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An Analysis of the Role of Midbrain Dopamine Systems in the Suppression of Tonic Pain

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ABSTRACT

An Analysis of the Role of Midbrain Dopamine Systems
in the Suppression of Tonic Pain

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It has been considered for some time that the activation of the midbrain ascending dopamine (DA) systems plays a role in the suppression of tonic or persistent pain. For instance, earlier work has shown that substance P (SP), a tachykinin neuropeptide released during pain and stress, acting in the midbrain induces analgesia in the formalin test for tonic pain. The present thesis explored in more depth the role of SP-DA interactions in the suppression of tonic pain, and examined whether the activation of midbrain DA neurons by endogeneous SP might be a mechar n underlying stress-induced analgesia. A first series of experiments were designed to examine the effects of agonists with different affinities for tachykinin NK-1 and NK-3 receptors in the formalin test for tonic pain and, for comparison, the tail-flick test for phasic pain, following infusions into either the ventral tegmental area (VTA) in the midbrain or nucleus accumbens (NAS) in the forebrain. Infusions into either the VTA or NAS of the NK-1 agonist, GR-73632, and the NK-3 agonist, senktide, induced analgesia in the formalin, but not the tail-flick test, suggesting that NK-1 and NK-3 receptors in the VTA and NAS are involved in mediating the inhibition of tonic pain. Pretreatment with the opioid receptor antagonist, naltrexone, did not attenuate the analgesic effects in the formalin test of intra-VTA or intra-NAS infusions of the SP analog, DiMe-C7, GR-73632, or senktide, suggesting that these effects are mediated independently from opioid mechanisms. The idea that exposure to stress inhibits tonic pain by causing, in part, the release of SP in the VTA was supported by the finding that intra-VTA pretreatment with the NK-1 selective receptor antagonist, RP 67580, blocks stress-induced analgesia in the formalin test. Another series of studies were designed to

explore the possibility that enhanced DA transmission in the NAS mediates the inhibition of tonic pain. It was found in these studies that intra-NAS pretreatment with DA receptor antagonists attenuates analgesia induced by intra-VTA DiMe-C7, intra-VTA morphine, and intra-NAS amphetamine in the formalin test. It was also found that reduced DA release in midbrain ascending systems, induced by a low autoreceptor-specific dose of apomorphine, prevents the analgesic effect of intra-VTA morphine. The findings revealed throughout the present thesis suggest that part of a pain-suppression system that serves to inhibit tonic pain depends, at least in part, upon the activation of the DA neurons innervating the NAS, and that this pain-suppression system is naturally triggered by exposure to stress and/or pain, through the release of opioids and SP.

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GENERAL INTRODUCTION

Pain is a complex multi-determined phenomenon that is characterized by both a sensory and an emotional experience and, as with pleasure, motivates behaviour in a powerful way. Descartes viewed, in 1664, the transmission of pain messages from the periphery to the brain as occuring in a straight-through channel, similar to a bell-ringing mechanism in a church and, in fact, this account is widely described in most medical textbooks today (see Melzack & Wall, 1988, for review). The transmission of pain signals in the central nervous system, however, has proved to be more complex than once envisioned by Descartes. Its suppression has proved to be an even a greater challenge. More and more, however, we are gaining a greater understanding of the neural mechanisms that are involved in mediating both the transmission of pain signals and the inhibition of pain.

Information about noxious painful stimulation is transmitted through sensory neurons to the spinal cord, where ascending neurons in turn relay the information to higher centers of the central nervous system through several pain-signalling pathways such as the spinoreticular, spinohypothalamic, and spinothalamic tract (see Besson & Chaouch, 1987, for review). These pathways have been categorized, based on physiological and anatomical properties, into two major pain-signalling pathways referred to as either the lateral or the medial system (Dennis & Melzack, 1979; Melzack, 1990). More specifically, the lateral system appears to transmit information about noxious painful information rapidly through myelinated, fast-conducting, thin fibres to the thalamus which, in turn, convey the information to the primary somatosensory cortex. The activation of this system is thought to give rise to phasic pain, which is sharp, transient, and sudden, and may function to provide an organism with immediate information about the location of the noxious stimulation. In contrast, the medial system

appears to transmit information about noxious stimulation through unmyelinated, slow-conducting, large diameter fibres to various brainstem areas and the thalamus, the latter of which, in turn, relays the information to several limbic sites such as the amygdala. The activation of the medial system is thought to be responsible for eliciting tonic or persistent pain, and may serve to remind an organism that an injury has occurred and that it requires special attention.

It is now known that exogeneous opioids inhibit pain by acting, in part, at spinal sites (Hylden and Wilcox, 1983; Siuciak & Advocat, 1987; Yaksh and Rudy, 1977; Yeung & Rudy, 1980). Numerous studies have also suggested that opioids inhibit the transmission of pain signals by activating spinally-projecting descending pathways that originate in the brainstem (see Advocat, 1988, for review). According to one model (Basbaum & Fields, 1984), opioids act in the periaqueductal gray of the brainstem. Upon the stimulation of opioid receptors at this site, neurons activate, through direct projections, serotonin-containing cells in the nucleus raphe magnus which, in turn, activate neurons that project, via the dorsolateral funiculus, to the dorsal horn of the spinal cord, where they inhibit axons that transmit information about noxious painful stimulation to higher centers of the central nervous system. There is considerable evidence in support of this model (e.g. Basbaum & Fields, 1984; Bennett & Mayer, 1976; Bodnar et al., 1988; Dickenson & Sullivan, 1986; Gebhart & Jones, 1988; Herz et al., 1970; Jacquet & Lajtha, 1973, 1974; Mayer et al., 1971; Mayer & Murphin, 1976; Mayer & Price, 1976; Tsou & Jang, 1964; Yaksh & Rudy, 1978). For instance, it has been reported that the analgesic effect of systemic morphine is reduced in spinally-transected animals relative to intact animals (Advocat, 1988). Presumably, this occurs because the spinally-projecting brainstem inhibitory systems are removed and the effect of systemic morphine is only expressed in the spinal cord.

This brainstem descending pain-suppression system, however, is more effective at inhibiting the transmission of phasic than tonic pain. In fact, several lines of evidence suggest that the neural systems that mediate the inhibition of phasic versus tonic pain are fundamentally different. This literature will be reviewed in the following paragraphs. Before this literature is considered, however, the following section describes the pain tests used in such studies.

Different Types of Pain Tests

Animal pain tests have been developed mainly to provide a screening device with which to test the analgesic effects of drugs, but have also been used to explore the neural mechanisms underlying pain suppression (Franklin & Abbott, 1989). Over 50 different types of pain tests are documented in the literature and numerous variations of these have been developed (see Franklin & Abbott, 1989, for review). The most commonly employed pain tests measure changes in the threshold at which a stimulus is first perceived as being noxious. The pain assayed by such tests is transient, short-lasting, rapidly rising and well-localized and thus has been termed phasic. The escape response to phasic pain serves to prevent or minimize tissue damage and enduring pain. One of the most popular phasic pain tests, the tail-flick test (D'Amour and Smith, 1941), measures the withdrawal response to thermal stimulation of the tail. The tail-flick response is organized at the level of the spinal cord, since it is elicited in spinally-transected rats (Carroll & Lim, 1960; Irwin et al., 1951). Although studies employing phasic pain stimuli have provided considerable information concerning the neural mechanisms of pain and analgesia, the nature of the pain assayed in these tests does not ressemble that encountered in the clinical situation. Thus the findings derived from phasic pain tests provide limited information about the effectiveness of pain management techniques (e.g. analgesic drugs) on pain of pathological origin.

The formalin test was devised by Dubuisson and Dennis (1977) to provide a model of injury-produced continuous (i.e., tonic) pain similar to that encountered in the clinical situation. Unlike phasic pain tests, the formalin test assesses the behavioural recuperative responses to inescapable pain generated by a subcutaneous injection of dilute formalin into an animal's fore- or hindpaw. Pain responses last approximately 90 minutes and are characterized by favouring, raising and licking of the injured paw. Human volunteers who have sustained a formalin injection describe the pain as being poorly localized, moderate in intensity, burning and throbbing with a time-course corresponding to the behavioural responses in animals (Alreja et al., 1984; Dubuisson & Dennis, 1977; Franklin & Abbott, 1989). These qualities, except for burning sensations, are similar to those produced by postsurgical pain in humans (Franklin & Abbott, 1989). Together, these findings lend credibility to the idea that this test adequately serves its purpose as an animal model of clinical pain.

