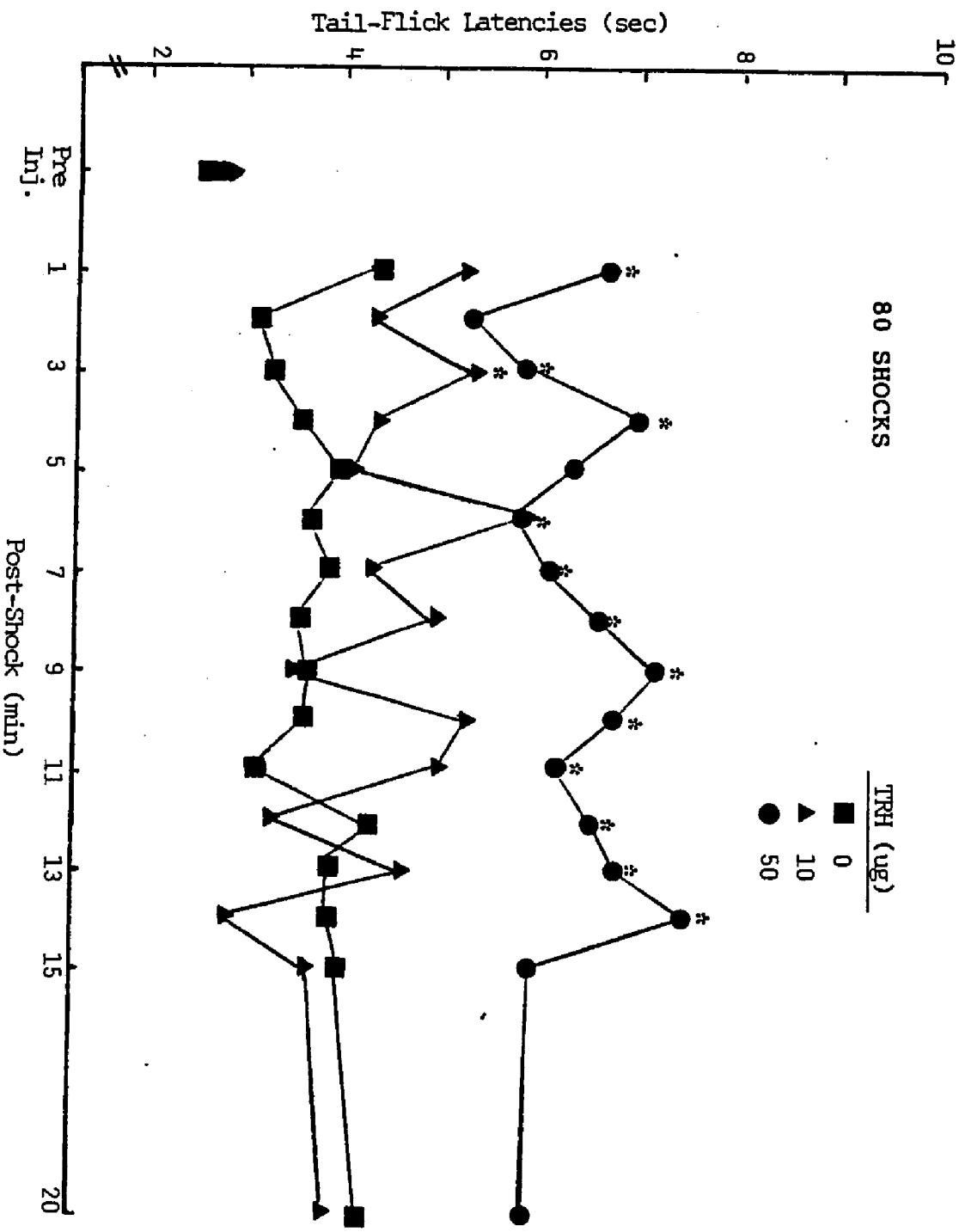

20 SHOCKS
TRH (ug)
0
10
50
Tail-Flick Latencies (sec)
10
8
6
4
2
Pre Injection
1
3
5
7
9
11
13
15
20
Post-Shock (min)

Figure 2. Centrally administered TRH significantly potentiated analgesia induced by 80 footshocks on the tail-flick test as compared to vehicle treatment.
* Dunnett comparison, $p<0.05$.


Tail-Flick Latencies (sec)
2
4
6
8
10
Pre Inj.
1
3
5
7
9
11
13
15
20
Post-Shock (min)
80 SHOCKS
TRH (ug)
0
10
50

Experiment 1B: Effects of Central TRH Upon 80 Footshock Analgesia: Writhing Test.

This experiment examined whether TRH-induced potentiations in analgesia on the tail-flick test elicited by 80 foot shocks would also occur on the writhing test, which measures reactivity to visceral nociceptive stimuli (Giesler & Liebeskind, 1976; Levine et al., 1982).

Method

One month after participating in Experiment 1A, sixteen randomly-chosen rats served as subjects in this experiment. All animals received an initial baseline writhing test. Four days later, two groups of eight rats received an ICV injection of either 0 or 50 ug of TRH respectively. Exposure to 60 inescapable foot shocks (1 mA, 5 sec/min, VI-1 min) preceded the injections and exposure to 20 inescapable foot shocks followed the injections. Immediately after the last footshock, the hypertonic saline (1.5 ml 4% saline/kg body weight, IP) was administered and the latency, number, and duration of writhing responses were determined.

Results

TRH significantly potentiated foot shock analgesia on the writhing test as measured by the number and duration, but not the latency of writhing responses. The number of writhes differed significantly between groups ($F(1,14)=12.65$, $p<0.003$) and approached significance

between baseline and experimental conditions ($F(1,14)=3.52$, $p<0.082$), but the interaction between conditions and groups failed to achieve significance ($F(1,14)=2.58$). While the number of writhes in the baseline (mean=5.0) and shock (mean=4.9) conditions of vehicle-treated rats failed to differ from each other, the number of writhes observed when TRH (50 ug) was paired with 80 inescapable footshocks (mean=2.5) was significantly lower than its corresponding baseline condition (mean=4.1). Significant changes in writhing duration were observed between groups following exposure to 80 foot shocks (Fisher Exact Test, $p<.025$); while seven of the eight vehicle-treated rats exhibited longer-lasting (>4 sec) writhes following 80 shocks, seven of the eight TRH-treated rats displayed shorter-lasting (<4 sec) writhes following 80 shocks. Significant differences in writhing latencies failed to occur between conditions ($F(1,14)=1.81$), between groups ($F(1,14)=1.35$), or for the interaction between conditions and groups ($F(1,14)=1.56$).

Experiment 1C: Effects of Naltrexone Upon Analgesia Induced by Morphine or 20, 40, 60, or 80 Footshocks.

In contrast to the previous use of tail shocks to elicit analgesia (Grau et al., 1981), the protocols of Experiments 1A and 1B employed foot shock to elicit analgesia. Therefore, this experiment examined whether

the opiate receptor antagonist naltrexone would differentially affect analgesia induced by 20 or 80 footshocks in the same manner as it antagonized analgesia induced by 80 but not 20 tail-shocks (Grau et al., 1981). To confirm the opioid activity of naltrexone in our test situations, its action upon morphine analgesia was also examined at the same time intervals.

Method

Two groups of eight rats each, matched on the basis of baseline tail-flick latencies, received a subcutaneous (SC) injection of either naltrexone (14 mg/ml normal saline/kg body weight) or saline respectively. Twenty min later, each rat received 80 inescapable footshocks (1 mA, 5 sec/min, VI-1 min). Tail-flick latencies were determined before shock and immediately following 20, 40, 60, and 80 footshocks. Two other groups of eight rats each, also matched for baseline tail-flick latencies, received either saline or a 14 mg/kg dose of naltrexone respectively, 20 min before administration of morphine (10 mg/ml normal saline/kg body weight, IP). Tail-flick latencies were then determined 20, 40, 60, and 80 min later.

Results

While footshock significantly increased tail-flick latencies above baseline levels ($F(4,56)=11.43$, $p<0.001$), significant differences failed to occur between

naltrexone-treated and saline-treated rats ($F(1,14)=0.15$) or for the interaction between drug groups and test conditions ($F(4,56)= 1.18$). This indicated that naltrexone failed to alter analgesia elicited by 20, 40, 60, or 80 foot shocks (Table 1A). In contrast, examination of naltrexone effects upon morphine analgesia revealed significant differences between groups ($F(1,14)=14.33$, $p<0.001$), between baseline and experimental conditions ($F(4,56)=23.24$, $p<0.001$) and for the interaction between groups and conditions ($F(4,56)=8.03$, $p<0.001$). Table 1B indicates that naltrexone significantly reduced morphine analgesia at 20, 40, 60, and 80 min following opiate administration.

Experiment ID: Effects of Central TRH Upon Tail-flick Latencies.

Although TRH generally fails to alter basal pain thresholds (Holaday et al., 1978; Martin et al., 1977; Nemeroff et al., 1979; Osbahr et al., 1981; Sullivan, & Pert, 1981), analgesic effects have been observed at short post-injection intervals (5-15 min) (Boschi et al., 1983; Griffiths et al., 1981; Webster et al., 1983). To examine whether the foregoing potentiations by TRH of footshock analgesia could be merely due to TRH-induced shifts in basal pain thresholds, this experiment examined whether central (ICV) pretreatment with TRH would alter baseline tail-flick latencies.

TABLE 1A

FAILURE OF NALTREXONE TO ANTAGONIZE
ANALGESIA PRODUCED BY 20, 40, 60, OR 80 FOOTSHOCKS

		(Number of Shocks)			
Group	Baseline	20	40	60	80
		Tail-Flick Latencies (sec)			
Sal + FS	2.92	7.05	4.93	6.79	5.96
Nalt + FS	2.63	6.57	5.65	6.46	8.19

TABLE 1B

NALTREXONE ANTAGONIZES MORPHINE ANALGESIA AT
20, 40, 60, AND 80 MIN FOLLOWING OPIATE ADMINISTRATION

		Post-Morphine Inj. (min)			
Group	Baseline	20	40	60	80
		Tail-Flick Latencies (sec)			
Sal + Mor	2.80	9.12	9.06	8.11	6.87
Nalt + Mor	2.76	4.23*	4.76*	3.89*	4.05

*Significantly different from Saline Condition (Dunnett Comparison, $p<0.05$).

Method

Eight rats, matched for baseline tail flick latencies, received three ICV injections of 0, 10, or 50 ug of TRH spaced at least 72 h apart. Half of the rats received the TRH an ascending series of doses and the remainder received a descending series of doses. Tail-flick latencies were determined 5, 10, 20, 30, and 40 min following each injection. This time course was chosen because it: a) overlapped the intervals between injection and experimental manipulations in the previous experiments and b) included intervals in which other have reported analgesic effects (Boschi et al., 1983; Griffiths et al., 1981; Webster et al., 1983).

Results

The results of this experiment were analyzed in three phases: a) 5 min following injection, b) 10 min following injection, and c) 20, 30, and 40 min following injection. The reason for this was that subsets of animals receiving the 10 ug (n=3 at 5 min; n=4 at 10 min) and the 50 ug (n=1 at 5 min; n=2 at 10 min) doses of TRH displayed elevations of the tail ('straub tail') which precluded accurate measures of latencies at these intervals. Table 2 summarizes the TRH-induced increases in tail flick latencies at 5, but not at 10, 20, 30, or 40 min following injection. At 5 min post-injection, there were significant differences between baseline and post-

TABLE 2

TRH ALONE AND TAIL-FLICK LATENCIES

		Post-Inj (min)				
TRH (ug)	Baseline	5	10	20	30	40
		Tail-Flick Latencies (sec)				
0	2.80	2.98	2.71	2.61	2.77	2.65
10	2.97	4.45*	3.81	2.72	2.28	2.14
50	2.53	3.63*	3.18	2.88	2.44	2.68

*Significantly different from Baseline (Dunnett Comparison, $p<0.05$).

Note: Three separate anovas were performed due to differential tail elevation in the animals. One was done for the 5 min data, one for the 10 min data, and a third for the 20, 30, and 40 min data. Thus, while the 10 ug dose appears to produce significant analgesia at 10 min, this was not significant due to the lack of animals that could be tested at that time.

injection tail-flick latencies ($F(1,17)=23.74$, $p<0.001$), among doses ($F(2,17)=6.41$, $p<0.008$), and for the interaction between time and dose ($F(2,17)=4.33$, $p<0.03$). Both the 10 and 50 ug doses of TRH significantly increased tail-flick latencies above baseline at 5 min post-injection. At 10 min post-injection, differences approached significance between baseline and post-injection tail-flick latencies ($F(1,15)=3.94$, $p<0.066$), and failed to achieve significance among doses ($F(2,15)=2.09$), or across time ($F(2,15)=1.65$). At 20, 30, and 40 min post-injection differences in tail-flick latencies approached statistical significance across the time course ($F(3,63)=2.27$, $p<0.089$) and for the interaction between time and dose ($F(6,63)=2.18$, $p<0.056$), and no significant difference was found between doses ($F(2,21)=0.24$). Thus, administration of TRH alone increased tail-flick latencies only at 5 min after injection.

Discussion

These data indicate that central TRH pretreatment significantly increased the magnitude and duration of analgesia produced by 20 or 80 footshocks on the tail-flick test and by 80 footshocks on the writhing test. Administration of TRH alone also increased tail-flick latencies but did so only at 5 min after injection, which agrees with previous reports (Boschi et al., 1983;

Griffiths et al., 1981; Watkins et al., 1986; Webster et al., 1983). Since TRH alone failed to alter tail flick latencies at times (20-40 min) when it potentiated footshock analgesia, this suggests synergistic action between TRH and these two parameters of footshock analgesia and not merely an additive effect of two potential analgesic mechanisms. The two forms of footshock analgesia that were potentiated by central TRH pretreatment are opioid-insensitive in that they failed to be affected by pretreatment with the opiate receptor antagonist naltrexone. The TRH-induced potentiation of these non-opioid forms of footshock analgesia stands in marked contrast to its antagonism of opiate-insensitive neurotensin analgesia (Osbahr et al., 1981).

The TRH potentiation of footshock analgesia does not appear to be due to an impairment in the animal's ability to respond to noxious stimuli. If this effect were the result of general incapacitation, one would also expect TRH to produce hypoactivity. Yet, TRH administration typically increases locomotor activity and other hyperactive responses (Barlow et al., 1975; Ervin et al., 1981). Indeed, agents that elicit hyperactivity do not necessarily potentiate stress-induced analgesia. The dopamine receptor agonist apomorphine produces hyperactivity but reduces stress-induced analgesia (Bodnar et al., 1980a). Amphetamine also produces hyperactivity

(Ervin et al., 1981) yet fails to alter stress-induced analgesia (Bodnar et al., 1980a). Further, the potentiation of footshock analgesia by TRH was demonstrated on two pain tests in opposite ways. Analgesia on the tail-flick test, a measure of reactivity to thermal stimuli, was defined in terms of increases in latency to respond. Analgesia on the writhing test, a measure of diffuse visceral discomfort, was defined in terms of decreases in the number and duration of writhes. It is difficult for nonspecific actions of TRH to explain both effects. Finally, the tail-flick test measures a spinally-mediated reflex (Grossman et al., 1982), which would appear to be less affected by supra-spinal attentional mechanisms.

It was of interest to examine the effects of TRH on opioid footshock analgesia since studies have either found that TRH has no effect (Holaday et al., 1978; Horita et al., 1976; Kasson & George, 1983: Martin et al., 1977; Osbahr et al., 1981), antagonizes (Watkins et al., 1986), or potentiates (Holaday, 1983; Holaday & Faden, 1983; Watkins et al., 1986) opiate analgesia. The purpose of testing TRH effects upon analgesia produced by 20 and 80 footshocks was to explore whether differentiations could be made in terms of the effects of TRH on opioid and non-opioid forms of pain-inhibition since analgesia induced by 80, but not by 20, inescapable tail shocks was reversed by

naltrexone pretreatment (Grau et al., 1981). This procedure, as opposed to different patterns of shock (see: e.g. Lewis et al., 1980) was used because it allowed injections of TRH or vehicle to be administered at identical intervals before shock for both shock conditions, thereby precluding confounding in terms of injection-test intervals. However, in contrast to tail shock analgesia, naltrexone failed to alter footshock analgesia after either 20, 40, 60, or 80 shocks. The possibility that the naltrexone used in this experiment was ineffective was controlled for by the observation that it significantly reduced morphine analgesia in the same injection and test protocol. In retrospect, the failure to replicate the tailshock findings of Grau and co-workers (1981) with footshock is not entirely surprising. Opiate mediation of tail shock analgesia is hypothesized to occur when the animal learns over time that the shock is unavoidable (e.g. after 80, but not 20 tail shocks) (Maier, Drugan, & Grau, 1982; Maier, Sherman, Lewis, Terman, & Liebeskind, 1983). Delivery of 80 tail shocks elicits an opioid form of analgesia because the electrodes are attached directly to the tail, making shock inescapable. However, during delivery of 80 foot shocks, the animal may exert some control over the shock by rearing, jumping, and rolling onto its side, among other responses (Maier, personal communication). Hence, tail

shock, over which the animal has no control, and footshock over which the animal might exert control, can conceivably produce different and separable forms of analgesia which could explain the failure to produce an opioid-sensitive analgesia with 80 footshocks. Thus, TRH is capable of potentiating non-opioid forms of footshock analgesia but the failure of naltrexone to antagonize either form of footshock analgesia precludes a statement about the effects of TRH on opioid stress-induced analgesia. Finally, since little work has been done on the mechanisms of action of 20 and 80 tailshock analgesia and since tailshock and footshock analgesia may be qualitatively different, it is difficult to speculate, based on the tailshock literature, on how TRH may be potentiating 20 and 80 footshock analgesia.

Experiment 2A: Effects of Central TRH Upon Forepaw and Hindpaw Footshock Analgesia.