In addition to developing the formalin test for tonic pain, Dubuisson and Dennis (1977) provided a detailed description of a method used to quantify the intensity of the various behavioural recuperative response to formalin pain. More specifically, using this method, the different behavioural responses thought to reflect varying intensities of pain are rated according to four behavioural categories each associated with a specified weighted-pain score. Thus, an animal receives a score of 0 if it walks or sits normally on the injected paw; a score of 1 if it favours the injected paw (e.g., if it limps or rests the injected paw on the floor with little weight); a score of 2 if it raises the injected paw away from the floor an body; a score of 3 if it licks, chews, or bites the injected paw. An average pain intensity score is then calculated by multiplying the time spent in each behavioural category by the weighted score, summing these products, and dividing by the total time in a given time interval. The weighted-pain scores method of quantifying

the behavioural responses to formalin has been widely employed (e.g., Abbott et al., 1982; Cohen & Melzack, 1993; Lin et al., 1989; Matthies & Franklin, 1992; Pertovaara et al., 1991). Other methods that have been used to quantify pain in the formalin test involve the measurement of one single parameter, such as the time spent licking the injured paw, or the number of times the animal licks the injected paw (e.g., Famser et al., 1985; Hunkskaar et al., 1985; Sugimoto et al., 1986). It has been argued by several researchers that the weighted-pain scores method is reliable and more accurately describes the overall multi-dimensional experience of tonic pain by measuring several pain-related behaviours, rather than one single behaviour, (e.g., Coderre et al., 1993; TjØlsen et al., 1992).

A recent study has confirmed the validity of the weighted-pain scores method of quantifying the behavioural responses to tonic pain in showing that the different weighted-pain scores assigned to the different behavioural categories accurately reflect varying levels of pain intensities (Coderre et al., 1994). More specifically, it was found in this study that escalating concentrations of formalin injected into a paw cause progressive increases in weighted-pain scores. The ordinal nature of the behavioural categories was also evident when the time spent in each category was examined. Thus, as the concentration of formalin increased, animals spent progressively more time in the behavioural categories reflecting more intense pain (i.e., scores 2 and 3), and progressively less time in those reflecting less intense pain (i.e., scores 1 and especially 0). Using a constant formalin concentration of 5.0 %, it was also found that, as the dose of morphine escalates, weighted-pain scores progressively decrease and animals spend progressively less time in the behavioural categories reflecting more intense pain and progressively more time in those reflecting less intense pain. Thus, all these findings suggest that the weighted-pain scores method of quantifying the behavioural responses to tonic pain is both valid and meaningful.

Formalin produces a distinct biphasic behavioral (TjØlsen et al., 1992) and electrophysiological (Dickenson & Sullivan, 1987) response and there appear to be fundamental differences between the two phases. An early transient pain phase begins immediately following the formalin injection and is characterized by vigorous chewing, shaking, and licking of the injured paw. These pain responses subside 5 minutes later and give way to a period lasting 10-15 minutes in which an animal is relatively insensitive to the injury. A late pain phase begins approximately 20 minutes following the formalin injection, remains relatively steady for about 20 minutes and then gradually dissipates. Centrally-acting narcotic drugs such as morphine, codeine, meperidine, buprenorphine and pentazocine inhibit pain responses during both the early and late phase (Dubuisson & Dennis, 1977; Hunskaar et al. , 1986; Hunskaar & Hole, 1987; Shibata et al. , 1989; Vaccarino et al., 1989). Several lines of evidence suggest that the early and late pain phases of the formalin test are mediated by independent physiological processes (see TjØlsen et al., 1992, for review). The early pain phase appears to be due to direct stimulation of nociceptors and C-fibers since capsaicin pretreatment, which selectively destroys SP-containing unmyelinated sensory neurons, produces analgesia during the early but not the late phase (Shibata et al., 1989). The late pain phase, in contrast, appears to result from ensuing inflammatory processes. Peripherally-acting non-steroidal (e.g. aspirin, indomethacin) and steroidal (e.g hydrocortisone, dexamethasone) anti-inflammatory drugs attenuate the late pain phase while leaving the early pain phase unaffected (Hunskaar et al., 1986; Hunskaar & Hole, 1987; Rosland et al., 1990; Shibata et al., 1989).

The neural structures that are involved in mediating the behavioural recuperative responses to formalin appear to lie at ipsilateral forebrain sites. More specifically, it has been reported that unilateral knife cuts made through the medial forebrain bundle,

hypothalamus, medial internal capsule, and thalamus attenuate formalin-induced pain responses in the ipsilateral but not contralateral forepaw when both forepaws are injected with formalin (Amodei & Paxinos, 1980). There is some evidence, however, to suggest that sites lying more caudally in the brainstem are sufficient for the expression of pain responses to formalin. Matthies and Franklin (1992) examined the behavioural responses to formalin in decerebrated rats and found that transections rostral or caudal to the pons fail to alter the rats' ability to display pain behaviour.

In summary, the formalin pain test differs from the more commonly used phasic pain tests, such as the tail-flick test, in that (1) pain in the formalin test is inescapable and long-lasting, whereas pain in phasic tests is escapable and short-lasting, (2) pain responses in the formalin test appear to be organized at supraspinal sites, whereas those in phasic pain tests appear to lie at more caudal sites in the spinal cord, and (3) pain responses in the formalin test are generated by tissue injury whereas those in phasic pain tests are usually elicited by nondamaging stimuli.

The neural mechanisms underlying analgesia in the formalin test are not well understood, but are known to differ, as mentioned previously, fundamentally from those involved in phasic pain tests. The following section reviews this literature.

Different Neural Substrates Mediate Analgesia in Different Pain Tests

Studies that have examined the effects of lesions of the brainstem descending pain-suppression systems indicate that they play a different role in the mediation of analgesia in phasic and tonic pain tests. For instance, it has been reported that lesions of the nucleus raphe magnus, caudal periaqueductal gray, or dorsal lateral funiculus attenuate morphine analgesia in the tail-flick test but are without effects on morphine

analgesia in the formalin test (Abbott & Melzack, 1982a; Abbott et al., 1982b; Ryan et al., 1985). Similarly, it has been shown that nucleus raphe magnus lesions disrupt stimulation-produced analgesia (in the midbrain) in the tail-flick, but not in the formalin test (Abbott & Melzack, 1983).

Pharmacological studies also reveal that the neurochemical systems subserving analgesia in phasic pain tests differ fundamentally from those involved in tonic pain tests. For instance, it has been reported that drugs which either decrease the synthesis of the neurotransmitter serotonin, such as p-chlorophenylalanine (i.e., PCPA), or block serotonin receptors, such as methysergide, attenuate morphine analgesia in the tail-flick test but potentiate this effect in the formalin test (Dennis & Melzack, 1979). In contrast, administration of the serotonin precursor L-tryptophan, which enhances serotonergic activity, has been found to potentiate morphine analgesia in the tail-flick test but diminish morphine analgesia in the formalin test (Abbott & Young, 1988). Similar findings have been reported in the clinical situation. More specifically, it has been found that the amount of morphine required by patients to control post-operative pain is lower when plasma tryptophan levels are low, suggesting that decreased serotonergic activity is associated with enhanced relief from persistent pain (Franklin et al., 1990).

Studies that manipulate noradrenergic systems reveal a similar dissociation of the neural mechanisms underlying the inhibition of phasic versus tonic pain. Dennis and Melzack (1980) have reported that the alpha-adrenergic agonist, clonidine, and the beta-adrenergic agonist, isoproterenol, enhance morphine analgesia in the formalin test but have no effect in the tail-flick. In another study, clonidine was found to produce analgesia in both the formalin and the tail-flick tests, but substantially higher doses were required to elevate tail-flick latencies (Tchakarov et al., 1985).

Support for the view that different neural systems mediate analgesia in different types of pain tests is also provided by the finding that tests which assay phasic versus tonic pain are differentially sensitive to the analgesic effects of opioid administration. Several findings suggest that, although exogeneous opioids inhibit both tonic and phasic pain, they are more effective against the former than the latter type of pain. For instance, it has been shown that morphine, in the doses used clinically, is more effective at alleviating the continuous, intense, burning pain produced by exercising an ischemic limb in human experimental studies employing the tourniquet technique (Smith et al., 1966) than at suppressing phasic pain produced by non damaging stimuli such as pricking, pinching, or radiant heat (Beecher, 1968). These findings parallel those of clinical reports indicating that exogeneous opioids relieve postoperative pain but fail, except at high doses, to suppress the sensations (e.g., twinges, tingling) caused by the surgical wound (Dennis & Melzack, 1979; Jaffe & Martin, 1980). Similarly, it has been reported that morphine relieves clinical postoperative pain without affecting psychophysical ratings of sensory intensity and quality of pain (Franklin et al., 1990). As in the clinical situation, animal studies indicate that exogeneous opioids are more effective at alleviating tonic rather than phasic pain. For instance, Cohen et al. (1984) found that a relatively low dose range (2.5 - 10.0 μg) of morphine infused into the lateral ventricle elicits analgesia in the formalin test whereas much higher doses (50.0 - 200.0 μg) are required to raise pain thresholds in the foot-flick test. In another study, it was found that morphine microinjected into the habenula produces analgesia in the formalin, but not in the foot-flick test (Cohen & Melzack, 1985).

The evidence that repeated administration of morphine affects the analgesic response differently depending on the nature of the pain involved provides another line of evidence that different neural substrates mediate analgesia in different types of pain tests. When animals are administered morphine repeatedly, using a given dose, and

tested for pain sensitivity, a typical finding is that morphine analgesia manifests tolerance (Adams et al., 1969; Advocat, 1989; Bardo & Hughes, 1979; Kayan et al., 1973; Mucha et al,1979; Siegel, 1975, 1976; Siuciak & Advocat, 1989). That is, the analgesic effect of a given dose of morphine declines progressively in response to repeated administration such that, in order to maintain the same amount of analgesia, escalating doses of the opioid are required. Although the pain assayed in these studies is phasic, it is widely assumed that the results derived from such studies apply to the types of pain encountered in the clinical situation and, therefore, it is misbelieved that tolerance to morphine analgesia is likely to be a practical problem in the management of clinical pain (Melzack, 1990). As a result, the use of morphine for the management of clinical pain is not only limited but also ill-tailored to suit the needs for both pain relief and avoidance of aversive side-effects such as mental cloudness and nausea (Melzack, 1990). There is considerable evidence, however, to suggest that when opioids are taken on a long-term basis solely for the relief of clinical pain, tolerance to morphine analgesia is either nonexistent or minimal (Melzack, 1990; Isbell et al., 1947; Mount et al., 1976; Twycross, 1974, 1978). Abbott et al. (1981, 1982) took a closer look at this problem by comparing the effects of repeated morphine administration on analgesia in the formalin and tail-flick tests. In accordance with findings obtained from clinical studies, they found that little tolerance develops to morphine analgesia in the formalin test, whereas morphine analgesia in the tail-flick test shows rapid tolerance.

In summary, it appears that the neural substrates underlying the inhibition of tonic pain differ from those underlying the inhibition of phasic pain. This is supported by the findings that analgesia in these two types of pain is dissociable with respect to manipulations of serotonergic, noradrenergic, and opioidergic systems and with respect to the phenomenon of tolerance to morphine's analgesic effects. The neurotransmitter dopamine has also been found to play a different role in the mediation of analgesia in

phasic and tonic pain tests. This evidence will be reviewed and will be followed by a description of some recent findings suggesting that part of the neural substrates underlying the inhibition of tonic pain depends on activity in dopamine-containing neurons originating in the midbrain and projecting to various forebrain sites. A clear understanding of this literature, however, requires an initial description of the neuroanatomy of these midbrain ascending dopamine-containing neurons.

Neuroanatomy of Midbrain Ascending Dopamine Systems

Detailed neuroanatomical mapping studies have shown that there are several pathways in the central nervous system containing the neurotransmitter dopamine (DA; Ungerstedt, 1971; Dahlstrom & Fuxe, 1965; Thierry et al., 1973; Lindvall & Bjorklund, 1974; Berger et al., 1976; Jacobowitz, 1978). The neurons of some of these pathways originate in the ventromedial mesencephalon and project to various forebrain sites. More specifically, these neurons arise from the continuum of cell bodies located in the ventral tegmental area (VTA), substantia nigra (SN) and retrorubral field and send axons ipsilaterally through the medial forebrain bundle to various forebrain sites (Dahlstrom & Fuxe, 1964). Subpopulations of midbrain DA ascending neurons are classified and referred to as nigrostriatal, mesocortical, and mesolimbic, according to their site of origin and innervation.

The neurons comprising the nigrostriatal pathway originate in the SN and project rostrally to innervate the striatum (which includes the caudate nucleus and the putamen) and the globus pallidus (Bjorklund & Lindvall, 1984). Mesocortical DA neurons arise from the VTA and project rostrally to innervate the prefrontal, entorhinal, anterior cingulate, and piriform cortices (Berger et al., 1976; Fuxe et al., 1974; Lindvall et al., 1978; Thierry et al, 1973). Mesolimbic DA neurons arise from the VTA and project

rostrally to various limbic structures such as the nucleus accumbens septi (NAS; also known as the ventral striatum), habenula, olfactory tubercle, medial portion of the lateral septum, amygdala, bed nucleus of the stria terminalis, anterior olfactory nuclei and, to a much lesser extent, the olfactory bulb and the nuclei of the diagonal band (Bjorklund & Lindvall, 1984; Swanson, 1982). Relatively recent studies indicate that the NAS, whose role in analgesia was examined in the present studies, contains three distinct subterritories referred to as the " core ", " shell ", and " rostral pole " (for review, see Heimer & Alheid, 1991; Zahm & Brog, 1992; Deutch et al., 1993). These subterritories have been reported to differ with respect to somatodendritic morphology (Meridith et al., 1992a), synaptic organization (Zahm, 1992; Meridith et al., 1992b), DA metabolism (Deutch & Cameron, 1991), electrophysiological properties (Pennartz et al., 1991), patterns of afferent (Brog et al., 1993) and efferent (Zahm & Heimer, 1990, 1993; Heimer et al., 1991) projections, and distributions of various transmitters, peptides, and receptors (Zaborsky et al., 1985; Phelps & Vaughn, 1986; Zahm & Heimer, 1988; Meredith et al., 1989; Voorn et al., 1989; Churchill et al., 1990; Lee et al., 1990).

The midbrain DA ascending neurons appear to project topographically. More specifically, it appears that different portions of the ventromedial mesencephalon give rise to different ascending DA projections (Fallon, 1988). For instance, whereas mesolimbic neurons innervating the olfactory tubercle and amygdala derive their cell bodies from the dorsal VTA, those innervating the NAS and lateral septum derive their cell bodies from the medial and ventral VTA, respectively. It has also been reported that neurons located in both the VTA and SN contribute to nigrostriatal, mesocortical, and mesolimbic ascending systems, although neurons in either cell group are predominantly implicated in one system over the other (Fallon, 1988). For instance, the majority of mesocortical neurons innervating the frontal, anterior cingulate and suprarhinal cortices originate from the VTA but a minority of these also derive from

discrete portions of the SN. Similarly, the caudate nucleus is innervated primarily by neurons originating in the SN but it also receives a few inputs from neurons originating in the ventral portion of the VTA. More detailed reviews of the neuroanatomy of midbrain ascending DA systems are provided by Bjorklund and Lindvall (1984), Fallon (1988), Oades and Halliday (1987), and Fallon and Loughlin (1987).

Only a few years ago, DA was thought to induce its effects by interacting in the central nervous system with two DA receptor subtypes, termed D-1 and D-2. In recent years, studies using molecular biological techniques have identified and cloned five different DA receptors (Andersen et al., 1990; Seeman & Van Tol, 1994; Sibley & Monsma, 1992; Sokoloff & Schwartz, 1995; Wolfarth & Ossowska, 1995). The DA D-1-like receptors, D-1 and D-5, and the DA D-2-like receptors, D-2, D-3, and D-4 differ from each other in terms of their intracellular signalling systems, pharmacology, localization, and sequence (Schwartz et al., 1994; Seeman & Van Tol, 1993; Vallar & Meldolesi, 1989; Waddington & Deveney, 1996). For simplicity, D-1-like and D-2-like receptors will be referred to as D-1 and D-2 receptors in the remaining sections of this thesis.

An enormous amount of research has been conducted on DA since the relatively recent discovery in the mid 1950s that it acts as a neurotransmitter. The findings revealed by numerous behavioural, pharmacological, and biochemical studies indicate that midbrain ascending DA systems play a cardinal role in mediating cognitive, emotional, motivational, and motor processes. The activation of these systems elicits a wide spectrum of goal-directed behaviours such as exploration, locomotion, drug-seeking, sniffing, and heightened attention to environmental stimuli (e.g. D'Angio et al; 1988; Joyce & Iversen, 1979; Kalivas et al., 1983; Kelley et al., 1985; Stewart, 1991; Stewart & Vezina, 1987, 1988, 1989; Stinus et al., 1978; Vezina et al., 1987, 1989;

Vezina & Stewart, 1984, 1989, 1990). There is also substantial evidence indicating that midbrain ascending DA systems are implicated in the pathophysiology of psychiatric (e.g. psychosis; Crow, 1980; Fuxe et al., 1974; Kalivas & Stewart, 1991) and neurological (e.g Parkinson's and Huntington's disease; Crow, 1980) disorders. Recently, it has also been discovered that, as mentioned previously, midbrain ascending DA systems play a role in mediating the inhibition of tonic pain. The following section reviews this evidence.