The present experiment examined the effects of central TRH upon neurally-mediated opiate-sensitive forepaw and opiate-insensitive hindpaw footshock analgesia (Watkins et al., 1982a; 1982d). Since it was not possible to assess the effects of TRH on opioid analgesia in Experiment 1, the present experiment was also aimed at examining this. A within-subjects design was employed in which the effects of TRH upon forepaw shock analgesia was

assessed, followed three weeks later by assessment of TRH effects upon hindpaw shock analgesia. Since analgesia can be classically conditioned to forepaw shock (Watkins, Cobelli, & Mayer, 1982b), it was important to determine whether the present protocol produced any classical conditioning to forepaw shock, thereby inadvertantly affecting hindpaw shock analgesia. Hence, three conditions were examined: forepaw shock, the forepaw shock paradigm in the absence of shock (conditioning test), and hindpaw shock.

Method

In the first condition, three groups of eight rats, matched for baseline tail flick latencies, received an ICV injection of either 0, 10, or 50 ug of TRH respectively. Twenty min following injection, each rat was suspended in the experimental chamber by a piece of cloth tied around the hindquarters to insure that only the forepaws came in contact with the grids. Shock (1.6 mA) was delivered continuously to the forepaws for 90 sec (e.g. Watkins et al., 1982a). Tail-flick latencies were determined at 0, 1, and 2 min and again at 2-min intervals for up to 20 min following shock. In the second condition, which occurred two weeks later, twelve rats were chosen randomly from the three groups. Following baseline tail-flick latency determinations, each rat was suspended in the experimental chamber for 90 sec by a piece of cloth so that only the

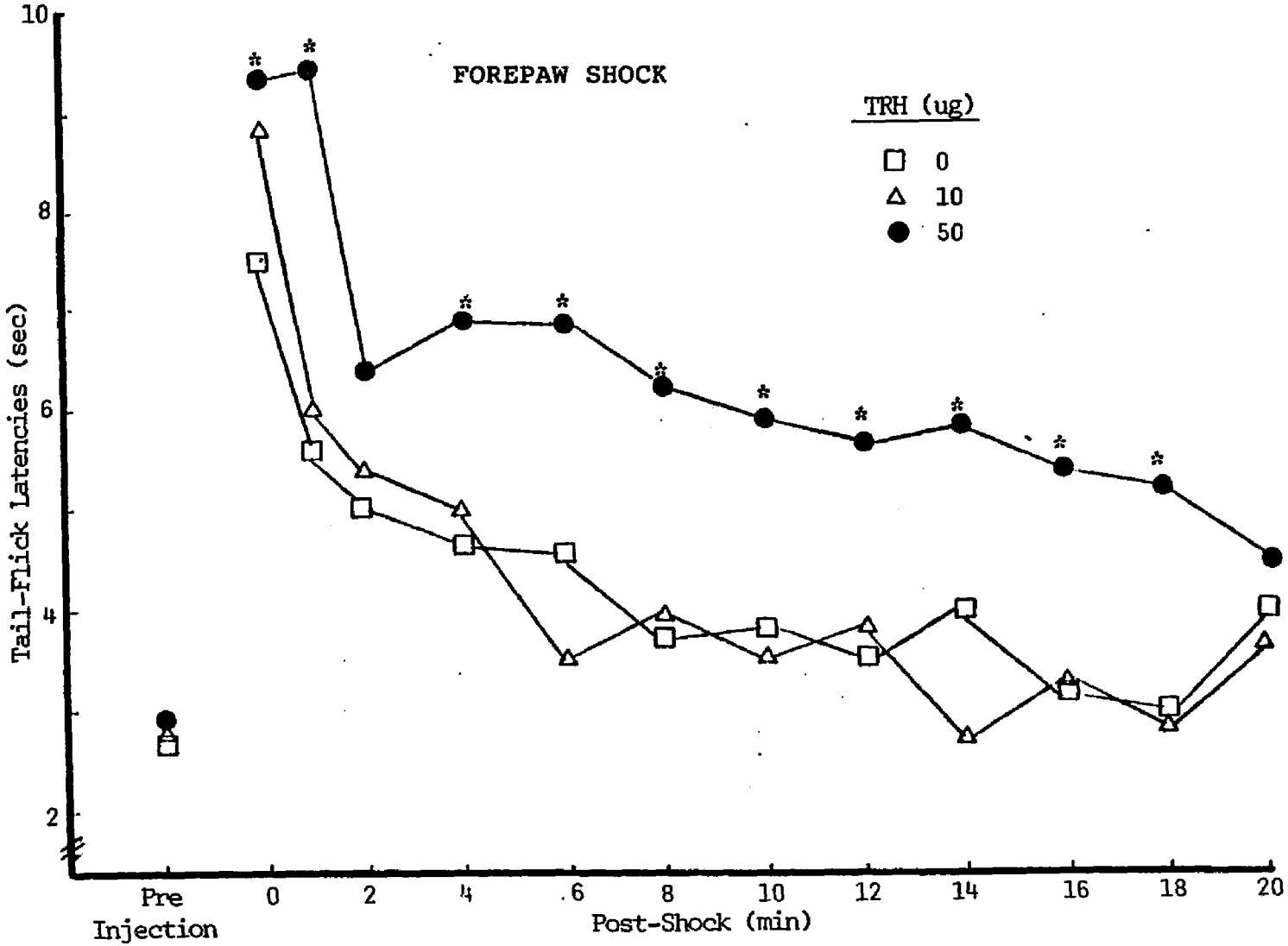
forepaw were in contact with the grid, in the absence of shock, but in the presence of all of the other environmental cues. The remaining twelve rats were treated identically except that all four paws came in contact with the grids. Tail-flick latencies were determined across the same time course. In the third condition, which occurred one week later, the three groups received an icv injection of either 0, 10, or 50 ug of TRH respectively. Twenty min later, each rat suspended by a piece of cloth tied around the chest to insure that only the hindpaws came in contact with the grids. Shock (1.6 mA) was delivered continuously to the hindpaws for 90 sec. The same time course of tail-flick latency determinations was then performed.

Results

Forepaw Shock Condition: Figure 3 displays the significant potentiation in the magnitude of forepaw shock analgesia following central pretreatment with the 50, but not the 10 ug dose of TRH. Significant differences were observed across the time course ($F(12,252)=22.14$, $p<0.001$), among doses ($F(2,21)=9.02$, $p<0.002$), but not for the interaction between test times and doses ($F(24,252)=1.29$). Exposure to forepaw shock significantly elevated tail-flick latencies over pre-injection levels for 2 min following shock in vehicle-treated rats, for 4 min following shock in rats receiving the 10 ug TRH dose,

Figure 3. Centrally administered TRH significantly potentiated forepaw shock analgesia on the tail-flick test as compared to vehicle treatment.

* Dunnett comparison, $p<0.05$.


FOREPAW SHOCK
TRH (ug)
0
10
50
Tail-Flick Latencies (sec)
10
8
6
4
2
Pre
Injection
0
2
4
6
8
10
12
14
16
18
20
Post-Shock (min)

and for 18 min following shock in rats receiving the 50 ug TRH dose. Rats receiving the 10 ug TRH dose failed to display any changes in forepaw shock analgesia relative to vehicle-treated rats. In contrast, rats receiving the 50 ug dose of TRH displayed significant potentiations in forepaw shock analgesia relative to vehicle-treated rats for up to 18 min (1, and 3-18 min) after shock.

Classical Conditioning Test: Figure 4 illustrates the transient increase in tail-flick latencies elicited by exposure to the shock-related environmental cues in the absence of shock. Significant differences occurred across the time course ($F(12,264)=3.13$, $p<0.001$), but not between conditions ($F(1,22)=0.28$), or for the interaction between time and condition ($F(12,264)=1.10$). Contact of all four paws with the grids in the absence of shock significantly increased tail-flick latencies over baseline values immediately following exposure. Elevation of the hindpaws and contact of the forepaws with the grids in the absence of shock increased tail-flick latencies above baseline for up to 6 min (0, and 2-6 min) following exposure.

Hindpaw Shock Condition: Figure 5 illustrates the failure of central TRH to potentiate hindpaw shock analgesia. Significant differences were observed across the time course ($F(12,252)=18.96$, $p<0.001$), approached statistical significance between doses ($F(2,21)=2.94$, $p<0.075$) but failed to occur for the interaction between

Figure 4. Exposure to the conditioned stimulus produced transient significant increases in tail-flick latencies as compared to baseline levels.

* Dunnett comparison, $p<0.05$.

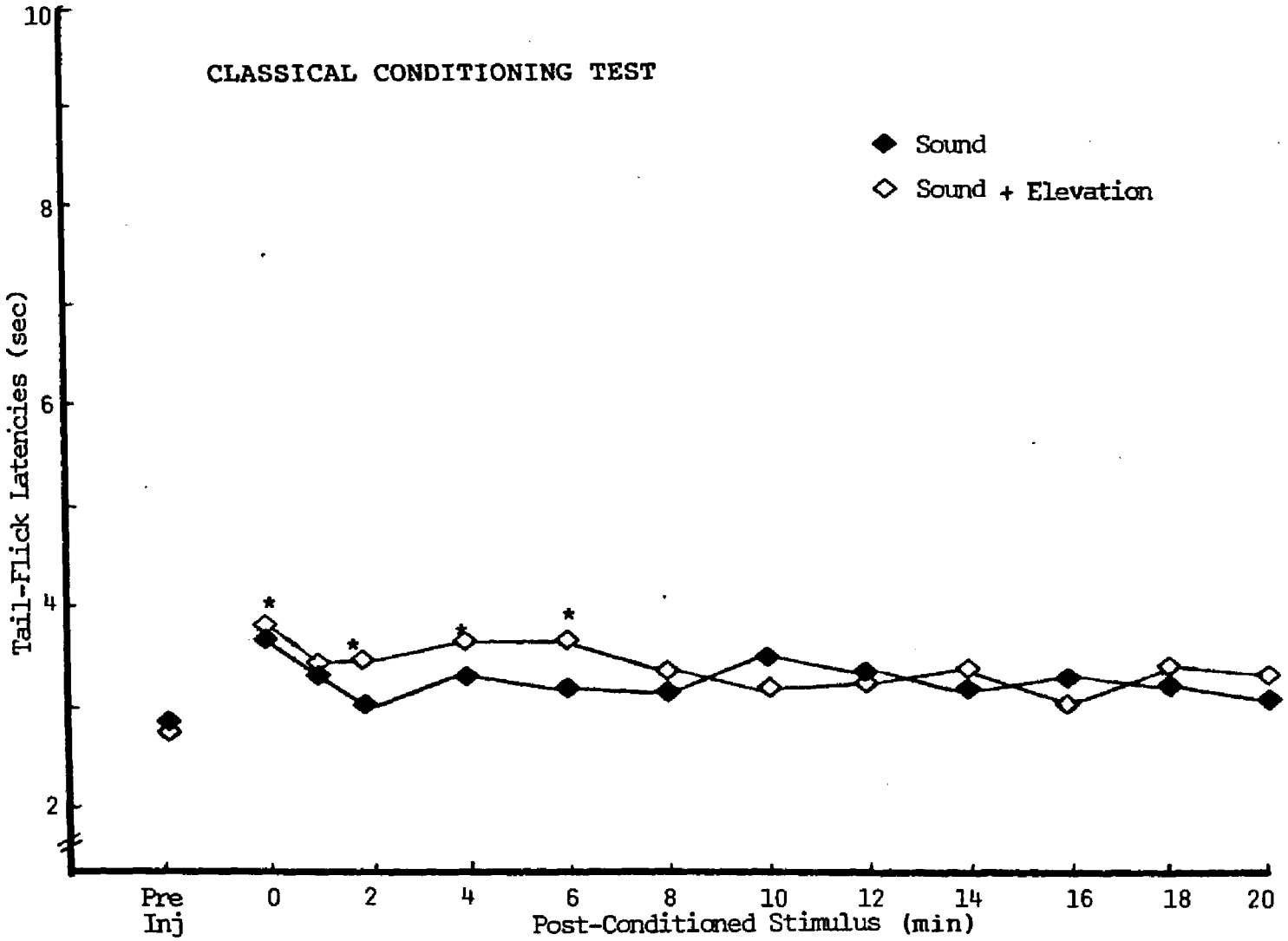
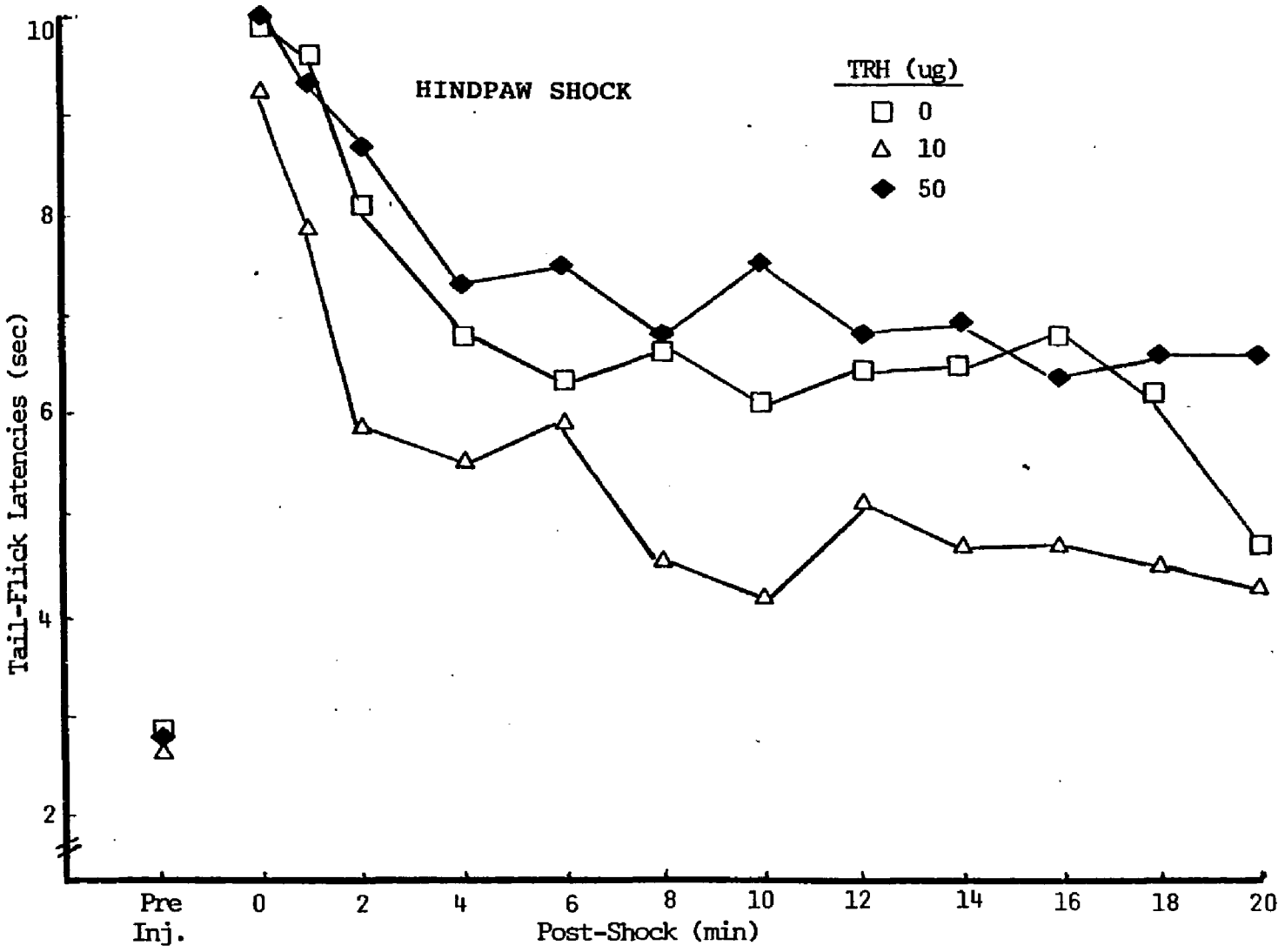

CLASSICAL CONDITIONING TEST
Sound
Sound + Elevation
Tail-Flick Latencies (sec)
10
8
6
4
2
Pre Inj
0
2
4
6
8
10
12
14
16
18
20
Post-Conditioned Stimulus (min)

Figure 5. Centrally administered TRH failed to potentiate hindpaw shock analgesia on the tail-flick test as compared to vehicle treatment.


HINDPAW SHOCK
TRH (ug)
0
10
50
Tail-Flick Latencies (sec)
10
8
6
4
2
Pre Inj.
0
2
4
6
8
10
12
14
16
18
20
Post-Shock (min)

test times and doses ($F(24,252)=.69$). Exposure to hindpaw shock significantly elevated tail flick latencies relative to pre-injection levels for 18 min following shock in vehicle-treated rats, for 6 min following shock in rats receiving the 10 ug dose of TRH, and for 20 min following shock in rats receiving the 50 ug dose of TRH. Differences between doses failed to occur as indicated by the lack of a significant effect among doses or for the interaction between time and dose.

Experiment 2B: Effects of Naloxone Upon the Interaction Between Central TRH and Analgesia Induced by Forepaw or Hindpaw Shock.

Although Watkins and Mayer (1982b) demonstrated that opiate-sensitive and opiate-insensitive forms of analgesia could be induced by forepaw and hindpaw shock respectively, a subsequent study (Cannon, Terman, Lewis, & Liebeskind, 1984) found that the opioid nature of forepaw shock could be eliminated by subtle changes in shock intensity. Thus, this experiment had two aims: 1) to determine the effects of naloxone upon forepaw and hindpaw shock analgesia using seemingly identical parameters to that of Watkins and Mayer (1982b) in our shock apparatus, and 2) to examine the effects of naloxone on TRH-induced changes in forepaw and hindpaw shock analgesia.

Method

Four groups of eight rats each, matched for baseline

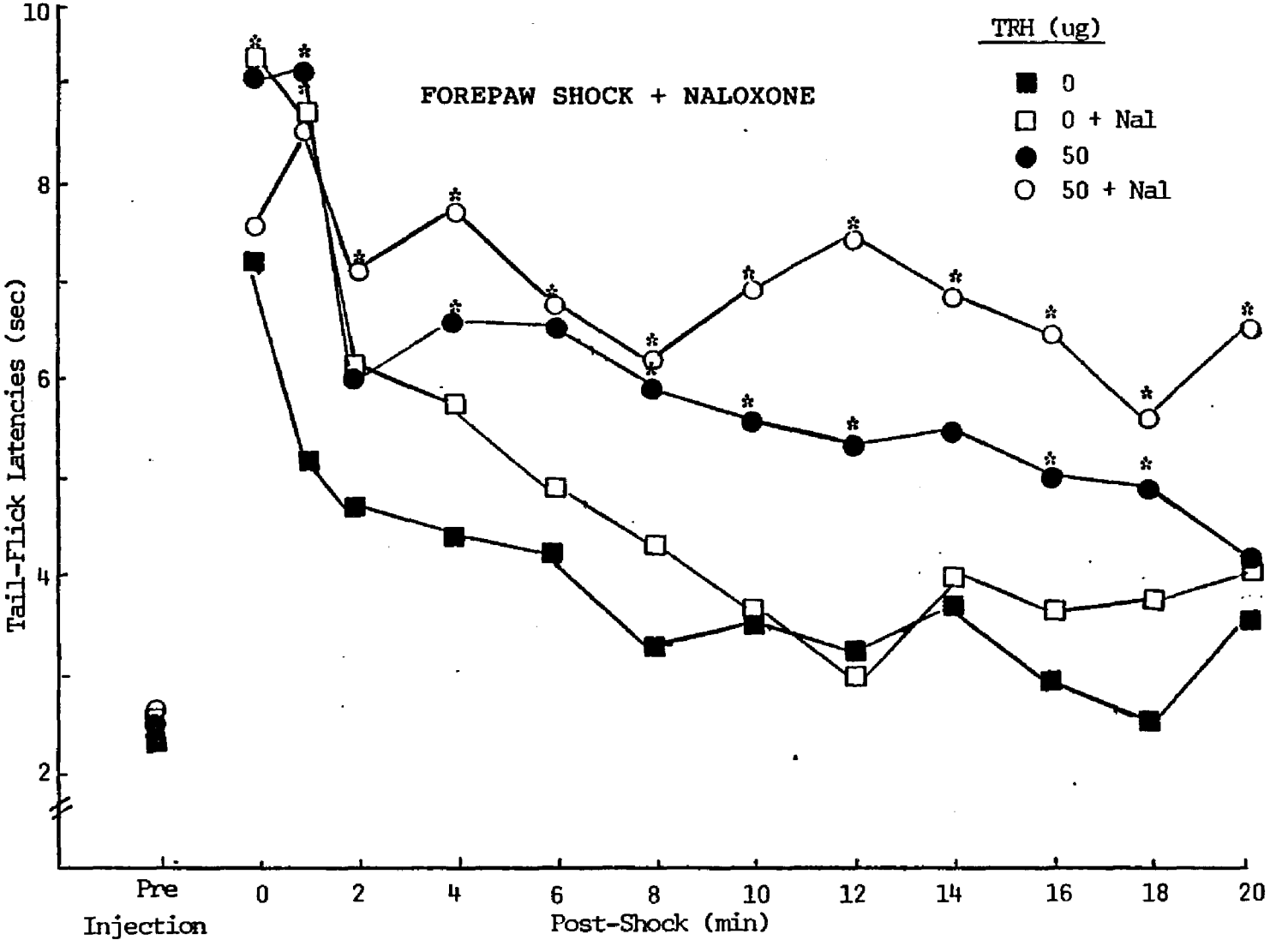
tail-flick latencies, received one of the following injection combinations: a) vehicle (ICV)/vehicle (IP), b) vehicle (ICV)/naloxone (10 mg/kg, IP), c) TRH (50 ug, ICV)/vehicle (IP), and d) TRH (50 ug, ICV)/naloxone (10 mg/kg, IP). Two intraperitoneal injections were administered at 15 and 20 min after the ICV injection as per Watkins and co-workers (1982a). Immediately following the last injection, shock (1.6 mA) was delivered continuously to the forepaws for 90 sec. Tail-flick latencies were determined at 0, 1, and 2 min, and then at 2-min intervals for up to 20 min following shock. Three weeks later, all groups were tested in identical fashion except that shock (1.6 mA) was delivered continuously to the hindpaws for 90 sec. To confirm naloxone activity, a separate group of six rats received injections of vehicle (n=3), or a 10 mg/kg dose of naloxone (n=3) 5 min prior to a morphine (10 mg/kg, sc) injection. Tail-flick latencies were determined at 30, 60, and 90 min after opiate administration. Each rat then received the other treatment or vehicle injection paired with morphine one week later.

Results

Forepaw Shock Condition: Figure 6 shows that rather than antagonizing forepaw shock analgesia, both naloxone and naloxone paired with TRH potentiated forepaw shock analgesia. Significant differences were observed across

Figure 6. Rather than antagonizing forepaw shock analgesia, both naloxone and naloxone paired with TRH potentiated forepaw shock analgesia on the tail-flick test as compared to vehicle treatment.

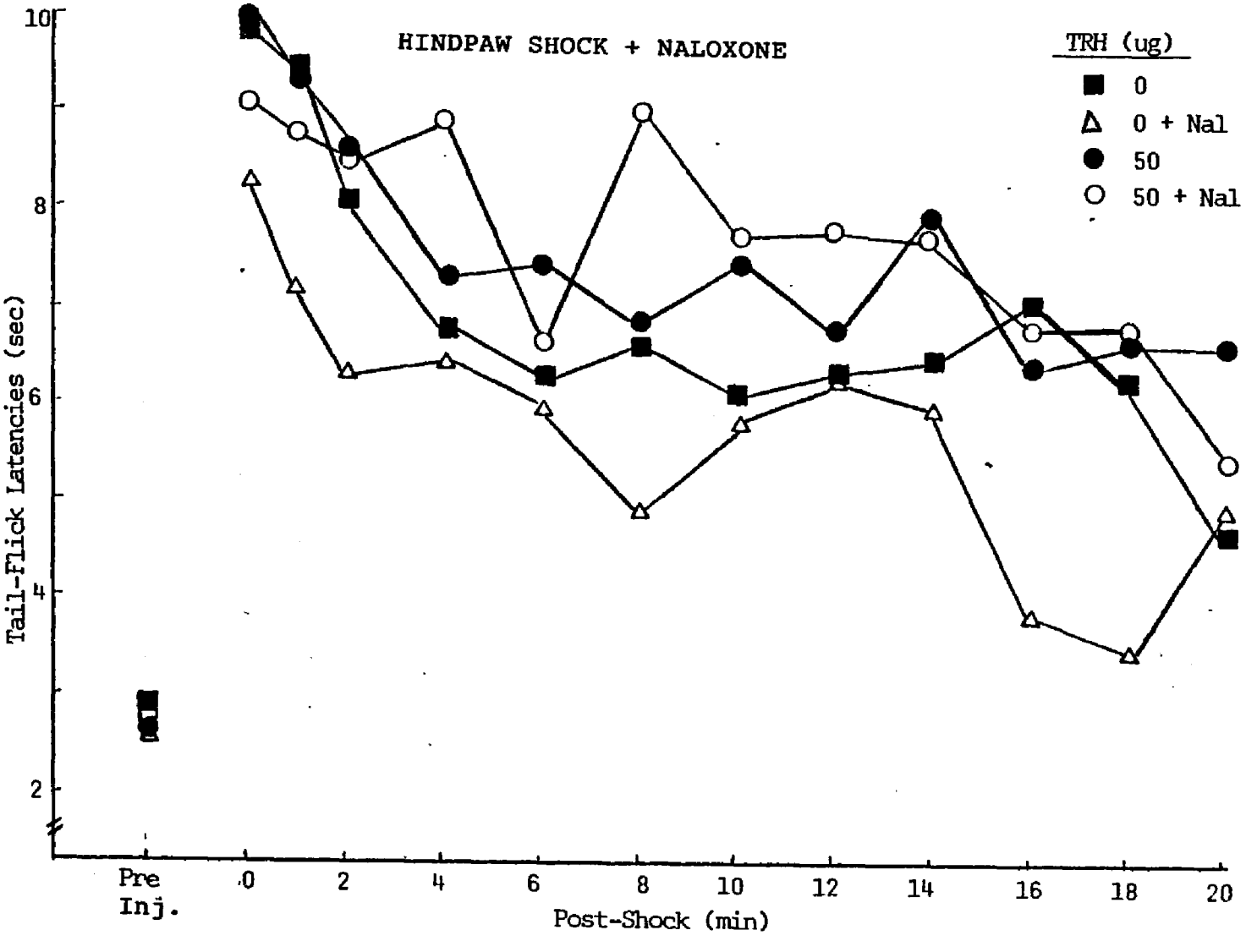
* Dunnett comparison, $p<0.05$.


FOREPAW SHOCK + NALOXONE
TRH (ug)
0
0 + Nal
50
50 + Nal
Tail-Flick Latencies (sec)
10
8
6
4
2
Pre
Injection
0
2
4
6
8
10
12
14
16
18
20
Post-Shock (min)

the time course ($F(12,336)=26.65$, $p<0.001$), among injection conditions ($F(3,28)=5.99$, $p<0.003$), and for the interaction between test times and conditions ($F(36,336)=1.85$, $p<0.003$). Tail-flick latencies following forepaw shock were significantly elevated over baseline values for 2 min in vehicle-treated rats, for 6 min in naloxone-treated rats, for 18 min in TRH-treated rats, and for 20 min in naloxone and TRH-treated rats. Potentiated forepaw shock analgesia was noted relative to vehicle-treated rats in naloxone-treated rats (1 min), in TRH-treated rats (1, 4-12, 16, and 18 min), and in TRH and naloxone-treated rats (1-20 min).

Hindpaw Shock Condition: Figure 7 indicates the relative failure of TRH and/or naloxone to alter hindpaw shock analgesia. Significant differences were found across the time course ($F(12,336)=20.32$, $p<0.001$), and approached statistical significance between doses ($F(3,28)=2.88$, $p<0.053$), but not for the interaction between time and dose ($F(36,366)=.96$). Tail-flick latencies following hindpaw shock were significantly elevated over baseline values for 18 min in vehicle-treated rats, for the first 6 min and then again at 10, 12, and 14 min post-shock in naloxone-treated rats, for 20 min in TRH-treated rats, and for 18 min in TRH and naloxone-treated rats. Significant differences between groups failed to occur, as indicated by the lack of a significant effect between doses or for

Figure 7. Naloxone and naloxone paired with TRH failed to significantly alter hindpaw shock analgesia compared to vehicle treatment.


HINDPAW SHOCK + NALOXONE
TRH (ug)
0
0 + Nal
50
50 + Nal
Tail-Flick Latencies (sec)
10
8
6
4
2
Pre Inj.
0
2
4
6
8
10
12
14
16
18
20
Post-Shock (min)

the interaction between time and dose.

Morphine Condition: Table 3 summarizes the ability of naloxone to antagonize morphine analgesia. Significant differences were found across the time course ($F(3,30)=71.98$, $p<0.001$), between vehicle and naloxone conditions ($F(1,10)=140.93$, $p<0.001$), and for the interaction between test times and conditions ($F(3,30)=29.81$, $p<0.001$). Significant increases in tail-flick latencies following morphine occurred 30, 60, and 90 min in vehicle-pretreated rats, and 60 and 120 min in naloxone-pretreated rats. The latter group displayed significantly less morphine analgesia at 30, 60, and 90 min after injection than rats receiving vehicle pretreatment.

Experiment 2C: Intravenous TRH and Analgesia Induced by Forepaw Shock.

Some of the actions of TRH such as its hypotensive effect have occurred following peripheral (particularly IV) administration (Holaday et al., 1981). Further, intravenous administration of TRH elicits an almost immediate rise in plasma TSH levels (Fleischer, Burgus, Vale, Dunn, & Guillemin, 1970; Metcalf, Dettmar, Lynn, Brewster, & Havler, 1981). Therefore, this experiment examined whether the effects exerted by central TRH upon forepaw shock analgesia would also occur following IV

TABLE 3

THE EFFECT OF NALOXONE ON MORPHINE ANALGESIA

Group	Baseline	Post Inj. (min) 30	60	120
		Tail-Flick Latencies (sec)		
Morphine	2.80	5.83	6.00	6.00
Morphine + Naloxone	2.69	3.24*	3.51*	3.34*

*Significantly different from Morphine Condition (Dunnett Comparison, $p<0.05$).

administration.

Method

Three groups of eight rats, matched for baseline tail-flick latencies, received an iv injection of either 0, 2, or 8 mg/kg of TRH respectively through a jugular catheter. Shock (1.6mA) was then delivered to the forepaws for 90 sec. Tail-flick latencies were determined at 0, 1, and 2 min, and at 2-min intervals for up to 20 min following shock.

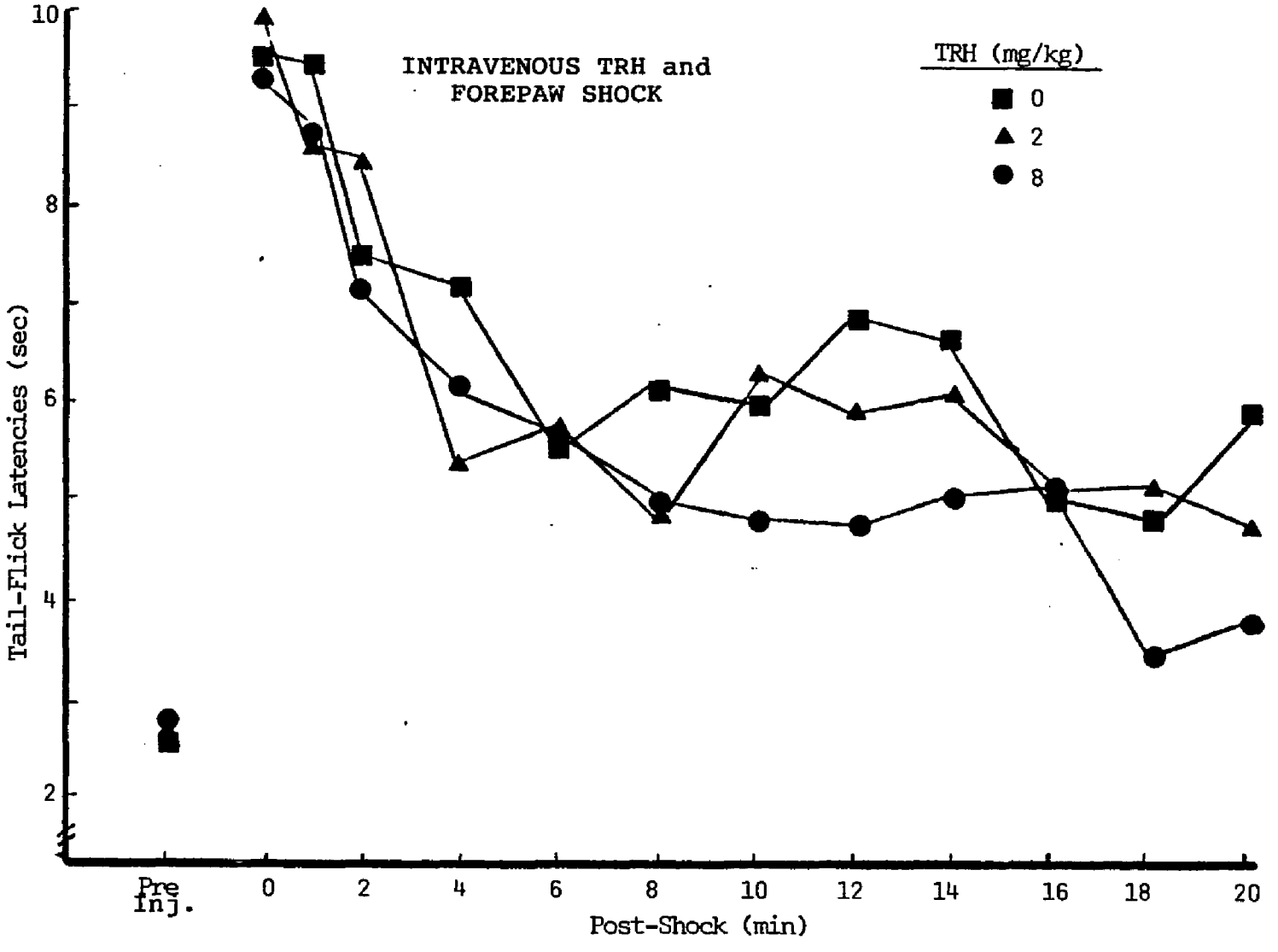
Results

Figure 8 displays the failure of intravenous administration of TRH to potentiate forepaw shock analgesia. Significant differences were found across the time course ($F(12,252)=26.42$, $p<0.001$), but not among doses ($F(2,21)=.69$), or for the interaction between test times and doses ($F(24,252)=.87$). Exposure to forepaw shock significantly elevated tail-flick latencies relative to pre-injection levels in vehicle-treated (0-14, and 20 min) rats, and in rats receiving the 2 mg/kg (0-6, 10-81 min), and 8 mg/kg (0-6 min) doses of TRH. Significant differences between groups failed to occur as indicated by the lack of a significant difference among doses or for the interaction between time and dose.

Discussion

The potentiation of forepaw shock analgesia following central TRH pretreatment in Experiment 2 is similar to the

Figure 8. Intravenous administration of TRH failed to potentiate forepaw shock analgesia on the tail-flick test as compared to vehicle treatment.


INTRAVENOUS TRH and
FOREPAW SHOCK
TRH (mg/kg)
0
2
8
Tail-Flick Latencies (sec)
10
8
6
4
2
Pre
Inj.
0
2
4
6
8
10
12
14
16
18
20
Post-Shock (min)

TRH potentiation of analgesia elicited by 20 and 80 footshocks in Experiment 1. This potentiation was selective in that central TRH potentiated the magnitude and duration of forepaw, but not hindpaw, shock analgesia. As in Experiment 1, this potentiation appears to be due to synergistic action between TRH and forepaw shock since the analgesia elicited by TRH alone does not last as long as the observed potentiations of forepaw shock analgesia.