Role of Midbrain DA Systems in Analgesia

As mentioned previously, DA has been shown to produce different effects on pain responsiveness depending on the type of pain test employed. In the formalin test, DA agonists such as cocaine, amphetamine, apomorphine and quinpirole elicit analgesia (Clarke & Franklin, 1992; Dennis & Melzack, 1983; Lin et al., 1989; Morgan & Franklin, 1990; 1991; Skaburskis, 1980). The analgesic effect of cocaine in this test is blocked by pretreatment with either the mixed DA D-1 and D-2 receptor antagonist, chlopromazine, the selective DA D-1 receptor antagonist, SCH 23390, or the selective DA D-2 receptor antagonist, eticlopride (Lin et al., 1989). Similarly, amphetamine-induced analgesia in the formalin test is blocked by pretreatment with either the mixed DA D-1 and D-2 receptor antagonist, flupenthixol, the DA D-1 receptor antagonist, SCH 23390, or the selective D-2 receptor antagonist, pimozide (Morgan & Franklin, 1991; Skaburskis, 1980). In contrast, when phasic pain tests are employed, DA agonists such as amphetamine, cocaine, bromocriptine, and apomorphine have either no effect on withdrawal latencies or elicit hyperalgesia (i.e., enhanced responsiveness to pain; Ben-Sreti et al., 1983; Carroll & Lim, 1960; Dennis & Melzack, 1983; Dunai-Kovács & Székely, 1977; Gonzales et al., 1980; Hernandez et al.,1986; Misra et al., 1987; Nott,

1968; Pertovaara et al., 1988; Robertson et al., 1981; Tocco & Maickel, 1984; Tocco et al., 1985; Tulunay et al., 1976; Witkin et al., 1961).

Inferences from the literature on reward and on stimulation-produced analgesia led Morgan and Franklin (Franklin, 1989; Morgan, 1990; Morgan & Franklin, 1990) to speculate that midbrain ascending DA systems might be implicated in mediating the analgesic effects of drugs such as morphine and amphetamine in the formalin test. With respect to the literature on reward, they speculated that the neural substrates underlying the rewarding effects of drugs such as amphetamine or opioids might be the same as those involved in mediating the inhibition of persistent pain because of the findings that all drugs that are rewarding are also effective against clinical pain (Franklin, 1989; Morgan, 1990). Thus, because psychostimulant drugs are known to induce their rewarding effect by activating midbrain ascending DA neurons (e.g. Bozarth, 1987; Bozarth & Wise, 1986; Broekkamp et al., 1976, 1979; Fibiger & Philipps, 1989; Jenck et al., 1987; Phillips & LePiane, 1980; Smith et al., 1985; Spyraki et al., 1982; Stewart, 1984; Stewart et al., 1984; Wise, 1988; Wise & Bozarth, 1987; Yokel & Wise, 1975, 1976), these systems became likely candidates. They also suspected that these systems might mediate psychostimulant-induced analgesia in the formalin test because of the evidence indicating that all the sites at which electrical stimulation inhibits the responses to pain in the vocalization-after-discharge test project to the VTA, and because pain in this test is associated with significant negative affect, as is the case for pain in the formalin test (Morgan, 1990).

Based on these lines of indirect evidence, Morgan and Franklin (1990) designed an experiment to investigate the role of midbrain ascending DA systems on drug-induced analgesia in the formalin and, for comparison, the tail-flick test. In this study, they found that DA-depleting 6-hydroxydopamine lesions of the ventral mesencephalon,

which contains the cell bodies of the neurons that give rise to ascending forebrain projections, abolish the analgesic effects of systemic morphine and amphetamine in the formalin test, but not in the tail-flick test. Their findings suggested, for the first time, that midbrain ascending DA systems are involved in mediating the inhibition of tonic but not phasic pain. In support of these findings, they reported subsequently that infusions of morphine directly into the VTA elicit analgesia in the formalin test (Manning et al., 1994; Morgan, 1990; Franklin, 1989).

Interestingly, exposure to stress selectively activates DA transmission in midbrain ascending DA neurons (see Deutch & Roth, 1990, for review) and this effect has been shown to be dependent upon opioid mechanisms in the VTA (Kalivas & Abhold, 1987). More specifically, it has been found that intra-VTA infusions of the opioid receptor antagonist, naltrexone methylbromide, prevent the stress-induced activation of DA metabolism in midbrain ascending neurons and that exposure to stress causes the release of met-enkephalin into the VTA (Kalivas & Abhold, 1987). These findings, combined with those indicating that exposure to stress inhibits tonic pain (e.g., Abbott et al., 1986; Fanselow, 1984; Fanselow & Baackes, 1982; Vaccarino et al., 1992) and that morphine infused into the VTA induces analgesia in the formalin test for tonic pain (Franklin, 1989; Manning et al., 1994; Morgan, 1990) suggest that the stimulation of opioid receptors in the VTA by endogeneously released opioids might be a mechanism underlying the stress-induced inhibition of tonic pain. In support of this idea, it was reported recently that infusions of the opioid receptor antagonist, naltrexone methylbromide, into the VTA attenuate stress-induced analgesia in the formalin test (Altier & Stewart, 1996).

Several lines of evidence suggest that release of the tachykinin neuropeptide substance P (SP) in the VTA might similarly play a role in mediating the suppression of

tonic pain by stress. Indeed, there is considerable biochemical, behavioural, and neuroanatomical evidence to suggest that SP interacts with midbrain ascending DA systems in an excitatory way, and that the effects of SP on these systems are triggered naturally by stress. The remaining sections will review in greater depth these lines of evidence. First, however, an overview of the literature on stress-induced analgesia is presented.

Stress-Induced Analgesia

Two important discoveries in the field of pain and analgesia were made during the late 1960s and early 1970s. One of these was made in 1969 when it was found that electrical stimulation in the brainstem of rats elicits analgesia and that this effect is profound enough to permit abdominal surgery without the use of any anesthetic drugs (Reynolds, 1969). In 1971, another group that was unaware of Reynolds' findings discovered the same phenomenon (Mayer et al., 1971). This phenomenon, which has come to be known as stimulation-produced analgesia, was subsequently replicated in the rat (Mayer & Liebeskind, 1974) and extended to other species including the cat (Oliveras et al., 1974), monkey (Goodman & Holcombe, 1976; Ruda et al., 1976) and human (Adams, 1976). During these same years, several researchers discovered the opioid receptors (Goldstein et al., 1971; Pert et al., 1974; Pert & Snyder,1973) and the endogeneous opioid peptides (Hughes et al., 1975; Terenius & Wahlstrom, 1975). All of these discoveries suggested for the first time that endogeneous pain-suppression mechanisms exist in the central nervous system. Based on these findings, pain researchers began to speculate that endogeneous pain-suppression systems might have evolved because they serve an important biological function. More specifically, they proposed that, while pain is adaptive (see Melzack & Wall, 1988, for review), there might be certain conditions under which pain inhibition might be even more adaptive. In

the years that followed the discovery of endogeneous pain-suppression systems, researchers devoted much effort searching for the environmental conditions that might naturally trigger these systems.

Initial studies on environmentally-induced analgesia were carried out almost simultaneously in three separate laboratories (Akil et al., 1976; Hayes et al., 1976, 1978a,b; Rosecrans & Chance, 1976). It was found that analgesia could be produced by such diverse environmental stimuli as exposure to inescapable footshock given acutely (Akil et al., 1976; Hayes et al., 1976, 1978a,b) or chronically (Rosecrans and Chance,1976), to centrifugal rotation and to intraperitoneal saline injections (Hayes et al., 1976, 1978a,b). These treatments appeared to affect pain responses specifically, as responses to tactile stimulation were unaffected (Hayes et al., 1978a). Interestingly, although all of the environmental manipulations found to induce analgesia are stressors (as defined by activation of the hypothalamus-pituitary-adrenal axis), not all are effective. Indeed, neither exposure to ether vapors nor horizontal oscillation induce analgesia (Hayes et al., 1978b).

Since these initial observations, a wide variety of stressors have been reported to inhibit responses to painful stimulation. Some of these include cold (Bodnar et al., 1978b) and warm water swimming (Vaccarino et al., 1992a,b; Willow et al., 1980), restraint (Amir & Amit, 1978), environmental novelty (Abbott et al., 1986), aggressive confrontation and defeat with a conspecific (Miczek et al., 1982, 1985), insulin injections (Bodnar et al., 1979), exposure to a predator (Lester & Fanselow, 1985), hypoglycemia (Bodnar et al., 1978a), body pinch (Ornstein & Amir, 1981), food deprivation (Bodnar et al., 1978), odors released by stressed conspecifics (Fanselow & Sigmundi, 1986), tail pinch (Levine et al., 1982) and footshock (e.g. Chance et al., 1977; Chance & Rosecrans, 1979; Cheser & Chan, 1977; Fanselow & Baackes, 1982;

Fanselow & Bolles, 1979a,b; Hayes et al., 1978b; MacLennan et al., 1980; Ross & Randich, 1985; Watkins et al., 1982). Exposure to stress has been observed to inhibit responses to a variety of noxious painful stimuli, such as application of heat to the tail (e.g. Cannon et al., 1983; Drugan et al., 1985; Lewis et al., 1983) or paws (e.g. Amir & Amit, 1978; 1979a,b; Blair et al., 1982) and subcutaneous injections of formalin into a paw (Abbott et al., 1986; Fanselow, 1984; Fanselow & Baackes, 1982; Fanselow et al., 1988; Fanselow et al., 1989a,b; Fanselow & Helmstetter, 1988; Fanselow & Sigmundi, 1986; Helmstetter, 1992; Helmstetter & Fanselow, 1987; Lester & Fanselow, 1985; Maier et al., 1984; Vaccarino et al., 1992 a,b).