This experiment indicates possible mechanisms of action of the TRH-induced potentiations of stress-induced analgesia. While low (10 and 50 ug) central doses of TRH significantly potentiated forepaw shock analgesia, neither intravenous dose (2 or 8 mg/kg) significantly altered the magnitude or duration of forepaw shock analgesia. Although IV vehicle pretreatment appeared to produce a larger analgesia than ICV vehicle pretreatment, it is unlikely that this factor was responsible for the failure of IV TRH to potentiate forepaw shock analgesia since none of the IV conditions paired with forepaw shock produced tail-flick latencies at the cut-off of 10 sec. The IV TRH doses chosen are effective in producing such effects as hypertension (Holaday et al., 1981) and inducing TSH release (Metcalf et al., 1981). Thus, peripheral doses of TRH which are active in producing these effects failed to affect forepaw shock analgesia. This suggests that TRH-induced potentiations of forepaw shock analgesia following

ICV administration may be due to a central mechanism of action.

It was again not possible to determine whether TRH would potentiate an opiate form of analgesia since naloxone failed to antagonize forepaw shock analgesia as it did in the hands of Watkins and co-workers (1982a). However, the ability of TRH to potentiate forepaw, but not hindpaw, shock analgesia may provide information pertaining to possible mechanism(s) of action of TRH since Watkins and Mayer (1982b) have described a number of differences between hindpaw and forepaw shock analgesia besides the opiate distinction. It is important to note that since the naloxone antagonism of forepaw shock analgesia could not be replicated here, the other mechanisms of action described for forepaw and hindpaw shock analgesia may not be applicable. To briefly review, Watkins and co-workers found that both forepaw and hindpaw shock analgesia are neurally, not homonally mediated (Watkins et al., 1982d). In terms of the neural substrate, since neither form of shock analgesia is affected by PAG lesions or decerebration, forebrain areas are not involved (Watkins et al., 1983a). Forepaw shock analgesia is dependent on pathways descending in the DLF from the RVM and is mediated by an opioid synapse in the spinal cord (Watkins et al., 1982c; 1983b). Hindpaw shock analgesia is mediated by descending pathways in the DLF which

originate only partly in the RVM, and by intraspinal pathways and is not dependent on an opioid synapse in the spinal cord (Watkins et al., 1982c; Watkins et al., 1983b). Finally, forepaw shock analgesia is opioid mediated (Watkins et al., 1982a) while hindpaw shock analgesia has a cholinergic component (Watkins et al., 1984b). Thus, if these mechanisms of action of forepaw and hindpaw shock analgesia are applicable, the selective TRH potentiation of forepaw shock analgesia would not appear to be due to involvement of forebrain areas, or of the cholinergic system, and could be due to TRH interactions with systems originating in the RVM and descending in the DLF. TRH involvement with such systems is not improbable since TRH is found in the medullary raphe nuclei (Hokfelt et al., 1975; Johannson et al., 1981). Further, the lack of effect of IV TRH on forepaw shock analgesia agrees with the CNS rather than hormonal mediation of forepaw shock analgesia found by Watkins and co-workers (1982d). It would be of interest to determine whether TRH modulation of neurons in the RVM or pathways descending in the DLF underlies its potentiation of forepaw shock analgesia.

Exposure to environmental cues in the absence of shock two weeks after receiving the forepaw shock condition produced small, though significant, immediate analgesia when all four paws came in contact with the grids and analgesia for 6 min when only the forepaws came

in contact with the grids. This suggests that either the analgesia was due to specific conditioning to hindquarter elevation, or elevation itself for 90 sec may have been stressful enough to produce analgesia for 6 min. The latter explanation is more feasible since the present level of analgesia following hindquarter elevation was much lower than the levels of classically conditioned analgesia obtained by Watkins and co-workers (1982b). Further, since extinction occurred five days after three CS-UCS pairings (Watkins et al., 1982b), and since only one CS-UCS pairing occurred in Experiment 2, the small significant analgesia 14 days later appears to be due to the stressful effects of hindquarter elevation per se. Therefore, previous exposure to the forepaw condition does not appear to have produced classically conditioned analgesia that could have affected hindpaw shock analgesia.

Both similarities and differences are present in the forepaw and hindpaw shock analgesia observed in this experiment and the findings of Watkins and Mayer (1982b). Like Watkins and co-workers (1982a), the magnitude of hindpaw shock analgesia was greater than that of forepaw shock analgesia. However, the failure of central TRH to potentiate hindpaw shock analgesia did not appear to be due to a ceiling effect since the 10 sec cut-off value was reached only immediately after shock when TRH was paired

with hindpaw shock. Also, like Watkins and co-workers (1982a), naloxone failed to alter hindpaw shock analgesia. However, unlike Watkins and co-workers (1982a), the magnitude and duration of both forepaw and hindpaw shock analgesia were lower in the present study. Further, naloxone failed to antagonize the putatively opioid-sensitive (Watkins et al., 1982a) forepaw shock analgesia. Indeed, a brief, immediate potentiation of this form of analgesia occurred. The failure of naloxone to antagonize forepaw shock analgesia is not due to any lack of activity since it significantly decreased morphine analgesia over a 90 min time course. Further, procedural and temporal injection variables (5 and 0 min pre-shock) were identical to that used by Watkins and co-workers (1982a) to successfully antagonize forepaw shock analgesia. In retrospect, the failure of naloxone to decrease forepaw shock analgesia in the present experiment is not surprising in light of the recent studies (Cannon, et al., 1984; Terman, Shavit, Lewis, Cannon, & Liebeskind, 1984) showing that small changes in procedure can alter the opioid or non-opioid nature of footshock analgesia. For instance, both front and hindpaw footshock analgesia can be opiate or non-opiate depending on the level of shock intensity used, with moderate levels (2 mA) and higher levels (3.5 mA) of shock to the hindpaws or forepaws producing opiate-sensitive and opiate-insensitive forms of

analgesia, respectively (Cannon et al., 1984). Thus, slight differences in equipment and actual levels of shock delivered could account for the present failure to produce opioid-mediated forepaw shock analgesia. Indeed, in this experiment, naloxone increased the magnitude and duration of forepaw shock analgesia and naloxone paired with TRH increased the magnitude of forepaw shock analgesia at more test times than did TRH. The naloxone potentiation of both forepaw shock analgesia and of the TRH potentiation of forepaw shock analgesia may be due to collateral inhibition. According to the collateral inhibition model, opioid analgesia is potentiated by increased levels of endogenous opioids and reduced by decreased levels (Kirchgessner et al., 1982). Non-opioid analgesia is modulated in an opposite manner, with low levels of opioids enhancing it, due to disinhibition, and high levels inhibiting it. Thus, decreasing opioid analgesia should increase non-opioid analgesia and vice versa. Indeed, non-opiate CWS analgesia is decreased by HPX or administration of d-phenylalanine while these manipulations increased morphine analgesia (Alleva et al., 1980; Bodnar et al., 1979a; Bodnar, Kelly, Mansour, & Glusman, 1979b; Bodnar et al., 1980b). Further, naloxazone decreases morphine analgesia and increases CWS analgesia (Kirchgessner et al., 1982). The naloxone potentiation of both non-opioid forepaw shock analgesia and the TRH

potentiation of forepaw shock analgesia seen in Exp. 2 provides further support for the collateral inhibition hypothesis.

Experiment 3A: Effects of Central TRH Upon Cold Water Swim (CWS) Analgesia.

The first two experiments examined the modulatory role of TRH upon forms of foot shock analgesia which have a relatively short (15-20 min) duration of action. As reviewed previously, CWS analgesia is typically opiate-insensitive (Bodnar et al., 1978b,d) and is longer-acting (60-90 min: Bodnar et al., 1978a). Therefore, the purpose of this experiment was to determine the effects of central TRH pretreatment upon CWS analgesia.

Method

Three groups of ten rats each received an ICV injection of either 0, 10, or 50 ug TRH respectively 20 min prior to a no swim condition and swims in bath temperatures of 21, 15, 8, and 2°C. The 3.5 min swim conditions occurred in descending order with a one-week interval separating each condition to minimize adaptation effects (Bodnar et al., 1978b; Spiaggia, Bodnar, Kelly, & Glusman, 1979). Rectal temperatures were determined immediately prior to and then at 0 and 15 min following each condition. Tail-flick latencies, jump thresholds, and rectal temperatures were determined 30, 60, and 120 min

following each condition.

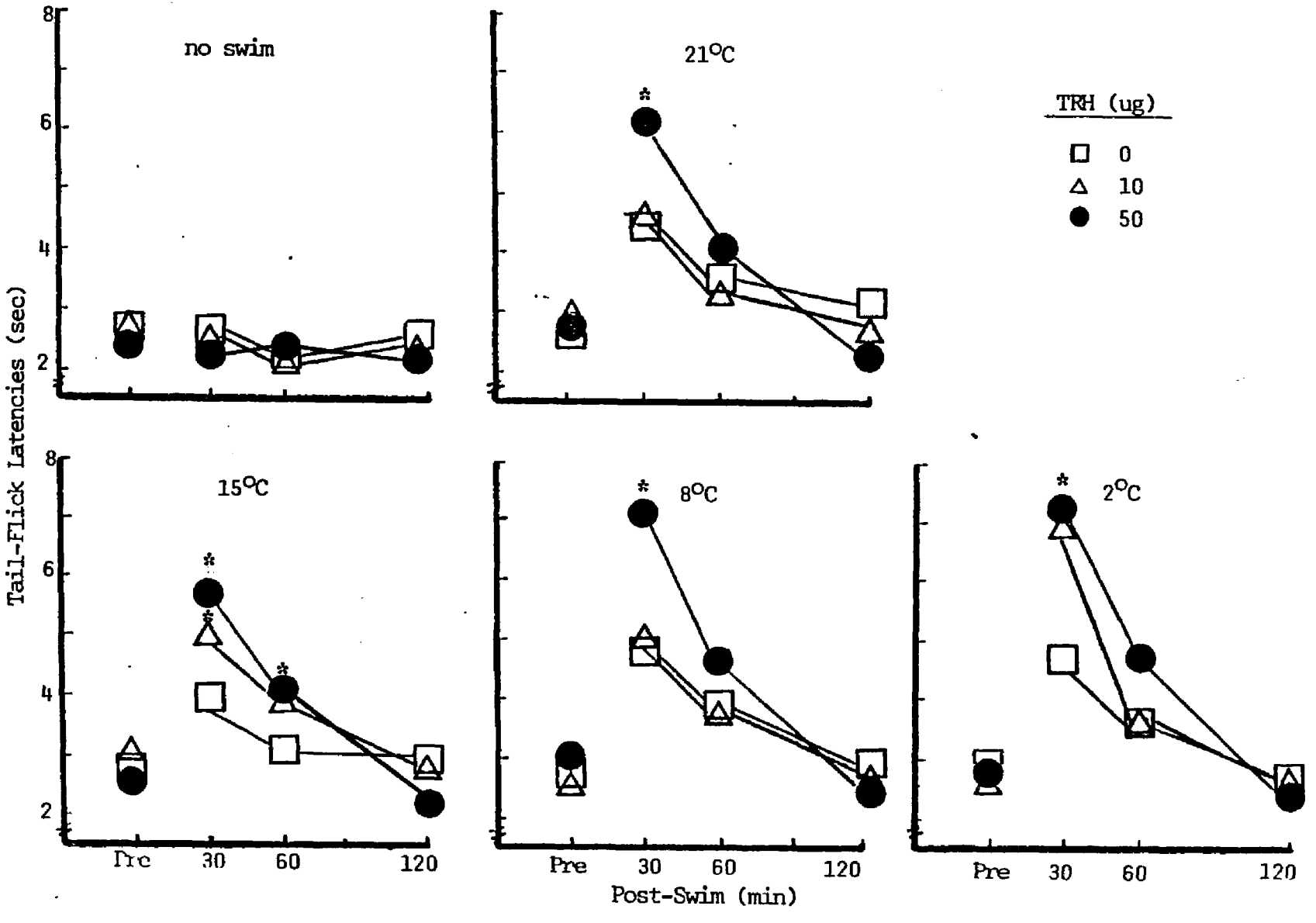
Results

CWS Analgesia: Tail-Flick Test: Figure 9 summarizes the TRH-induced potentiation of swim-induced analgesia on the tail-flick test. For the no-swim condition, significant differences in tail-flick latencies were observed across the time course ($F(3,81)=7.29$, $p<0.001$) but failed to occur among doses ($F(2,27)=1.29$), and for the interaction between test times and doses ($F(6,81)=.81$). Tail-flick latencies were significantly decreased from baseline values in vehicle-treated rats (60 min), and in rats receiving the 10 ug (60 min) and 50 ug (120 min) doses of TRH.

For the 21^{o}C swim condition, significant differences were observed across the time course ($F(3,81)=65.61$, $p<0.001$), and for the interaction between test times and doses ($F(6,81)=5.76$, $p<0.001$), but not among doses ($F(2,27)=2.04$). Tail-flick latencies were significantly elevated over baseline values in vehicle-treated rats (30 and 60 min), and in rats receiving the 10 ug (30 min) and 50 ug (30 and 60 min) doses of TRH. Significant potentiations in analgesia relative to vehicle-treatment occurred 30 min after the 21 C swim in rats receiving the 50 ug, but not the 10 ug, dose of TRH.

For the 15^{o}C swim, significant differences were observed across the time course ($F(3,81)=57.54$, $p<0.001$),

Figure 9. Centrally administered TRH potentiated analgesia following each of the swim conditions on the tail-flick test as compared to vehicle treatment.
* Dunnett comparison, $p<0.05$.


no swim
21°C
15°C
8°C
2°C
TRH (ug)
0
10
50
Tail-Flick Latencies (sec)
8
6
4
2
Pre
30
60
120
Post-Swim (min)
*

and for the interaction between test times and doses ($F(6,81)=6.58$, $p<0.001$), but not among doses ($F(2,27)=1.48$). Tail-flick latencies were significantly elevated over baseline values in vehicle-treated rats (30 min), and in rats receiving the 10 ug (30 and 60 min) and 50 ug (30 and 60 min) doses of TRH. Significant potentiations in analgesia relative to vehicle-treatment occurred 30 and 60 min after the 15°C swim following pretreatment with both TRH doses.

For the 8°C swim, significant differences were observed across the time course ($F(3,81)=83.84$, $p<0.001$), among doses ($F(2,27)=4.46$, $p<0.021$), and for the interaction between test times and doses ($F(6,81)=6.70$, $p<0.001$). Tail-flick latencies were significantly elevated over baseline values at 30 and 60 min following the swim in all groups. Significant potentiations in analgesia relative to vehicle treatment rats occurred 30 min after the 8°C swim in rats receiving the 50 ug, but not the 10 ug, dose of TRH.

For the 2°C swim, significant differences were observed across the time course ($F(3,78)=88.66$, $p<0.001$), for the interaction between test times and doses ($F(6,78)=6.31$, $p<0.001$), and approached significance among doses ($F(2,26)=3.17$, $p<0.059$). Tail-flick latencies were significantly elevated over baseline values in vehicle-treated rats (30 min) and in rats receiving the 10 ug (30

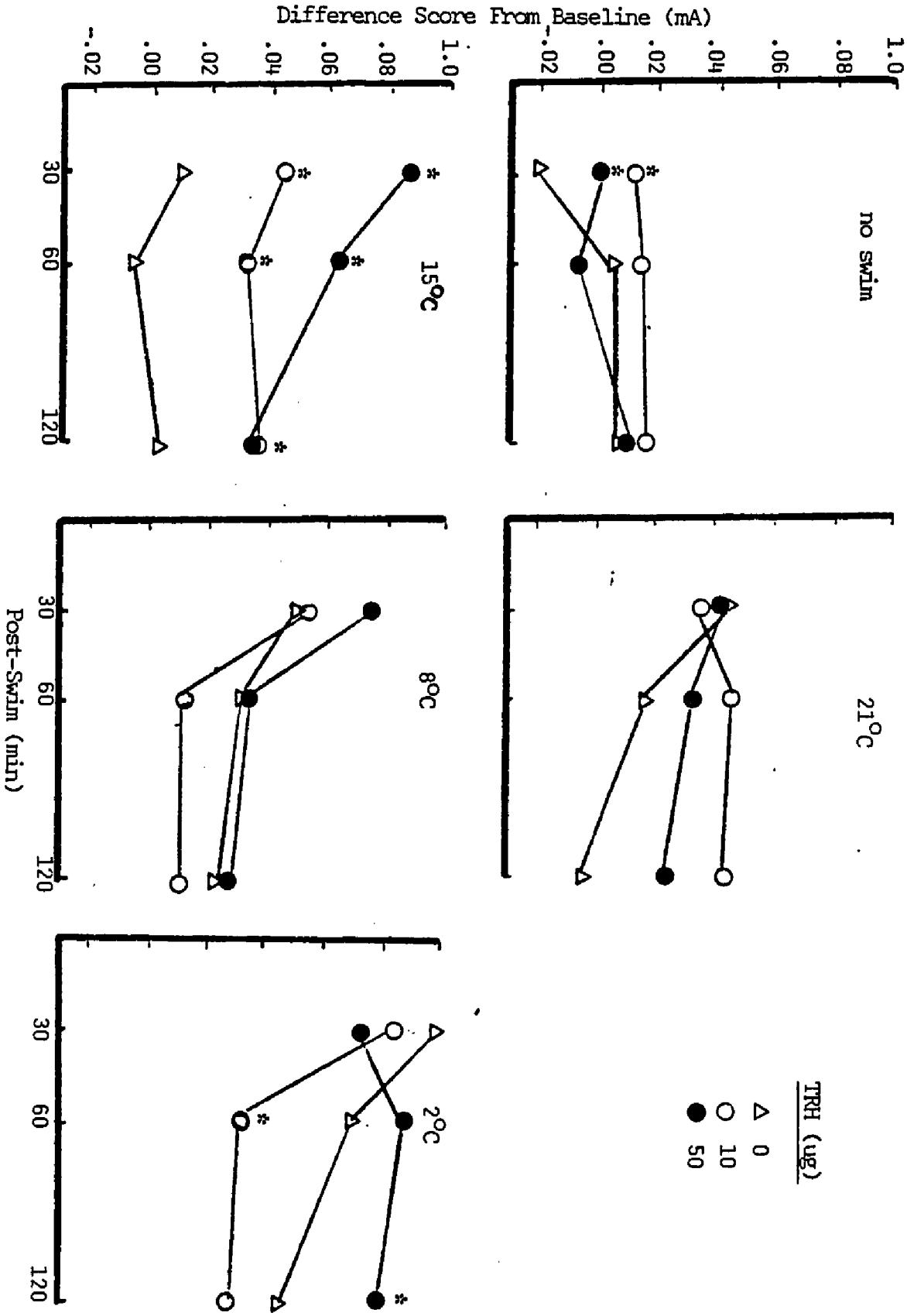
min) and 50 ug (30 and 60 min) doses of TRH. Significant potentiations in analgesia relative to vehicle treatment occurred 30 min after the 2°C swim following pretreatment with both TRH doses. Thus, TRH produced dose-dependent potentiations in swim analgesia on the tail-flick test across bath temperatures.

CWS Analgesia: Jump Test: Figure 10 shows that TRH potentiated swim analgesia on the jump test on some, but not all of the swim temperatures. For the no-swim condition, significant differences in jump thresholds were observed across the time course ($F(3,81)=3.02$, $p<0.035$) and for the interaction between test times and doses ($F(6,81)=2.27$, $p<0.045$), but not among doses ($F(2,27)=2.02$). However, comparisons failed to reveal significant differences between baseline and post-injection jump thresholds. After partialling out a significant difference in baseline jump thresholds, it was found that both doses of TRH alone significantly increased jump thresholds 30 min following injection as compared to corresponding vehicle values.

For the 21°C swim condition, significant differences were observed across the time course ($F(3,81)=10.38$, $p<0.001$), among doses ($F(2,27)=5.35$, $p<0.011$), and approached statistical significance for the interaction between test time and doses ($F(6,81)=1.85$, $p<0.099$). Jump thresholds were significantly elevated over baseline

Figure 10. Centrally administered TRH potentiated swim analgesia following the 15 and 2 ^{0}C swim conditions on the jump test as compared to vehicle treatment.

* Dunnett comparison, $p<0.05$.


Difference Score From Baseline (mA)
-.02
.00
.02
.04
.06
.08
1.0
30
60
120
Post-Swim (min)
no swim
15°C
21°C
8°C
2°C
TRH (ug)
Δ 0
○ 10
● 50

values in vehicle-treated rats (30 min) and in rats receiving the 10 ug (30, 60, and 120 min), and 50 ug (30 min) doses of TRH. After partialling out significant differences in baseline jump thresholds, it was found that neither dose of TRH significantly potentiated jump thresholds as compared to vehicle treatment.

For the 15°C swim condition, significant differences were observed across the time course ($F(3,81)=13.69$, $p<0.001$), among doses ($F(2,27)=5.97$, $p<0.007$), and for the interaction between test times and doses ($F(6,81)=4.03$, $p<0.001$). Jump thresholds were significantly elevated over baseline levels in rats receiving the 10 ug (30 and 120 min) and 50 ug (30, 60, and 120 min) doses of TRH, but not in vehicle-treated rats. After partialling out significant differences in baseline jump thresholds, it was found that both doses of TRH potentiated analgesia for all 120 min following the 15°C swim as compared to the corresponding vehicle values.

For the 8°C swim condition, significant differences were observed across the time course ($F(3,81)=18.29$, $p<0.001$), approached statistical significance among doses ($F(2,27)=2.71$, $p<0.085$), but failed to occur for the interaction between test times and doses ($F(6,81)=.61$). Jump thresholds were significantly elevated over baseline values at 30 min for all groups.

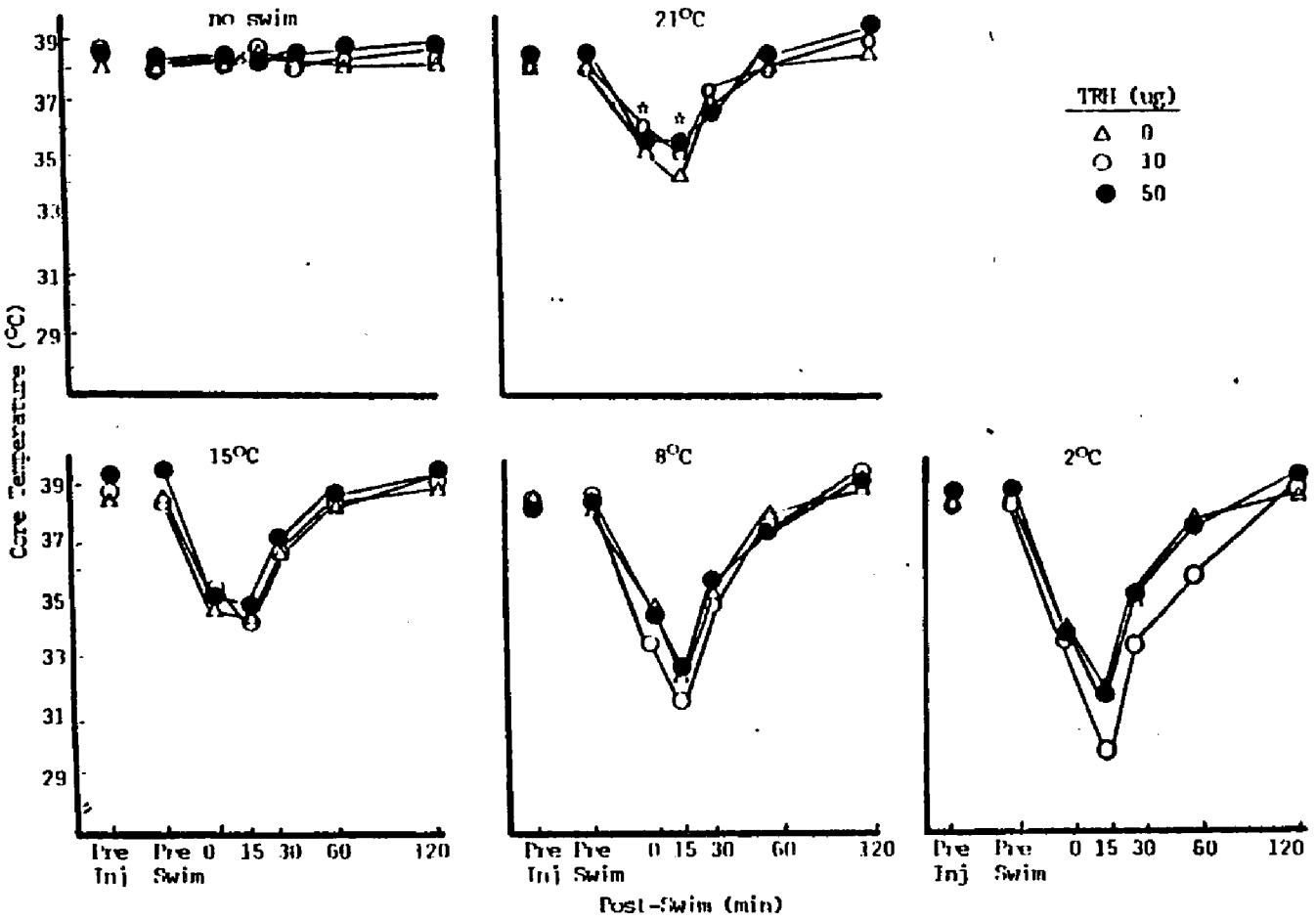
For the 2°C swim condition, significant differences

were observed across the time course ($F(3,81)=17.14$, $p<0.001$), among doses ($F(2,27)=4.00$, $p<0.030$), but not for the interaction between doses and test time ($F(6,81)=1.66$). Jump thresholds were significantly elevated over baseline values in vehicle-treated rats (30 and 60 min) and in rats receiving the 10 ug (30 min) and 50 ug (30, 60, and 120 min) doses of TRH. A significant potentiation in analgesia relative to vehicle treatment occurred following pretreatment with the 50 ug (120 min) dose of TRH while a significant decrease relative to vehicle treatment occurred following pretreatment with the 10 ug (60 min) dose of TRH. Thus, while clear dose-dependent TRH-induced potentiations of analgesia occurred across the temperature bath range on the tail-flick test, TRH induced potentiations on the jump test occurred only after the 15 and 2^{o}C swims.

CWS Hypothermia: Figure 11 shows that core temperatures of the TRH and vehicle-treated rats differed from each other following only the 21^{o}C swim. For the no swim condition, significant differences were observed across the time course ($F(6,138)=3.97$, $p<0.001$), they failed to occur among doses ($F(2,23)=0.20$), and for the interaction between times and doses ($F(12,138)=1.73$). The 50 ug dose of TRH increased core temperatures above baseline levels at 120 min while treatment with vehicle or 10 ug TRH failed to change core temperatures from baseline

Figure 11. Core temperatures of TRH- and vehicle-treated rats differed from each other following only the 21 °C swim.

* Dunnett comparison, $p<0.05$.


no swim
21°C
15°C
8°C
2°C
Core Temperature (°C)
39
37
35
33
31
29
TRH (ug)
0
10
50
Pre Inj
Pre Swim
0 15 30 60 120
Post-Swim (min)

values.

For the 21°C swim condition, significant differences were observed across the time course ($F(6,162)=247.39$, $p<0.001$), and for the interaction between test times and doses ($F(12,162)=2.84$, $p<0.001$), but not among doses ($F(2,27)=1.04$). Core temperatures were significantly decreased relative to baseline values at 0, 15, and 30 min after the swim for all three groups and then were significantly elevated over baseline levels at 120 min after the swim for the groups receiving the 10 or 50 ug doses of TRH. Significant decreases in swim hypothermia relative to vehicle-treatment occurred following pretreatment with the 10 ug (0 and 15 min) and 50 ug (15 min) doses of TRH. For the remaining swim conditions, significant differences were observed across the time course (15°C: $F(6,162)=244.60$, $p<0.001$; 8°C: $F(6,162)=253.13$, $p<0.001$; 2°C: $F(6,156)=134.89$, $p<0.001$) but not among doses (15°C: $F(2,27)=.77$; 8°C: $F(2,27)=.14$; 2°C: ($F(2,26)=1.19$), or for the interaction between time and dose (15°C: $F(12,162)=.81$; 8°C: $F(12,162)=1.24$; 2°C: $F(12,156)=1.42$). The 15, 8, and 2°C swim conditions, like the 21°C swim condition, decreased core temperature relative to baseline values at 0, 15, and 30 min in all three groups. The lack of effect among doses or for the interaction between time and dose indicates that following the 15, 8, and 2°C swims, there were no significant

differences in core temperature between TRH and vehicle-treated rats.

Experiment 3B: Intravenous TRH and CWS Analgesia.

It was found in Experiment 2 that central, but not intravenous, administration of TRH potentiated forepaw shock analgesia. To determine whether the TRH-induced potentiation of CWS analgesia was also centrally mediated, the effects of intravenous injections of TRH on CWS analgesia were examined.

Method

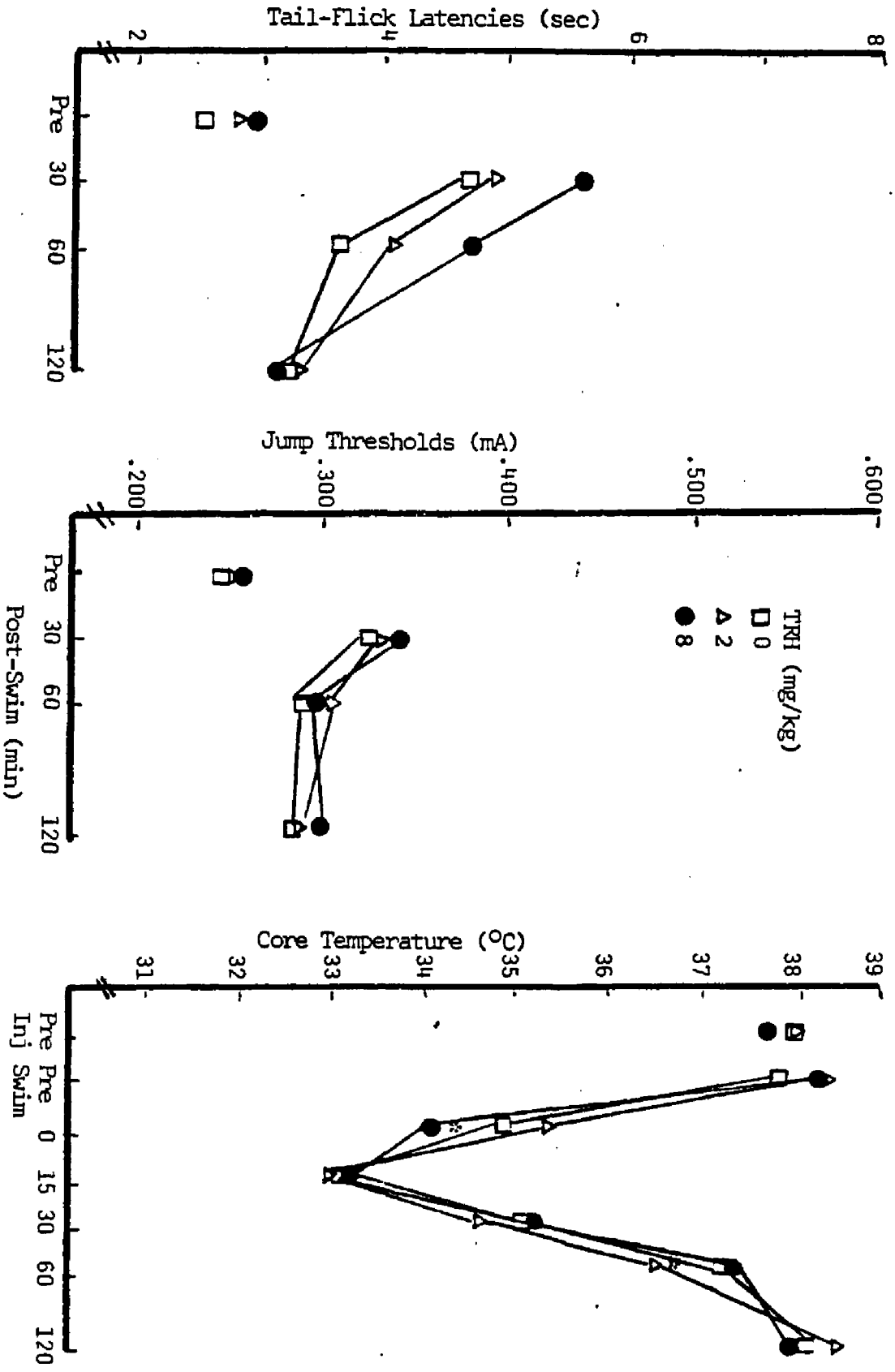
Three groups of eight rats each, matched for baseline tail flick latencies and jump thresholds, received an IV injection of either 0, 2, or 8 mg/kg of TRH respectively through an intravenous jugular catheter. Twenty min following the injection, each rat received a 21°C CWS for 3.5 min, and tail-flick latencies, jump thresholds, and rectal temperatures were determined in identical fashion as described in Experiment 3A.

Results

Figure 12 shows that IV administration of TRH failed to potentiate CWS analgesia on either the tail-flick or jump tests, and transiently potentiated swim hypothermia. Significant differences on the tail flick test following the 21°C swim were observed across the time course ($F(3,63)=51.61$, $p<0.001$), but not among doses ($F(2,21)=2.12$) and for the interaction between time and

Figure 12. Intravenous administration of TRH failed to potentiate swim analgesia on the tail-flick and jump tests and transiently decreased core temperature as compared to vehicle treatment.

* Dunnett comparison, $p<0.05$.


Tail-Flick Latencies (sec)
2
4
6
8
Pre
30
60
120
Jump Thresholds (mA)
.200
.300
.400
.500
.600
Pre
30
60
120
Post-Swim (min)
TRH (mg/kg)
0
2
8
Core Temperature (°C)
31
32
33
34
35
36
37
38
39
Pre
Pre
0
15
30
60
120
Inj
Swim

dose ($F(6,63)=1.36$). Tail-flick latencies were significantly elevated over baseline values at 30 and 60 min after the swim for all three groups. The lack of effect among doses or for the interactions between time and dose indicates that IV administration of TRH failed to potentiate CWS analgesia as compared to vehicle treatment on the tail-flick test.

Significant differences on the jump test were observed across the time course ($F(3,63)=36.31$, $p<0.001$), but not among doses ($F(2,21)=.75$), or for the interaction between dose and time ($F(6,63)=.50$). Jump thresholds were significantly elevated over baseline values in vehicle-treated rats (30 and 60 min), and in rats receiving the 2 mg/kg (30 and 60 min) and 8 mg/kg (30, 60, and 120 min) doses of TRH. Significant differences failed to occur among groups.

Significant differences in core temperature were observed across the time course ($F(6,108)=391.42$, $p<0.001$), and for the interaction between time and dose ($F(12,108)=4.14$, $p<0.001$), but not among doses ($F(2,18)=.06$). Core temperatures were significantly decreased from baseline values in vehicle-treated rats (0, 15, 30, and 60 min), and in rats receiving the 2 mg/kg (0, 15, 30, and 60 min), and the 8 mg/kg (0, 15, and 30 min) doses of TRH. Transient, though significant decreases in core temperature relative to vehicle-treated rats occurred

in rats receiving the 2 mg/kg (60 min) and the 8 mg/kg (immediately post-swim) doses of TRH.