The neurochemical nature of stress-induced analgesia

Early observations indicated that analgesia produced by exposure to stressful stimuli was similar to that induced by exogeneous opioids. These studies suggested that stress-induced analgesia might be mediated by the recently discovered endogeneous opioid peptides, endorphins, enkephalins and dynorphins (Akil et al., 1984; see Bodnar et al., 1980, for review). Akil et al. (1976) and Chesher and Chan (1977), for instance, studied the analgesic effect of footshock stress and found that the opioid antagonist, naloxone, could block the response. In addition, other studies indicated that, as in the case with repeated administration of opioids, tolerance develops to the analgesic effects of repeated exposure to stress and cross-tolerance occurs between opioids and stress (Chesher & Chan, 1977; Lewis et al., 1981; Terman et al., 1986). Naloxone-sensitive stress-induced analgesia has also been reported in humans (Willer et al., 1980). Other studies, however, found that, under some conditions, naloxone failed to reverse stress-induced analgesia (see Amit & Galina, 1988, and Lewis, 1986, for review), suggesting that at least two neurochemically discrete forms of stress-induced analgesia exist, one mediated by opioids and one that is not. Many studies

followed in which the determinants of opioid and non-opioid stress-induced analgesia were sought using a single and reliable stressor, inescapable footshock stress.

Lewis et al. (1980) observed that exposure to footshock of a constant intensity produces opioid or non-opioid forms of stress-induced analgesia depending on the temporal parameters of the stressor. More specifically, they found that 20-minute exposure to a 2.5 mA prolonged, intermittent footshock applied once every 5 seconds causes analgesia that is blocked by naloxone at a dose as little as 0.1 mg/kg, suggesting opioid involvement. In contrast, exposure to a 2.5 mA footshock applied continuously for 3 minutes causes equipotent analgesia which is unaffected by even high doses of naloxone. The naloxone-sensitive analgesia produced by prolonged, intermittent footshock also satisfies other criteria for opioid involvement. It shows complete tolerance after 14 days of exposure to this stressor (Lewis et al., 1981; Mayer & Price, 1976) and shows cross-tolerance with morphine in that the analgesia produced by this stressor is markedly reduced in morphine-tolerant rats (Mayer & Price, 1976). The naloxone-insensitive analgesia produced by brief, continuous footshock, on the other hand, fails to manifest tolerance or cross-tolerance with morphine (Mayer & Price, 1976).

In summary, exposure to prolonged, intermittent footshock stress produces an opioid form of stress-induced analgesia whereas exposure to brief, continuous footshock stress elicits a non-opioid form of stress-induced analgesia. Terman et al. (1984) subsequently extended these findings and observed that the neurochemical nature of stress-induced analgesia produced by continuous footshock depends on shock severity (intensity x duration). Based on their findings, these investigators suggested that the neurochemical basis of analgesia produced by exposure to continuous footshock stress follows a coulometric (intensity x duration) relation such that stress-induced

analgesia is opioid-mediated if the product of these variables is below 7.5 (2.5-mA shocks for 3 minutes) but non-opioid if the coulometric product is at or above 7.5. Stress severity appears to play a similar role in determining the opioid versus non-opioid nature of stress-induced analgesia from cold water swim stress (Terman et al., 1986) and from conditioned fear (Fanselow, 1984).

Interestingly, exposure to the stressors that inhibit pain has been shown, in other studies, to selectively activate DA transmission in midbrain ascending neurons. For instance, exposure to stressors such as footshock, restraint, or conditioned fear have all been reported to enhance DA metabolism in terminals regions of midbrain ascending systems (see Deutch et al., 1990, for review). The following section reviews this evidence and will be followed by a description of the evidence indicating that SP in the VTA plays an important role in mediating the stress-induced activation of midbrain ascending DA systems.

Stress and Midbrain Ascending DA Systems

Several studies indicate that exposure to certain stressors such as mild intermittent footshock consistently activates DA transmission in midbrain ascending neurons, as reflected by increased levels of the DA metabolite dihydroxyphenylacetic acid (DOPAC; Glowinski, 1984; Roffler-Tarlov et al., 1987; Roth et al., 1976) in terminal projection fields. Thierry et al. (1976) provided the first evidence for this selective stress-induced activation of midbrain ascending DA neurons. They reported that exposure to mild intermittent footshock stress (1.6 mA) caused a pronounced increase in DA utilization in the prefrontal cortex, and a smaller but significant increase in DA utilization in the NAS. These findings were subsequently confirmed by other investigators, using various footshock parameters (Deutch et al., 1985 b; Deutch et al.,

1990; Fadda et al., 1978; Herman et al., 1982; Lavieille et al., 1978; Tissari et al., 1979). Exposure to stressors other than footshock stress has similarly been documented to enhance DOPAC levels in the prefrontal cortex and NAS. These include conditioned fear (Deutch et al., 1985 b; Deutch & Roth, 1990; Herman et al., 1982; Roth et al., 1988), restraint (Deutch & Roth, 1990; Imperato et al., 1991; Kennedy et al., 1980; Roth et al., 1988), tail pinch (Abercrombie et al., 1989; Bertolucci-D'Angio et al., 1990 a,b), swim stress (Knorr et al., 1984; Yang et al., 1985), forced locomotion (Bertolluci-D'Angio et al., 1990 a,b) and environmental novelty (Tassin et al., 1980).

Whereas mesocortical DA neurons appear to consistently respond to even minor forms of stress, the stress-induced activation of mesolimbic DA neurons innervating the NAS appears to be more variable (Deutch et al., 1985 b; Deutch et al., 1990; Lavieille et al., 1978; Roth et al., 1988). Using footshock as a single and reliable stressor, it has been suggested that stress severity plays an important role in determining whether mesolimbic DA neurons, in addition to mesocortical DA neurons, will respond to stress. More specifically, it has been found that, as the intensity or duration of footshock stress increases, DA metabolism is enhanced in the prefrontal cortex, followed by the NAS (Deutch et al., 1990; Deutch & Roth, 1990; Roth et al., 1988). For instance, exposure to 0.2 mA footshock stress increases DOPAC concentrations in the prefrontal only, whereas exposure to slightly more intense footshock (0.26 mA) increases this measure in the NAS (Deutch & Roth, 1990). Still, more severe stressors have been found to enhance DOPAC levels in terminal projection sites of nigrostriatal neurons (Cabib et al., 1988; Deutch & Roth, 1990; Dunn, 1988; Roth et al., 1988; Speciale et al., 1986).

Stress and SP

Several findings suggest that SP release in the VTA plays a critical role in the stress-induced activation of midbrain ascending DA neurons. Bannon et al. (1983) found that pretreatment with a monoclonal SP antibody infused into the VTA completely prevented the stress-induced biochemical activation of midbrain DA neurons innervating the prefrontal cortex. In addition, it has been reported that SP levels in the VTA are decreased during and following exposure to footshock stress, suggesting enhanced release of the peptide by the stressor (Bannon et al., 1986; Deutch et al., 1985a; Lisoprawski et al., 1981). Finally, there is considerable indirect evidence suggesting that SP release in the VTA mediates the stress-induced activation of midbrain DA neurons. This indirect evidence is based on the findings of numerous studies indicating that infusions of SP into the VTA mimic the biochemical activation of midbrain ascending DA systems induced by stress. The following section reviews this evidence in more detail.

Interactions Between SP and Midbrain Ascending DA Systems

Several studies report that SP infused directly into the cell bodies of the VTA activates DA metabolism in mesocortical and mesolimbic neurons. For instance, Cador et al. (1989) found that intra-VTA SP infusions dose-dependently increase the DOPAC/DA ratio in the prefrontal cortex and, to a lesser extent, in the NAS. Likewise, Deutch et al. (1985a) reported that DOPAC levels in the prefrontal cortex are enhanced following infusions of SP into the VTA. Similar increases in DA metabolism in midbrain terminal projection sites have been reported following intra-VTA infusions of the enzyme-resistant and, therefore, long-acting SP analog, DiMe-C7 (Elliott et al., 1986). Similarly, there is also some recent evidence indicating that the highly selective

SP (NK-1) receptor agonist, GR-73632, increases DA metabolism in the NAS (Elliott et al., 1991). Finally, it has been reported more recently that SP or DiMe-C7 administered systemically enhance extracellular levels of DA and its metabolites in studies employing in vivo microdialysis in the freely-moving rat (Boix et al., 1992a,b).

Evidence for a close functional relationship between SP and midbrain ascending DA systems is also provided by the findings that infusions of SP into the VTA stimulate DA-mediated behaviours. A number of studies have shown that DA agonists increase locomotor activity and that a primary event underlying this effect is the release of DA from terminals of mesolimbic neurons innervating the NAS. Thus, increased locomotor activity is produced by the systemic administration of the indirect DA agonists amphetamine and cocaine (Kelly & Iversen, 1976), the DA precursor L-DOPA, the DA receptor agonist apomorphine (Kelly et al., 1975), and numerous other DA-enhancing drugs (Cole, 1978; Isaacson et al., 1978). In support of the idea that enhanced DA activity in the NAS mediates this behavioural response, it has been shown that the locomotor-activating effects of amphetamine are greatly reduced by DA-depleting 6-hydroxydopamine lesions of the NAS (Kelly & Iversen, 1976; Kelly et al., 1975) and by infusions of the DA receptor antagonist haloperidol in the NAS (Pijnenburg et al., 1975), but are unaffected by a lesion to the PFC (Simon et al, 1981). Furthermore, it has been reported that infusions of amphetamine, DA, and other DA agonists directly into the NAS stimulate locomotor activity (Kelly, 1977; Pijnenburg et al., 1976; Staton & Solomon, 1984).

Stinus et al. (1978) were the first to report that infusions of SP into the VTA stimulate locomotor activity in rodents. This behavioural effect of SP was subsequently replicated by other investigators (Kelley et al., 1985; Kelley et al., 1979), and was also observed following intra-VTA infusions of the enzyme-resistent SP analog, DiMe-

C7 (Eison et al. , 1982 a,b; Elliott & Iversen , 1986; Naranjo & Del Río, 1984). Likewise, there is also evidence that intra-VTA infusions of agonists with different affinities for tachykinin receptors stimulate locomotor activity (Elliott et al., 1991, 1992; Stoessl et al., 1991). As observed with DA agonists, enhanced DA activity in the NAS appears to be an important mechanism underlying the behavioural-activating effects of SP. More specifically, it has been shown that the systemic administration of the DA receptor antagonist, haloperidol (Eison et al., 1982a; Naranjo & Del Rio, 1984) blocks the locomotor-stimulant effects of SP and DiMe-C7, whereas that of the DA agonist, amphetamine, potentiates the response (Eison et al., 1982a; Stinus et al., 1978). More convincing evidence is provided by the findings that the locomotor-activating effects of SP infused into the VTA are blocked either by intra-NAS infusions of the DA receptor antagonist, haloperidol, or by 6-hydroxydopamine lesions of the NAS (Kelley et al., 1979).

Interestingly, there are also reports indicating that SP interacts with midbrain DA ascending systems by acting into the NAS. Indeed, SP and SP receptors are present in the NAS (Ljungdahl et al., 1978 a,b; Pickel et al., 1988; Saffroy et al., 1988), and several studies indicate that SP acts in the NAS to enhance DA transmission. For instance, it has been reported that infusions of SP into the NAS augment DA metabolism at this site (Kalivas & Miller, 1986). There is also some evidence indicating that intra-NAS infusions of a selective SP (NK-1) agonist increases locomotor activity and that this effect is prevented by the systemic administration of the DA receptor antagonist, haloperidol (Elliott et al., 1992).

In summary, there is considerable evidence suggesting that SP interacts with midbrain ascending DA systems in an excitatory way and that the effects of SP on DA transmission in these systems are triggered naturally during exposure to stress. This

close functional relationship between SP and midbrain ascending DA systems is further supported by the findings that SP terminals make direct synaptic contacts with DA-containing cell bodies in the VTA (Tamiya et al., 1990) and that SP receptors are located directly on these cell bodies in the midbrain (Stoessl, 1992). The evidence that exposure to stress both inhibits tonic pain and causes the SP-mediated activation of midbrain ascending DA neurons, and that the activation of these neurons by morphine inhibits tonic pain in the formalin test suggests that SP release in the VTA might play a role in mediating the inhibition of tonic pain by stress. Additional evidence in support of this idea is provided by the finding that infusions of the SP analog, DiMe-C7, into the VTA induce analgesia in the formalin test for tonic pain (Altier & Stewart, 1993). More specifically, in this study, it was found that intra-VTA DiMe-C7 decreased formalin pain responses immediately following the infusions during approximately 30 minutes. Importantly, it was found that infusions of DiMe-C7 made 1.0 mm dorsal to the VTA did not affect formalin pain reseponses, indicating that the SP analog induces analgesia by acting at receptors within the VTA.

Rationale of the Present Experiments

The role that midbrain SP plays in the inhibition of tonic pain was further explored in the present thesis. Because SP interacts with at least three tachykinin receptor subtypes, the purpose of Experiment 1 was to examine the effects of selective tachykinin receptor agonists infused into the VTA in the formalin test for tonic pain. Thus, animals were tested in the formalin test following intra-VTA infusions of either the NK-1 selective agonist, GR-73632, or the NK-3 selective agonist, senktide. Based on the evidence that SP terminals and receptors are present in the NAS and that SP interacts at this site with DA in an excitatory way, Experiment 2 was designed to examine the role of SP acting in the NAS in the inhibition of tonic pain. Thus, animals

were tested in the formalin test following infusions into the NAS of either the NK-1 selective agonist, GR-73632, or the NK-3 selective agonist, senktide.

Previous studies have reported that the analgesic effects induced by SP administered either intracerebroventricularly or systemically are mediated via endogenoeus opioids. On the basis of these findings, Experiment 3 was designed to examine whether opioids might similarly mediate the analgesic effects induced by either the SP analog, DiMe-C7, the NK-1 selective agonist, GR-73632, or the NK-3 selective agonist, senktide, infused into the VTA or the NAS.

As mentioned previously, there is considerable evidence indicating that the neural systems mediating the inhibition of tonic versus phasic pain are fundamentally different. In addition, in a previous study, it was found that DiMe-C7 infused into the VTA elicits analgesia in the formalin test for tonic pain, but is without effect in the tail-flick test for phasic pain. Given these findings, the effects that were observed in the formalin test following infusions of tachykinin selective agonists into either the VTA or NAS (Experiments 1 and 2) were compared, in Experiment 4, to those in the tail-flick test. In addition, the effect of intra-VTA morphine in the tail-flick test was examined, using a dose found in previous studies to induce potent analgesia in the formalin test.

As described previously, there is considerable evidence suggesting that SP release in the VTA plays a role in the stress-induced activation of midbrain ascending DA systems. This evidence, together with that indicating that midbrain DA systems play are involved in mediating the suppression of tonic pain suggests that SP release in the VTA might play a role in the stress-induced inhibition of tonic pain. To examine this idea, Experiment 5 was designed to explore the effect of intra-VTA pretreatment with the

novel selective NK-1 receptor antagonist, RP 67580, on stress-induced analgesia in the formalin test.

Several lines of evidence suggest that the analgesic effects induced by SP, as well as by morphine and amphetamine in the formalin test are caused, at least in part, by enhanced DA transmission in mesolimbic neurons innervating the NAS. This idea was explored in the last series of experiments. Experiment 6 examined the effects of intra-NAS pretreatment with the DA receptor antagonists raclopride, SCH 23390, and flupenthixol on the analgesic effects induced by intra-VTA infusions of either DiMe-C7 or morphine, and intra-NAS infusions of amphetamine. Another approach taken to test this idea was to examine, in Experiment 7, the effects of reducing DA release in midbrain ascending DA neurons, by pretreating animals with a low autoreceptor-specific dose of apomorphine, on the analgesic effects of intra-VTA infusions of either SP or morphine, and intra-NAS infusions of amphetamine in the formalin test.

THE EXPERIMENTS

General Methods and Procedures

Because most of the experiments described in this thesis were conducted following the same basic procedures, these are described in the following section.

Subjects, housing and habituation

Naive male Wistar rats weighing 350-375 g (Charles River Canada, St-Constant, Quebec) served as subjects. Upon arrival, they were housed two by two in standard clear plastic cages with wire tops. They had continuous access to food and water and were maintained on a 12 h light-dark cycle (lights on at 9:00 pm and off at 9:00 am). All testing took place in an illuminated test room during the dark portion of the light-dark cycle. On the four days prior to testing, each rat was handled for one minute. On the two days prior to either formalin or tail-flick testing, each animal was handled and habituated to the various testing procedures exactly as it was to be treated on the test day, except that neither intracranial nor formalin injections were administered.