Discussion

The potentiations of CWS analgesia following central TRH pretreatment are similar to the TRH-induced potentiations of footshock analgesia seen in Experiments 1 and 2. TRH potentiated analgesia on the tail-flick test across swim temperatures, while only potentiating swim analgesia on the jump test following the 15 and 2°C swims. Potentiated swim hypothermia does not appear to be responsible for the TRH potentiation of swim analgesia since TRH and vehicle-treated rats failed to differ from each other on core temperature following any of the swim conditions except the 21°C swim condition in which TRH decreased swim hypothermia. As in Experiments 1 and 2, the TRH potentiation of swim analgesia was synergistic since TRH failed to produce analgesia in the no-swim condition on the tail-flick test. However, while TRH did significantly increase jump thresholds at 30 min in the no swim condition, this does not appear to be responsible for its potentiation of swim analgesia since the latter effect outlasted the former. Further, the TRH induced increase in jump thresholds at 30 min in the no-swim condition is not due to an analgesic effect of TRH since TRH treated animals did not have increased jump thresholds compared to baseline but rather as compared to vehicle-treated

animals. The latter effect was significant due to a decrease in jump thresholds at 30 min in the vehicle-treated animals rather than an increase in jump thresholds by the TRH-treated animals.

The differential effects of TRH on the jump test and tail-flick tests are not unexpected since these pain tests involve different mechanisms. The jump test involves supraspinal mechanisms while the tail-flick test involes spinal mechanisms. Like the present more frequent results on the tail-flick test, the early work on CWS analgesia showed a longer duration of 2°C swim analgesia on the tail-flick than on the jump test (Bodnar et al., 1978a). The TRH potentiation of swim analgesia on both the tail-flick and jump tests indicates that both spinal and supraspinal mechanisms are involved. However, since effects are more frequently seen on the tail-flick test, spinal mechanisms may be more important.

The TRH potentiation of swim analgesia indicates possible mechanisms of action for TRH. To briefly review the mechanisms of action of CWS analgesia, while forepaw and hindpaw shock analgesia are neurally mediated, CWS has a hormonal component (Bodnar et al., 1979a). Noradrenergic, dopaminergic, cholinergic, and vasopressinergic, but not opiate systems have all been implicated in 2°C swim analgesia (Bodnar et al., 1978b; Bodnar et al., 1980c; Bodnar et al., 1980a; Bodnar et al.,

1980d; Sperber, Kramer, & Bodnar, in press). Since IV administration of TRH failed to potentiate CWS analgesia, this suggests that even though CWS analgesia has a hormonal component, the TRH potentiation of it is neurally mediated. As in Experiments 1 and 2, TRH again appears to be potentiating a non-opiate form of analgesia. Since noradrenergic, cholinergic, dopaminergic, and vasopressinergic systems have been implicated in CWS analgesia, TRH may potentiate CWS analgesia by interacting with one or more of these systems. This is not implausible since TRH has been shown to interact with noradrenergic, dopamineric, and cholinergic systems (Bennett et al., 1983; Green & Grahame-Smith; Yarbrough, 1983). TRH may potentiate forepaw shock and CWS analgesia via the same or different mechanisms. Thus, while Experiment 2 indicated that the cholinergic system and forebrain areas may not be involved in the TRH potentiation of forepaw shock analgesia, they may be involved in the TRH potentiation of swim analgesia. The neural sites involved in CWS analgesia have not been worked out, which makes it difficult to speculate on CNS site(s) where TRH may be acting to potentiate swim analgesia. Further experiments are necessary to determine which neurotransmitter(s) and CNS site(s) are involved in the TRH potentiation of swim analgesia.

Experiment 4. Comparison of Central Administration of TRH, a TRH Metabolite, and a TRH Analogue Upon CWS Analgesia.

It has been suggested that some of the effects of TRH may be due to its metabolism to DKP (Prasad et al., 1977). In an effort to determine whether this is responsible for the TRH potentiation of swim analgesia, this experiment examined whether DKP exerts effects similar to TRH upon swim analgesia. The effects of the TRH analogue RX77368 on swim analgesia were also examined. This is a stable analogue which is not metabolized to DKP (Brewster, 1983) so that if metabolism to DKP is solely responsible for the TRH potentiation of swim analgesia, RX77368 should fail to produce this effect.

Method

Two groups of eight rats each, matched for baseline tail-flick latencies and jump thresholds, received an icv injection of 50 ug DKP or 50 ug RX77368 respectively followed 20 min later by the no-swim condition. Tail-flick latencies and jump thresholds were assessed in order to examine the effects of these agents upon basal pain thresholds. One week later each group received the same injection regimen followed 20 min later by a 21^{o}C swim for 3.5 min. In addition, two other groups of six rats each received vehicle or a 50 ug dose of TRH respectively followed by a 21^{o}C swim. Experimental conditions following the swim were identical to those described in

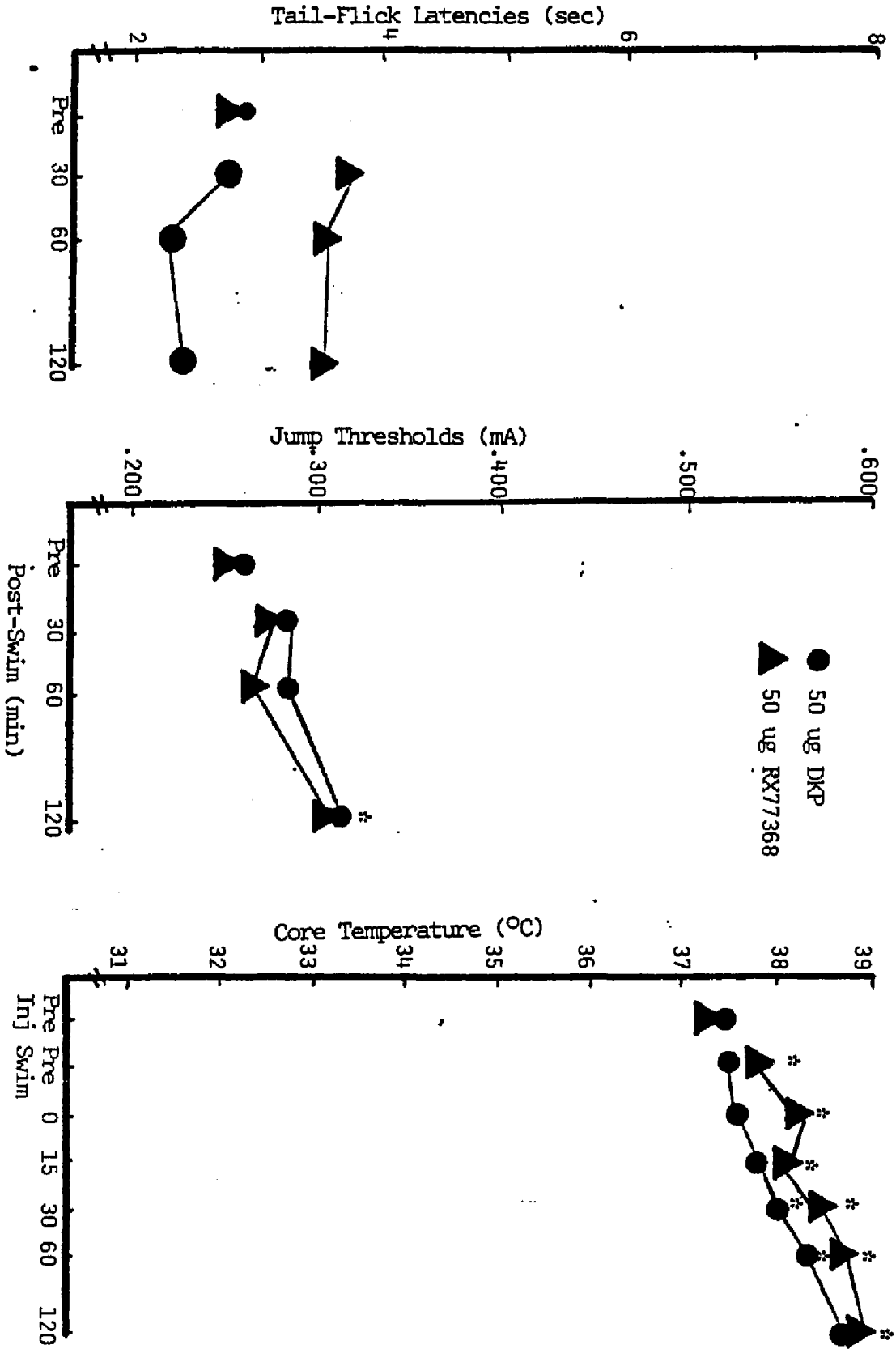
Experiment 3A. In addition, the presence or absence of sniffing, head stereotypy, chewing, grooming, wet dog shakes, and tail elevation were recorded for all rats for two consecutive 15 sec periods at 5, 10, and 15 min post-injection.

Results

Basal Thresholds: Figure 13 shows that RX77368 and DKP produced transient analgesia on the jump but not tail-flick test, and produced hyperthermia. Differences in tail-flick latencies approached statistical significance between drugs ($F(1,14)=3.73$, $p<0.074$), but significant differences failed to occur across the time course ($F(3,42)=.55$) or for the interaction between test times and drugs ($F(3,42)=1.22$). Thus, RX77368 and DKP alone were without effect on the tail-flick test. Significant differences in jump thresholds were observed across the time course ($F(3,42)=9.68$, $p<0.001$) but not betweem drugs ($F(1,14)=.24$), or for the interaction between time and drugs ($F(3,42)=.08$). Jump thresholds were significantly elevated over baseline levels at 120 min for rats receiving the 50 ug doses of DKP and RX77368. Significant differences in core temperature were observed across the time course ($F(6,84)=26.07$, $p<0.001$) and for the interaction between test times and drugs ($F(6,84)=2.42$, $p<0.033$), but not between drugs ($F(1,14)=2.88$). Core temperatures were significantly elevated over baseline

Figure 13. Centrally administered RX77368 and DKP produced transient analgesia on the jump but not the tail-flick test, and produced hyperthermia.

* Dunnett comparison, $p<0.05$.


Tail-Flick Latencies (sec)
2
4
6
8
Pre
30
60
120
Jump Thresholds (mA)
.200
.300
.400
.500
.600
Post-Swim (min)
50 ug DKP
50 ug RX77368
Core Temperature (°C)
31
32
33
34
35
36
37
38
39
Pre
Pre
Pre
0
15
30
60
120
Inj
Swim

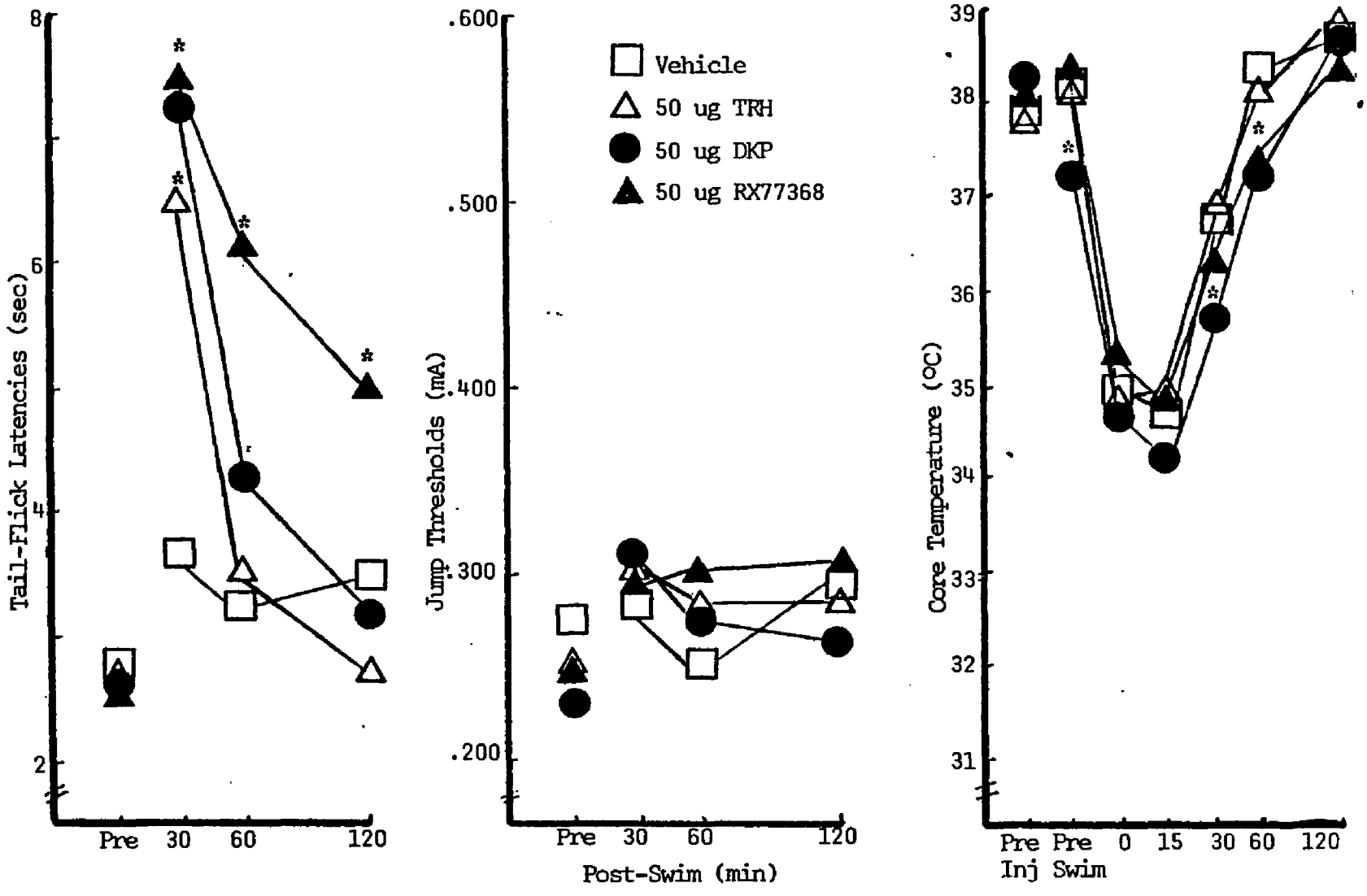
values across the time course following RX77368 and at 30, 60, and 120 min following DKP.

CWS Analgesia: Tail-Flick Latencies: Figure 14 shows that TRH, RX77368 and DKP each potentiated 21°C analgesia on the tail-flick, but not the jump test. RX77368 and DKP, but not TRH, also potentiated swim-induced hypothermia. Significant differences in tail-flick latencies were observed across the time course ($F(3,69)=55.43$, $p<0.001$), among conditions ($F(3,23)=5.70$, $p<0.005$), and for the interaction between test time and conditions ($F(9,69)=4.65$, $p<0.001$). Tail-flick latencies were significantly elevated over baseline values for rats receiving TRH (30 min), 50 ug RX77368 (30-60 min), or 50 ug DKP (30-120 min), but not for vehicle-treated rats. Significant increases in latencies over vehicle-treatment occurred following TRH (30 min), DKP (30 min) and RX77368 (30-120 min).

CWS Analgesia: Jump Thresholds: Significant differences in jump thresholds were observed across the time course ($F(3,72)=11.15$, $p<0.001$), approached significance for the interaction between test time and conditions ($F(9,72)=1.92$, $p<0.06$), and did not occur among conditions ($F(3,24)=.52$). Jump thresholds were significantly elevated over baseline in rats receiving TRH (30 min), DKP (30 and 60 min), and RX77368 (30-120 min), but not for vehicle-treated rats. Since significant

Figure 14. Centrally administered TRH, RX77368, and DKP each significantly potentiated 21 °C swim analgesia on the tail-flick but not the jump test compared to vehicle treatment. RX77368 and DKP also significantly potentiated swim hypothermia.

* Dunnett comparison, $p<0.05$.


Tail-Flick Latencies (sec)
8
6
4
2
Pre
30
60
120
Jump Thresholds (mA)
.600
.500
.400
.300
.200
Pre
30
60
120
Post-Swim (min)
Vehicle
50 ug TRH
50 ug DKP
50 ug RX77368
Core Temperature (°C)
39
38
37
36
35
34
33
32
31
Pre
Pre
0
15
30
60
120
Inj
Swim

differences failed to occur among conditions or for the interaction between time and conditions, it was concluded that TRH, DKP, and RX77368 failed to potentiate 21°C swim analgesia compared to vehicle treatment on the jump test.

CWS Hypothermia: Significant differences in core temperature were observed across the time course ($F(6,144)=154.88$, $P<0.001$), for the interaction between test times and conditions ($F(18,144)=1.81$, $p<0.03$), but not among conditions ($F(3,24)=.48$). Core temperatures were significantly decreased from baseline values for up to 30 min after the swim for all groups and also just prior to and 60 min after the swim in DKP-treated rats. Significant potentiations in hypothermia relative to vehicle treatment occurred following DKP (post-injection/pre-swim, 30, and 60 min) and RX77368 (60 min), but not following TRH.

Table 4 shows that while TRH and RX77368 elicited tail elevation, head stereotypy, and chewing, DKP only produced chewing. Significant differences in tail elevation occurred among groups (χ^2= 28, $p<0.001$) with more instances relative to vehicle treatment observed following TRH ($\chi^2=12$, $p<0.001$) and RX77368 ($\chi^2=14$, $p<0.001$), but not DKP. Significant differences in head stereotypy ocurred among groups ($\chi^2=13.59$, $p<0.003$) with more instances relative to vehicle treatment observed following TRH ($\chi^2=8.57$, $p<0.003$) and RX77368 ($\chi^2=10.5$,

TABLE 4

EFFECTS OF TRH, RX77368, AND DKP ON MOTOR BEHAVIORS

Group	Tail Elevation	Head Stereotypy	Chewing	Grooming	wet Dog Shakes	Sniffing
	(Percent of Animals Showing Behavior)					
Saline	0	0	0	0	0	100
TRH	100*	83.3*	66.7*	16.7	66.7	100
RX77368	100*	87.5*	100*	12.5	50	100
DKP	0	37.5	50*	0	50	100

*Significantly different from saline (χ^2 analysis, $p<0.05$).