Apparatus

The formalin test cubicles measured 30 x 30 x 30 cm and were made from clear Plexiglas. A mirror was mounted beneath the floor of the cubicle at a 45 ° angle to allow an unobstructed view of the rats' paws.

Surgery

Seven to ten days after arrival, rats were anesthetized with an injection of sodium pentobarbital (Somnotol, 65 mg/kg, i.p.; MTC Pharmaceuticals Ltd.,

Cambridge, Ontario). When required, anesthesia was maintained with Methoxyflurane (Metofane; Pitman-Moore, Missisauga, Ontario). Following immobilization of the animals in a stereotaxic frame, an incision was made along the midline and the skull was exposed. Guide cannulae (Plastics One, Inc.) were implanted, bilaterally, 1.0 mm above the target structure and anchored to the skull with 5 stainless steel screws and dental acrylic cement. Prior to surgery, animals were injected with 0.6 mg/kg given s.c. of atropine sulfate (Glaxo Laboratories, Montreal, Quebec) and 0.1 ml given i.m. of Penicillin G (Ayercillin; Ayerst, Montreal, Canada). Following surgery, 28 gauge stainless steel obturators (Plastics One, Inc.) were inserted into the guide cannulae. These extended 1.0 mm beyond the tip of the guide cannulae. Upon recovery under a heat lamp, rats were housed individually in standard clear plastic cages with wire tops and were allowed a 7-day recovery period before habituation to testing procedures was begun.

Intracranial microinjections

After removing the obturators, 28 gauge stainless steel internal injector cannulae extending 1.0 mm beyond the tip of the guide cannulae were inserted and held in place in the guide cannulae by a brass screw cuff. The injector cannulae were connected via polyethylene tubing to 1-µl Hamilton syringes. Compounds were administered in unrestrained rats in a volume of 0.5 µl/side over 60 seconds. The injectors remained in place for an additional 120 seconds to allow diffusion of the solutions around the injection site. Obturators were immediately replaced following removal of the injectors. To prevent intracranial infections, all internal injector cannulae and obturators were wiped with 70% alcohol and dried immediately prior to being inserted into the guide cannulae.

Histology

Following completion of the experiments, rats were deeply anesthetized with chloral hydrate (1 ml, i.p.) and perfused transcardially with the injectors in place with 0.9 % saline followed by 10% formalin. Brains were then removed and stored in 10% formalin for at least one week. Histological verification of cannulae tip placements was subsequently determined on 30 micron thionine-stained coronal sections. Data from an animal were included in the analyses only if the injector tips were located within 0.5 mm of the target structure.

The Formalin Test

On the two days prior to testing, each rat was habituated to the observation cubicle by being placed in it and left undisturbed for at least 60 minutes. On the test day, tonic pain was induced by a subcutaneous injection of 0.05 ml of 2.5 % formalin into the plantar surface of one hind paw. Animals were then placed in the observation cubicle and pain responses were recorded continuously once every 5 seconds for 60 minutes, using a time sampling procedure. Thus, 12 observations were recorded were made per minute. In the present studies, the weighted-pain scores method described by Dubuisson and Dennis (1977) was employed to rate the intensity of various pain-related behaviours because there is evidence that the different weighted-pain scores (e.g., score 3) associated with each behavioural category (e.g., paw-licking) accurately reflect varying levels of pain intensities (Coderre et al., 1993). Thus, the intensity of pain was rated according to 4 behavioral categories, using a scale of 0 to 3 : a score of 0 was recorded, if the rat walked or sat normally with weight placed equally on both hind paws; 1, if the rat favored the injured paw (e.g., if it limped); 2, if the rat held the injured paw off the floor, with at most the nails touching the floor; and 3, if the rat chewed or licked the injured paw. Average formalin pain scores were determined for 3-minute periods. If an animal failed to exhibit pain behavior at any time throughout a

session, its data were discarded. In all experiments, the observer was blind to which animal had been injected with a drug and which with the vehicle.

EXPERIMENT 1

Effects of Intra-VTA Infusions of the Tachykinin NK-1 Selective Agonist, GR-73632, or the Tachykinin NK-3 Selective Agonist, Senktide, in the Formalin test

As mentioned in the Introduction, it was found previously that infusions of the SP analog, DiMe-C7, into the VTA induce analgesia in the formalin test for tonic pain (Altier & Stewart, 1993). It was also shown, in that set of studies, that the effects of DiMe-C7 are restricted to the VTA, for control infusions of the peptide made 1.0 mm dorsal to the VTA are without effects on pain responses in this test. SP, as well as the structurally-related tachykinins, Neurokinin A and B, interact in the peripheral and central nervous system with at least three receptor subtypes (Watling, 1992). SP is the most potent natural ligand of the NK-1 receptor subtype, whereas Neurokinin A and B bind preferentially with the NK-2 and NK-3 receptor subtypes, respectively (Regoli et al., 1988). It must be kept in mind, however, that these tachykinin peptides likely exert their physiological and behavioural effects by interacting with all three receptors because their selectivity for their preferred receptor is poor (Watling, 1992).

Although SP displays the highest affinity for the NK-1 tachykinin receptor subtype, the SP analog, DiMe-C7, appears to bind preferentially to the NK-3 receptor subtype (Torrens et al., 1985). This finding suggests the analgesic effect induced by DiMe-C7 infused into the VTA in the formalin test might be due to the preferential stimulation at this site of NK-3 receptor subtypes. In support of this idea, autoradiographic studies indicate that NK-3 receptors are present in the VTA (Dam et

al., 1990; Stoessl and Hill, 1990; Stoessl, 1994) and it has been reported that intra-VTA infusions of the highly selective NK-3 agonist, senktide, increase locomotor activity (Elliott et al., 1991, 1992). It is likely, however, that DiMe-C7 infused into the VTA induces analgesia by stimulating NK-1, in addition to NK-3, receptor subtypes at this site. Indeed, although this SP analog displays higher affinity for the NK-3 receptor subtype, it is not a highly selective NK-3 agonist and thus probably also binds, like SP, to NK-1 receptor subtypes. The finding that intra-VTA infusions of the highly selective NK-1 agonist, GR-73632, cause behavioural activation (Elliott et al., 1991, 1992) supports this idea. On the other hand, it is unlikely that the stimulation of NK-2 receptor subtypes in the VTA mediates the analgesic effects of intra-VTA DiMe-C7 because there is, as of yet, no evidence for the existence of these receptors in the VTA and it has been reported that intra-VTA infusions of highly selective NK-2 agonists are without effects in behavioural studies (Elliott et al., 1991, 1992).

The purpose of Experiment 1 was to explore the relative contribution of NK-1 and NK-3 tachykinin receptor subtypes on analgesia in the formalin test for tonic pain. This was accomplished by examining the analgesic effects of intra-VTA infusions of the selective NK-1 receptor agonist, GR-73632 (Hagan et al., 1991) and the selective NK-3 receptor agonist, senktide (Laufer et al. 1986; Wormser et al. 1986).

Method

Surgery

21 mm long, 22 gauge guide cannulae (Plastics One, Inc.) were implanted, bilaterally, 1.0 mm above the VTA and aimed at the following coordinates : - 5.7 mm posterior to bregma, + 0.6 mm lateral from the midline, and - 7.4 mm ventral from the

skull surface (Paxinos and Watson, 1986). The stereotaxic arms were angled at 15 degrees from the perpendicular and the skull was level between lambda and bregma (i.e., flat skull position).

Drugs

The doses of the NK-1 agonist, GR-73632 {Ava-[L-Pro^9, N-Me-Leu^{10}] SP (7-11); Research Biochemicals, Inc., Natick, MA}, used in these experiments were 0.005, 0.05, 0.5 and 1.5 nmol/side. The doses of the NK-3 agonist, senktide {(Succinyl-[Asp^6, MePhe^8] SP (6-11)Peninsula Laboratories, Inc., Belmont, CA}, used were 0.005, 0.50, or 1.5 nmol/side. These doses of GR-73632 and senktide were reported previously to be effective at stimulating locomotor activity (Elliott et al., 1991; 1992). The compounds were dissolved in saline. Stock volumes (5.0 µl) of all compounds and vehicles were aliquoted into polypropylene vials and frozen at - 70 ° C until used. Solutions were thawed within 30 min of the infusions.

Design and Procedure

On the test day, animals received bilateral intra-VTA infusions of either the NK-1 agonist, GR-73632 (0.005, 0.05, or 0.5 nmol/0.5 µl/side), the NK-3 agonist, senktide (0.005, 0.5, or 1.5 nmol/0.5 µl/side), or saline, using a between-subjects design, immediately prior to a subcutaneous injection of 0.05 ml of 2.5 % formalin into the plantar surface of one hind paw. In another experiment, animals received bilateral intra-VTA infusions of either a combined solution containing GR-73632 and senktide (0.05 and 0.5 nmol/0.5 µl/side, respectively), or saline, using a counterbalanced within-subjects design. Thus, for this experiment, animals were tested twice (once with the combined solution, once with saline) in the formalin test, at a 1-week interval. Either the right or left hind paw was injected on successive tests.