$p<0.001$), but not DKP ($\chi^2=2.86$). Significant differences in chewing occurred among groups ($\chi^2=14.39$, $p<0.002$) with more instances relative to vehicle treatment observed following TRH ($\chi^2=6$, $p<0.02$), RX77368 ($\chi^2=14$, $p<0.001$), and DKP ($\chi^2=4.2$, $p<0.04$). Significant differences failed to occur among groups on grooming behavior ($\chi^2=2.24$), sniffing, or wet dog shakes ($\chi^2= 6.22$). However, though not a significant difference, while non of the vehicle-treated rats exhibited wet dog shakes, about half the rats in each of the drug groups did exhibit wet dog shakes. Thus, the RX77368 potentiation of swim analgesia appears to be characteristic of its ability to mimic a number of other TRH behaviors. However, the ability of DKP to mimic the TRH potentiation of swim analgesia but not a number of other TRH behaviors is similar to previous research (Prasad et al., 1977; Webster & Griffiths, 1983) showing it is less effective than RX77368 in mimicing TRH behaviors.

Discussion

Both the TRH analogue RX77368 and the TRH metabolite DKP produced transient analgesia on the jump, but not the tail-flick test, at 120 min after the "no-swim" condition. Given the increased potencies of RX77368 (Metcalf, 1983) and DKP (Prasad et al., 1977) as well as the increased CNS half-life (190 min) of RX77368 (Brewster, 1983) it is not surprising that the analgesic effect occurred so long

after injection. However, the reason for the failure of the analogue and metabolite to produce analgesia until this time is unclear. Thus, like TRH alone which produced significant analgesia at 5 min after injection on the tail-flick test (Exp. 1), RX77368 and DKP also appear to produce only transient analgesia. The little previous work done on the analgesic properties of these agents showed that RX77368 produced hyperalgesia on the mouse hot plate test (Burgess et al., 1983) while DKP had no effect from 5 to 30 min post-injection on the rat tail-flick test (Webster & Griffiths, 1983). While TRH alone only produced hyperthermia at 120 min in the no-swim condition (Exp. 3), RX77368 and DKP produced significant hyperthermia over the 120 min time course. Again, this is consistent with the greater potency of DKP and RX77368 as compared to TRH (Metcalf, 1983; Prasad et al., 1977). While this experiment indicates that RX77368 and DKP are potent hyperthermic agents when given alone, other data indicate that RX77368 fails to produce hyperthermia at a behaviorally-active, though unspecified dose (Metcalf, 1983) and that DKP produced hypothermia in a 2°C ambient temperature (Prasad et al., 1978).

Like TRH, both DKP and RX77368 potentiated the magnitude of analgesia induced by the 2°C swim on the tail-flick, but not jump tests. Like the TRH-induced potentiations of footshock and swim analgesia, the

potentiation of swim analgesia by RX77368 and DKP appears to be due to synergistic rather than additive actions since RX77368 and DKP failed to produce analgesia in the no-swim condition on the tail-flick test. While TRH and DKP only potentiated swim analgesia at 30 min, RX77368 did so from 30 to 120 min which is consistent with the increased potency of RX77368 (Metcalf, 1983) but not DKP (Prasad et al., 1977) as compared to TRH. Though the DKP-induced potentiation of swim analgesia (30 min) corresponded with its potentiation of swim hypothermia (30 min), the latter temperature changes were small (1^{o} C). Thus, the potentiated swim hypothermia may not have been responsible for the potentiated analgesia. However, further work is necessary to determine whether the DKP potentiation of swim hypothermia is responsible for its potentiation of swim analgesia. In contrast, the RX77368-induced potentiations of swim analgesia (30-120 min) and swim hypothermia (only at 60 min) had different durations, with the latter effect relatively weak in magnitude (1^{o}C). This indicates that the RX77368 potentiation of swim analgesia is probably not due to its potentiation of swim hypothermia.

If the effects of TRH are produced by the parent peptide and are not a result of its metabolism to DKP, then DKP should fail to mimic the effects of TRH. Further evidence that the effects of TRH are due to the parent

peptide would be provided by a failure of RX77368 to mimic the effects of TRH since RX77368 is not metabolized to DKP and the only major metabolite of RX77368 is behaviorally inactive (Brewster, 1983). On the other hand, if the effects of TRH are solely due to its metabolism to DKP, then DKP should be able to mimic the effect of TRH, and RX77368 should fail to do so. However, Expermiment 4 showed that both DKP and RX77368 mimiced the ability of TRH to potentiate swim analgesia. Thus, since DKP produced the effect, and if indeed the DKP potentiation of swim analgesia is not due to its potentiation of swim hypothermia, DKP may be involved in the TRH potentiation of swim analgesia. Since RX77368 also produced the effect, TRH itself must also be involved in producing the potentiation. Thus, it would appear that the TRH potentiation of swim analgesia may be a result of the combined effects of the parent peptide and of its metabolism to DKP. This could explain the fact that while TRH only has a half-life of 9 min in brain (Brewster, 1983), it's potentiation of swim analgesia lasted from 50 to 80 min post-injection in the present experiments.

The motor effects of TRH, RX77368 and DKP were compared to determine whether any potentiations in swim analgesia were part of a general effect in which these agents are all active in producing the same behaviors. The results showed that while RX77368 and TRH generally

produced similar changes in motor behaviors, DKP did so only in several instances. It was not surprising that RX77368 produced the same motor effects as TRH since it has been found to mimic almost all of the other behavioral effects of TRH (Burgess et al., 1983; Metcalf, 1983). The present results agree with a general pattern in which RX77368 mimics almost all of the effects of TRH while DKP only mimics some of the effects of TRH. Interestingly, DKP also occasionally antagonizes the effects of TRH (Webster & Griffiths, 1983). Thus, metabolism to DKP appears to be able to regulate the effects of TRH either by ending them or by prolonging them. In conclusion, the results of this experiment show that the TRH potentiation of swim analgesia seems to be an instance in which metabolism to DKP prolongs the effect of TRH.

Experiment 5: Central TRH Effects Upon Pilocarpine Analgesia.

As indicated previously, cholinomimetics alone produce analgesia (see Watkins et al., 1984b, for review) and the cholinergic system is also implicated in non-opiate forms of stress-induced analgesia since cholinergic antagonists decrease hindpaw shock (Watkins et al., 1984b) and CWS analgesia (Sperber, Kramer, & Bodnar, in press). Excitatory interactions of TRH with the cholinergic system have been found in a number of behavioral and biochemical

studies (Yarbrough, 1983). Thus, the present study examined whether TRH interactions with the cholinergic system could be extended to potentiation of cholinergic analgesia by TRH. This would provide preliminary, indirect evidence that the TRH potentiation of SIA may be due, at least in part, to TRH excitation of the cholinergic system.

Method

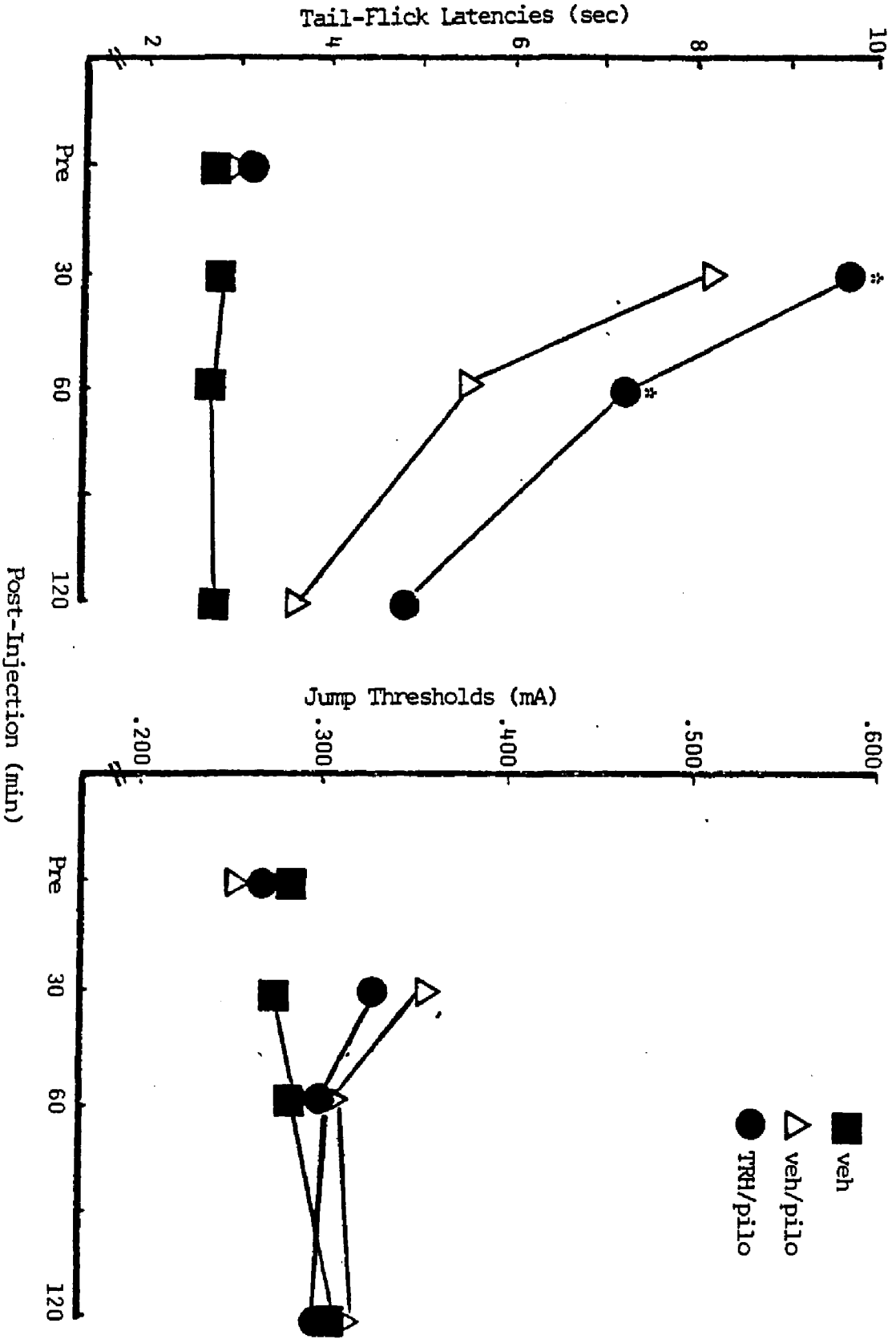
Nine rats, matched for baseline tail-flick latencies and jump thresholds, each received the following three experimental conditions according to a modified Latin Square design: vehicle (ICV)/vehicle (IP); vehicle (ICV)/pilocarpine (10 mg/kg, IP); and TRH (50 ug, ICV)/ pilocarpine (10 mg/kg, IP). The ICV injection preceeded the systemic injection by 20 min. Tail-flick latencies and jump thresholds were determined prior to the first injection and 15, 30, 60, and 120 min following the systemic injection.

Results

Figure 15 illustrates the TRH-induced potentiation of pilocarpine analgesia on the tail-flick, but not the jump test. Significant differences in tail-flick latencies were observed across the time course ($F(3,63)=63.10$, $p<0.001$), among conditions ($F(2,21)=40.40$, $p<0.001$), and for the interaction between test times and conditions ($F(6,63)=16.54$, $p<0.001$). Tail-flick latencies were

Figure 15. Centrally administered TRH significantly potentiated pilocarpine analgesia on the tail-flick but not the jump test.

* Dunnett comparison, $p<0.05$.


Tail-Flick Latencies (sec)
2
4
6
8
10
Pre
30
60
120
Post-Injection (min)
Jump Thresholds (mA)
.200
.300
.400
.500
.600
Pre
30
60
120
veh
veh/pilo
TRH/pilo

significantly elevated over baseline values up to 60 min after injection for the group receiving pilocarpine alone, up to 120 min after injection for the group receiving TRH and pilocarpine, but failed to change for vehicle-treated rats. TRH potentiated pilocarpine analgesia at 30 and 60 after injection.

Significant differences in jump thresholds were observed across the time course ($F(3,66)=11.63$, $p<0.001$), and for the interaction between test times and conditions ($F(6,66)=5.26$, $p<0.001$), but not among conditions ($F(2,22)=.42$). Jump thresholds were significantly elevated above baseline values up to 120 min after injection for the group receiving pilocarpine alone, at 30 min after injection for the group receiving TRH and pilocarpine, but failed to change for vehicle-treated rats. TRH failed to significantly potentiate pilocarpine analgesia on the jump test.

Discussion

TRH is capable of modulating cholinergic analgesia, as seen by its potentiation of analgesia induced by the muscarinic cholinergic receptor agonist pilocarpine on the tail-flick, but not on the jump test. This is similar to the ability of TRH, DKP, and RX77368 to potentiate swim analgesia more consistently on the tail-flick than on the jump test.

The CNS distribution of TRH and its receptors

combined with behavioral data indicate that the septum is an area where the TRH potentiation of cholinergic analgesia could occur. The septal area, including the septo-hippocampal cholinergic pathway, is involved in analgesia (Breglio, Anderson, & Merrill, 1970; Gol, 1975; Kasper, 1964; and Kelsey & Baker, 1983) and the septal area has one of the highest concentrations of TRH (Hokfelt et al., 1975; Brownstein, Palkovits, Saavedra, Bassiri, & Utiger, 1974) and TRH receptors (Simasko & Horita, 1982; Pilotte et al., 1984) in the rat brain. Further, there is evidence that TRH receptors in the septum are on cholinergic cell bodies which project to the hippocampus (Simakso & Horita, 1984). Finally, the septum is the site most sensitive to TRH antagonism of pentobarbital narcosis (Kalivas & Horita, 1980) which appears to be due to TRH interactions with cholinergic neurons (Kalivas & Horita, 1983). Thus, further experiments are needed to determine whether the septum or other sites are involved in the TRH potentiation of cholinergic analgesia.

Since the muscarinic cholinergic antagonist scopolamine decreases non-opiate swim analgesia (Sperber et al., in press) and since TRH potentiates swim analgesia, this experiment indirectly supports the hypothesis that the TRH potentiation of swim analgesia may be due, at least in part, to a TRH interaction with muscarinic cholinergic receptors. This interaction would

be one of TRH exciting cholinergic neurons since electrophysiological and biochemical evidence indicates that TRH interactions with the cholinergic system are excitatory (Yarbrough, 1983). Further, a TRH interaction with the cholinergic system in potentiating forepaw shock analgesia cannot be ruled out. This is because, though the cholinergic system was not found by Watkins and co-workers (1984b) to be involved in forepaw shock analgesia, the present form of forepaw shock analgesia may be qualitatively different since Experiment 2 failed to replicate the naloxone antagonism of forepaw shock analgesia found by Watkins and co-workers (1982a). There is not data to indicate or contraindicate cholinergic involvement in 20 or 80 tailshock or footshock analgesia, so involvement of the cholinergic system in the TRH potentiation of 20 and 80 footshock analgesia is also a possibility. Thus, further work is necessary to determine whether a TRH interaction with the cholinergic system underlies its potentiation of the forms of stress-induced analgesia studied here. Again, though forebrain areas are not involved in 20 and 80 tailshock analgesia (Watkins et al., 1984a) and forepaw shock analgesia (Watkins et al., 1983a), the analgesia obtained in Experiments 1 and 2 after 20 and 80 footshocks and forepaw shock may be qualitatively different from that originally found. Thus, futher experimentation is needed to determine whether the

septum, other forebrain areas, or hindbrain areas mediate the TRH potentiation of stress-induced analgesia and the possible cholinerigic involvement in it.

The TRH potentiation of SIA may also involve TRH interactions with a number of other systems since TRH interacts with noradrenergic, serotoninergic, and dopaminergic systems (Bennett et al., 1983; Biggins, Das, Dodd, Edwardson, Hardy, McDermott, & Smith, 1983; Heal, Pycock, Youdim, & Green, 1983; Hennies, Friedrichs, & Flohe, 1983; Lin, Chan, Chen, & Teh, 1983; Prange et al., 1979). However, TRH interactions with the cholinergic system were pursued here since its involvement with this system is well documented (Yarbrough, 1983). Further, the involvement of some several other neurotransmitters in the TRH potentiation of stress-induced analgesia is contraindicated or only weakly indicated. For instance, TRH has an excitatory effect on cholinergic neurons and cholinergic behaviors (Breese et al., 1975; Horita et al., 1976; Yarbrough, 1983) and activation of the cholinergic system produces analgesia (Houser, 1976). In contrast, though TRH potentiates dopaminergic behaviors and dopamine release (Bennett et al., 1983; Huidobro-Toro et al., 1974; Lin et al., 1983; Plotnikoff et al., 1972), stimulation of the dopamine system decreases CWS analgesia (Bodnar et al., 1980a). Thus, an interaction with the cholinergic system was the most likely way in which TRH could

potentiate stress-induced analgesia. The TRH potentiation of cholinergic analgesia provides preliminary support for this hypothesis.

General Discussion

This series of studies demonstrates that while TRH alone has only transient analgesic properties, it powerfully potentiates a number of forms of SIA, via a central mechanism of action. This potentiation is due to TRH itself, though its metabolism to DKP may also play a role.

The TRH potentiation of both SIA and cholinergic analgesia could be due to TRH-induced increases in blood pressure (Holaday et al., 1981) since hypertension produces analgesia (Zamir & Segal, 1979; Zamir & Shuber, 1980). However, this is unlikely since 2 and 8 mg/kg TRH, given intravenously, is effective in increasing blood pressure (Holaday et al., 1981), but not in potentiating forepaw or swim analgesia. Further, the analgesic effects of vasopressin have been dissociated from its pressor effects (Kordower & Bodnar, 1984) showing that the analgesic and pressor effects of a substance can occur independently.

Due to the lack of TRH antagonists and the lack of negative feedback of thyroid hormones or TSH to the CNS which could decrease TRH, it was not possible to determine whether TRH is normally involved in the production of SIA

(e.g. whether it is a physiological effect). Determining this would further the understanding of the mechanisms of action of various forms of stress-induced analgesia and of the effects of TRH. If it is a physiological effect, a further issue of interest is whether the role of TRH in SIA is that of a modulator or if it is a major component of the response. This issue arises because, as with a number of other peptides, it is unclear whether TRH is acting as classical neurotransmitters or as a modulator of neurotransmitter systems. While Metcalf (1983) has suggested TRH may act as a neuromodulator, the existence of specific TRH receptors suggests that it could also act as a classical neurotransmitter (Burt & Snyder, 1975; Pilotte et al., 1984; Sharif et al., 1983).

If the TRH potentiation of SIA does represent a physiological effect, it could be adaptive for the organism as it would aid in producing analgesia yet allow the animal to remain alert so that it could cope with an attack or other threatening situation, since TRH is also a CNS activating substance (Prange et al., 1974; 1975). However, even if this is not a physiological effect, it could have clinical relevance. For instance, there is some evidence that TRH potentiates opioid analgesia in rats and monkeys (Holaday, 1984; Holaday & Faden, 1983) and if this occurs in humans, the use of TRH in conjunction with morphine could increase the effectiveness

of the narcotic and perhaps allow a lower dose to be given. This would be beneficial since morphine has negative side effects such as constipation and respiratory depression and since it is necessary to increase the dose as tolerance develops. Interestingly, TRH antagonizes a number of opiate effects such as respiratory depression, hypothermia, and decreased blood pressure (Holaday, 1984; Holaday et al., 1978; Horita et al., 1976; Tache et al., 1977), so that if TRH were given with morphine it might actually be able to counteract some of the negative side effects while potentiating the analgesic effects. Since TRH also potentiates a number of non-opioid forms of SIA and cholinergic analgesia, it may also have clinical efficacy in potentiating the effects of nonopiate analgesics. Finally, use of TRH in conjunction with other pain relievers would enable the patient to remain alert while taking analgesics.

The ability of TRH to potentiate SIA is in keeping with the recent SIA and SPA studies showing that, while endogenous pain inhibitory systems were originally thought to be opioid mediated (Fields & Basbaum, 1978), nonopioid pain inhibitory systems, mediated by a number of different neurotransmitters and peptides, also exist (see Bodnar, 1984, fore review; Cannon et al., 1982). Thus, while TRH is not usually associated with pain inhibitory systems, the present series of studies shows that TRH can be added

to the list of peptides and classical neurotransmitters that mediate analgesia. Further, these studies are in agreement with previous studies showing that, while TRH alone has only transient analgesic properties (Boschi et al., 1983), it can modulate pain inhibitory systems. For instance, it antagonzies neurotensin analgesia (Osbahr et al., 1981), and potentiates (Holaday & Faden, 1983; Watkins et al., 1986), antagonizes (Watkins et al., 1986), or fails to affect opiate analgesia (Holaday et al., 1978; Horita et al., 1976; Martin et al., 1977). One reason that TRH decreases neurotensin analgesia but potentiate various forms of SIA may be that neurotensin and stress-induced analgesia are subserved by different mechanisms. Neurotensin analgesia is not antagonized by cholinergic, noradrenergic, or dopaminergic antagonists (Clineschmidt, McGuffin, & Bunting, 1979), while the cholinergic system is involved in footshock and CWS analgesia (Watkins et al., 1984b; Sperber et al., in press) and noradrenergic and dopaminergic systems have been implicated in CWS analgesia (Bodnar et al., 1980a; Bodnar et al., 1983a). Thus, for instance, TRH neuromodulation of the cholinergic system could underlie its potentiation of SIA, whereas it could interact with a different system to decrease neurotensin analgesia. Further, neurotensin analgesia and SIA differ in that neurotensin analgesia is seen on the rat hot plate, but not the rat tail-flick test

(Clineschmidt et al., 1979) whereas the TRH potentiation of SIA is seen on the rat tail-flick test. The conflicts in the literature concering the effects of TRH on opioid analgesia need clarification. As previously discussed, this would be helpful since it has possible clinical relevancy.

TRH could act at different CNS sites and with different systems to potentiate the various forms of SIA, or it could potentiate all of them via the same mechanism. At this point it is impossible to rule out any brain area based on previous research into the mechanisms of SIA since the forms of SIA obtained here may be qualitatively different from those previously examined. The distribution of TRH overlaps with a number of areas involved in pain inhibition, including the PVN, the septum, the nucleus tractus solitarius, the PAG, and the RVM (Breglio et al., 1970; zkasper, 1964; Basbaum & Fields, 1984). Thus, microinjecting TRH into or lesioning these areas prior to administering the anaglesic stressor, would be a next step in determining the site of action of TRH. Using agonists and antagonists, or lesioning neurotransmitter pathways would provide information concerning which neurotransmitter(s) TRH interacts with to potentiate SIA. As indicated in Exp. 5, the cholinergic system is one system that TRH may interact with to potentiate SIA.

In conclusion, these studies show that TRH potentiates several forms of SIA including 20 and 80 shock analgesia, forepaw shock analgesia, and analgesia induced by a range of swim temperatures. TRH also potentiates cholinergic analgesia. The TRH potentiation of these forms of SIA appears to be due to a central mechanism of action and not to involve the opioid system. Studies utilizing antagonists, microinjections and lesions of various brain regions are needed to determine the site(s) of action and neurotransmitter(s) with which TRH interacts to potentiate these forms of SIA. The ability of TRH to potentiate cholinergic analgesia suggests that an interaction with this system could be underlie its potentiation of SIA.

Appendix A: The CNS Distribution of TRH

Hypothalamic and Extrahypothalamic Cell Bodies: The studies cited in this appendix all used rats except where indicated. In an initial study (Hokfelt et al., 1975), cell bodies containing TRH were found only in the dorsomedial hypothalamic nucleus. However, Johansson and Hofkelt (1980) pretreated animals with colchicine and were able to visualize TRH in cell bodies in a number of hypothalamic areas and also in extra-hypothalamic areas. The hypothalamic regions included the preoptic suprachiasmatic nucleus, the basal hypothalamic-suprachiasmatic complex, the periventricular area, the paraventricular area, the perifornical area, the dorsomedial and ventromedial hyopthalamic nuclei, and the lateral hypothalamus. In extra-hypothalamic areas, Johannson and Hokfelt (1980) found cell bodies in the nucleus raphe magnus and mucleus raphe pallidus of the medulla. At the EM level, cell bodies in the areas dorsal to the optic chiasm and in the dorsomedial hypothalamic nucleus were found to contain several labeled, large dense core granular vesicles (Johannson et al., 1980).

Hypothalamic Procceses: TRH has not only been found in the hypothalamus, but in a number of other brain areas using both radioimmunassays (RIA) and immunohistochemistry (IHC). RIA studies agree that the greatest concentration

of TRH in the rat brain is in the hypothalamus (Brownstein et al., 1974; Jackson & Reichlin, 1974; Kardon, Winokur, & Utiger, 1977; Oliver, Eskay, Ben-Jonathan, & Porter, 1974; Winokur & Utiger, 1974). Hokfelt and co-workers (1975), examining the hypothalamus using indirect immunofluorescence, found the greatest concentration of TRH positive fibers in the medial part of the external layer of the median eminence and a dense plexus was found in the external layer of the stalk, extending slightly into the posterior pituitary. There was a high concentration of TRH positive fibers in the dorsomedial hypothalamic nucleus, the perifornical region, and the parvocellular part of the paraventricular nucleus. In part of the medial part of the ventromedial hypothalamic nucleus, the periventricular area, and the zona incerta a slightly dense network of TRH positive fibers were found. Single TRH positive fibers were found in several other hypothalamic areas, such as the medial forebrain bundle, the anterior hypothalamic nucleus, in parts of the preoptic area, in the magnocellular part of the paraventricular nucleus, and in the suprachiasmatic nucleus. Brownstein et al. (1974), using RIA, utilized the punch technique to examine a number of hypothalamic nuclei and the median eminence and found the highest concentrations of TRH in the same areas as did Hokfelt et al. (1975). Brownstein, Eskay, and Palkovits (1982) found

that almost all of the TRH in the median eminence originated from the paraventricular nucleus and much of the TRH in the arcuate nucleus originates from the paraventricular nucleus since bilateral paraventricular nucleus lesions produced an 87% delpletion of TRH from the median eminence, a 65% depletion of TRH from the arcuate nucleus, and no TRH depletion in the medial forebrain bundle. Johansson and co-workers (1980) used the peroxidase-antiperoxidase (PAP) method, and examined TRH in the rat brain at both the light and electron microscopic levels. At the EM level, almost all of the TRH in the processes was found in large, granular vesicles. No TRH was found in small, electron lucent vesicles, which are considered to be the classical synaptic vesicles. High concentrations of immunoreactive fibers were found in the medial part of the external layer of the median eminence, frequently approaching the blood vessels in the surface zone. Electron microscopy showed that several immunoreactive boutons in this area contained large granular vesicles with an electron dense core. Frequently these immunoreactive nerve terminals were directly in contact with non-immunoreactive nerve endings, and sometimes were close to the perivascular space, in a "secretion position". The immunoreactive, large granular vesicles had a diameter of 956 A and in boutons which also had small, synaptic

vesicles, the TRH immunoreactive large, granular vesicles made up about 16% of all the vesicles in the bouton. Johansson and co-workers (1980) localized TRH to boutons in the suprachiasmatic nucleus, the dorsomedial nucleus, and the parvocellular part of the paraventricular nucleus and TRH was again in large, granular vesicles. Immunoreactive boutons in the suprachiasmatic nucleus and paraventricular nucleus were often directly in contact with dendrites. Immunoreactive boutons in all three areas were often directly in contact with cell bodies and appeared to form axo-somatic synapses. Immunoreactive boutons in the dorsomedial nucleus were sometimes in contact with unlabelled boutons. Johansson and co-workers (1980) explained that the reason they did not find any TRH in classical synaptic vesicles may be because of TRH loss during tissue processing since TRH is a small peptide and may be highly soluble. Also, the content of TRH in the vesicles may not be high enough to be visualized with the procedure used, or the antibody may not penetrate into the small synaptic vesicls very well. Also using the PAP technique, Shioda and Nakai (1983) examined the arcuate nucleus and median eminence of rats. As in the Johansson et al. (1980) study, the greatest amount of TRH-LI in the median eminence was in the medial part of the external layer and at the EM level, the TRH-LI fibers and terminals in the arcuate nucleus and median eminence was found to be

in large granular vesicles and no TRH-LI was found in small, clear vesicles. No TRH-LI was found in mitochondria, in microtubules, or other organelles in the TRH-LI fibers and terminals.

Extrahypothalamic Processes: TRH has also been found in a number of extrahypothalamic areas. The dorsal part of the nuclues accumbens contained a high-density network of TRH positive fibers, while networks of moderate density were seen in the organum vasculosum lamina terminalis, the nucleus interstitialis stria terminalis, and in the ventral part of the lateral septal nucleus, in which they formed basket like structures around cell bodies (Hokfelt et al., 1975). Johansson and co-workers (1983), using indirect immunofluorescence in colchicine treated rats, describe the location of brainstem and spinal cord TRH fibers. In the mesencephalon, moderate density networks were found in the nucleus originis nervi oculomotorii, and low density networks were seen in the red nucleus and in the ventral, caudal part of the nucleus of the lateral lemniscus. Single fibers were found in the raphe region, in the paramedian reticular area, around the interpeduncular nucleus extending in a lateral direction, and in the substantia nigra and medial lemniscus. In the pons, moderate density networks were found in the nucleus originis nervi trigemini and in the ventral part of the lateral parabrachial nucleus. Low density networks were

seen in the nucleus tegmenti pedunculo-pontinis. Single fibers were found in the raphe region, in the pontine reticular formation, in the periaqueductal gray (especially in the ventral part), in the lateral lemniscus, in the nucleus tractus mesencephali, the locus coeruleus, and in the superior olivary nucleus. In the medulla, moderate density networks were found in the nucleus originis nervi facialis, the nucleus nervi hypoglossi, in peripheral parts of the NTS, in the border zone between the nucleus nervi hypoglossi and the caudal part of the nucleus dorsalis motorius nervi vagi, in the nucleus intercalatus, in the nucleus commissuralis, the nucleus ambiguus, and in a patchy manner in the ventrolateral and dorsomedial reticular formations. Low density networks were seen in the raphe region (especially the ventral parts, including the nucleus raphe magnus and pallidus), in the reticular formation medial and lateral to the nucleus originis nervi facialis, and in the caudal part of the nucleus nervi hypolgossi. Single fibers were seen in other parts of the reticular formation, in the medial and spinal vestibular nucleus, and in the medial lemniscus. Finally, in the spinal cord, moderate dinsity networks were seen in the most anterior, lateral and medial zones of the ventral horn mostly around motorneurons, in the zone around the central canal sometimes extending laterally, and in the sympathetic

lateral column but not in the dorsal horn. However in mice, using the PAP technique and a highly specific antiserum, TRH was found in the dorsal horn of mice, primarily in cell bodies and sometimes in processes in the superficial lamina, especially along the lamina II-III border (Coffield et al., 1984).

Since it has been found that vasopressin and oxytocin immunoreactivity in the intermediolateral column of the spinal cord comes soley from cell bodies in the paraventricular nucleus and since TRH has been found in fibers close to sympathetic preganglionic neurons in the intermediolateral column of the lateral horn and in fibers close to alpha motor neurons, Lechan, Snapper, and Jackson (1983) wanted to determine whether any spinal TRH containing neurons originated in the paraventricular nculeus. The paraventricular nuclei of male rats were electrolytically lesioned and the median eminence and spinal cord later removed. They found a significant decrease in the TRH content of the median eminence following the paraventricular nucleus lesion but no decrease in spinal cord TRH was found.

Co-existence of TRH With Other Peptides and Transmitters: IHC techniques have been used to investigate the coexistence of TRH, 5-HT, and SP. Johansson and co-workers (1981), using IHC in colchicine treated rats, found immunoreactive 5-HT, TRH, and SP cell bodies found

in the nucleus raphe magnus (NRM), nucleus raphe pallidus (NRP), nucleus raphe obscuris (NRO), and in areas dorsal, lateral, ventral, and within the pyramidal tract, with 5-HT neurons being more numerous than the other two. Examining consecutive sections of the same cell bodies showed that 5-HT and TRH immunoreactivity coexisted in the NRM, NRO, NRP, parapyramidal, and paraolivar regions. Further, three consecutive sections of the same cell showed that TRH, 5-HT, and SP coexisted in the NRP. Elution and restaining experiments provided evidence that TRH, 5-HT, and SP all coexisted in one cell body in the NRM, NRO, and NRP, and parapyramdial region and that other cell bodies in these areas contain 5-HT and SP or TRH and SP, or only one of the three. In the medulla, there are about twice as many 5-HT neurons (56%), as TRH (23%), or SP (21%) neurons. This proportion holds for the NRM and NRO, indicating that there are 5-HT neurons in the medulla which do not also contain TRH and SP. However, in several series of NRP neurons, almost identical numbers of the three exist, supporting the finding that many of these cells contain all three. In the rat lumbar and thoracic spinal cord, a similar distribution of TRH, SP, and 5-HT immunoreactivity was seen in the ventral horn with immunoreactive fibers close to motorneurons, dendrites, and within the surrounding neuropil and in the sympathetic lateral column. Again, there were more immunoreactive 5-

HT fibers than TRH and SP fibers. 5-HT and SP, but not TRH, were also found in the dorsal horn. Gilbert et al. (1982) found that, unlike 5-HT and SP, only a few isolated TRH fibers were in the dorsal horn, non of which were in the substantia gelatinosa. A few fibers were also seen around the central canal. Dense TRH, 5-HT, and SP fibers all were seen around neurons in the sympathetic lateral column and bands of fibers radiated toward the central canal. Dense 5-HT, SP, and TRH fibers were in the ventral horn around motor neurons. Two studies found that, following treatment with 5,7-DHT or 5,6-DHT, which are 5-HT neurotoxins, almost all of the TRH, SP, and 5-HT immunoreactivity was gone from the ventral horn and lateral sympathetic column and 5-HT was also gone from the dorsal horn, though SP was unaffected there (Gilbert et al., 1982; Johannson et al., 1981). However, using RIA, there was a 25% decrease in SP in the dorsal horn after 5,6-DHT, implying that there is a descending 5-HT SP pathway from the medulla to the dorsal horn (Gilbert et al., 1982). This provides evidence that the peptides coexist, either singly or together, with 5-HT in neurons that project from the medulla to the ventral horn of the spinal cord and that 5-HT and SP may coexist in neurons projecting to the dorsal horn.

Bowker and co-workers (1982) used a different technique and found slightly different results regarding

the location of TRH cell bodies. Following horseradish peroxidase (HRP) injection into the lumbosacral spinal cord of rats retrogradely labeled cell bodies were found in the NRO, NRP, NRM, and in the nucleus reticularis gigantocellularis (an adjacent area of the reticular formation). After the peroxidase histochemistry the same brainstem sections were incubated with antisera to 5-HT, TRH, SP, or leucine- or methionine-enkephalin (ENK) using the PAP technique. Of the raphe neurons that were found by HRP to project to the lumbosacral spinal cord, 82.3% contained 5-HT, 55% contained SP, 10-15% contained TRH, and 10-20% contained enkephalin. 5-HT cells that project to the cord (eg. were double labeled by HRP and 5-HT) were found in the NRM, NRP, and NRO and also in the nucleus reticularis gigantocellularis. Spinally projecting SP cells were found in the NRM, NRP, and NRO. In contrast to the previous study, spinally projecting TRH cells were found only in the NRP and in the ventral part of the reticular formation lateral to the inferior olive. Thus, the most likely place for 5-HT, TRH, and SP to coexist or for 5-HT and TRH to co-exist was the NRP. Enkephalin projection to the cord was found in the NRM and ventral parts of the nucleus gigantocellularis. 5-HT extended as far rostral as the trapezoid body, whereas TRH and SP did not.

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