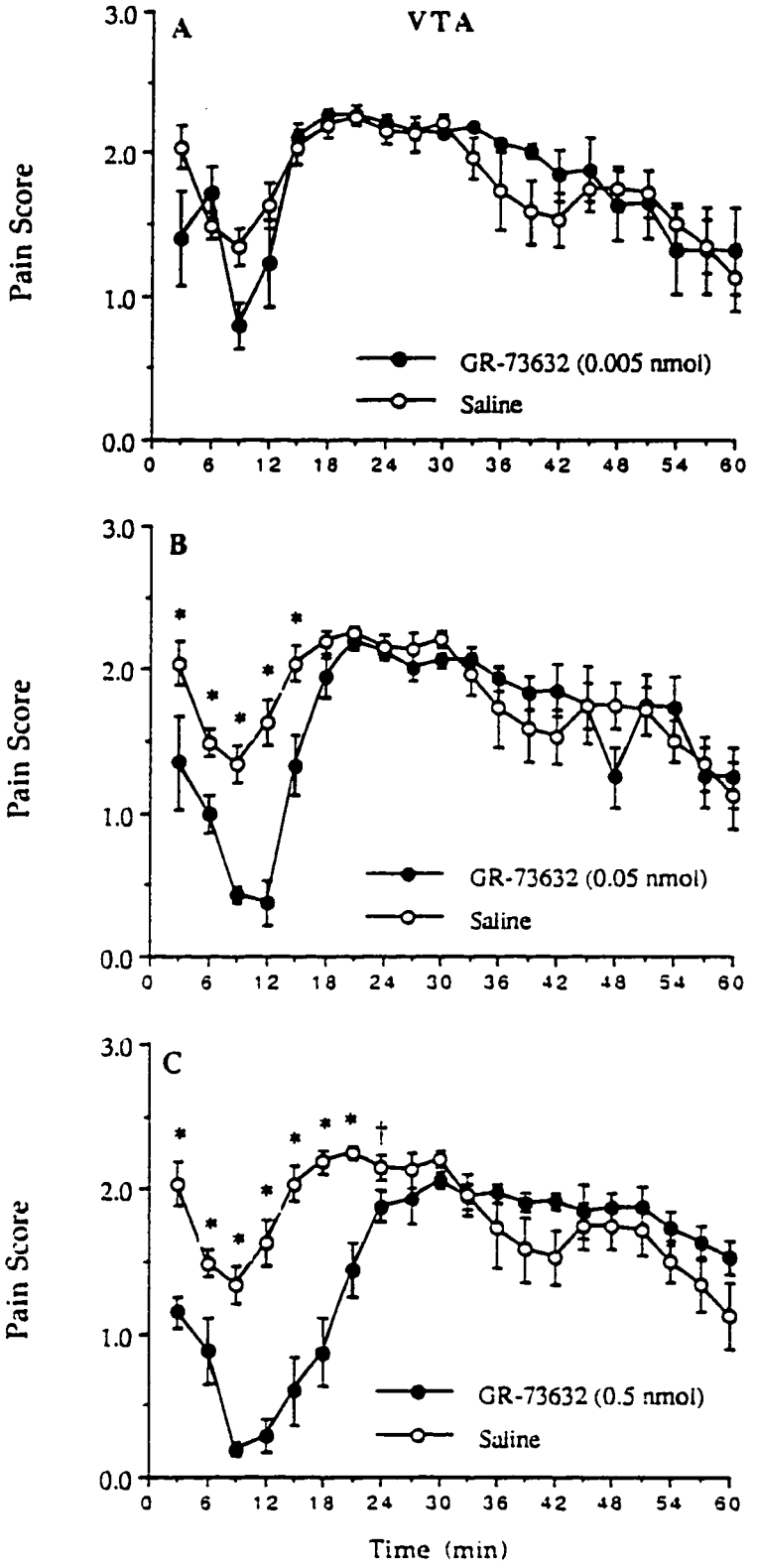
Statistical Analyses

Data on pain scores were analyzed by two-way analyses of variance (ANOVA) with Treatment as either the between-subjects variable (Figures 1, 2, and 3; drug vs saline; 4 levels) or the within-subjects variable (Figure 4; 2 levels) and Time (10 post-infusion time points) as the within-subjects variable; because most of the effects of the drugs occurred within the first 30 minutes of testing, only the first 10 post-infusion time-points were included in the analyses. It could be argued that because formalin pain scores are measured on an ordinal scale, a non-parametric statistical test should be used to analyze the data. There is, however, no non-parametric analog of the two-way analysis of variance. Therefore, in addition to carrying out the two-way ANOVAs, one-way ANOVAs were conducted to analyze the differences in the time spent in each behavioural category following various doses of GR 73632 infused in the VTA. All analyses were followed, if appropriate, by Tukey's post-hoc test for overall differences between conditions. Tests for simple main effects were used, when appropriate, to analyze the differences at each time point between a control and treatment condition. Data from an animal were included in the analyses only if the injector tips were located within 0.5 mm of the target area.

Results

Figures 1 a, b, and c show the effect of intra-VTA infusions of the NK-1 agonist, GR-73632 (0.005, 0.05, or 0.5 nmol/0.5 µl/side), or saline, in the formalin test. As shown in saline-treated animals, formalin injected into one hind paw typically produced a biphasic pain response. An early pain phase developed immediately following the injection and lasted approximately 5 minutes. This was followed by a period in which rats exhibited less pain reactivity. Approximately 15 minutes after the formalin injection, pain responses returned gradually, lasting until the end of the test

Figure 1. Mean formalin pain scores (± S.E.M.) following bilateral intra-VTA infusions of the NK-1 agonist, GR-73632, using a) 0.005, b) 0.05, or c) 0.5 nmol/0.5 µl/side). Significantly different from saline : * $p < 0.01$; † $p < 0.05$. Rats (n = 5-9 per group) were tested in a between-subjects design. For clarity, the three doses were plotted in different graphs, with the same control group in each.


A
VTA
B
C
Pain Score
Pain Score
Pain Score
3.0
2.0
1.0
0.0
0 6 12 18 24 30 36 42 48 54 60
GR-73632 (0.005 nmol)
Saline
GR-73632 (0.05 nmol)
Saline
GR-73632 (0.5 nmol)
Saline
Time (min)

session. The ANOVA yielded a significant effect of Treatment, $F(3, 24) = 23.9$, $P < 0.0001$. The NK-1 agonist caused significant analgesia following intra-VTA infusions of the intermediate and highest doses used ($P < 0.05$). Pain scores were also significantly different between the lowest and highest, lowest and intermediate, and intermediate and highest doses of GR-73632 tested ($Ps < 0.05$).

Figure 2 shows the effect of intra-VTA infusions of GR-73632 (0.0, 0.005, 0.05, or 0.5 nmol/0.5 µl/side) on the time spent in score a) 0, b) 1, c) 2, and c) 3. There was a significant effect of Treatment in a) $F(3, 23) = 21.63$, $P < 0.0001$, c) $F(3, 23) = 3.5$, $P < 0.05$, and d) $F(3, 23) = 4.44$, $P < 0.05$, but not b) $F(3, 23) = 0.612$, $P = 0.62$. Figure 2 a shows that animals spent progressively more time in score 0 as the dose of GR-73632 increased. The time spent in this behavioural category was significantly longer following GR-73632 infusions of 0.05 and 0.5 nmol/side than following saline ($Ps < 0.05$). There was no relationship, however, between escalating doses of GR-73632 and the time spent in score 1, as shown in Figure 2 b. Figure 2 c shows that animals spent progressively less time in score 2 as the doses of GR-73632 increased. There was a significant difference in the time spent in this behavioural category between saline and a GR-73632 dose of 0.5 nmol/side ($P < 0.05$). Similarly, animals spent progressively less time in score 3 as the dose of the NK-1 agonist increased, as seen in Figure 2 d. The time spent in this behavioural category was significantly shorter following a dose of 0.05 and 0.5 than following saline ($Ps < 0.05$).

Figures 3 a, b, and c show the effect of the NK-3 agonist, senktide (0.005, 0.5 , or 1.5 nmol/0.5 µl/side), or saline, infused into the VTA. There was a significant effect of Treatment, $F(3, 29) = 5.45$, $P < 0.0005$. Each of the senktide groups differed significantly from the saline group ($Ps < 0.05$), but there were no significant differences in pain scores between the three doses of senktide tested.

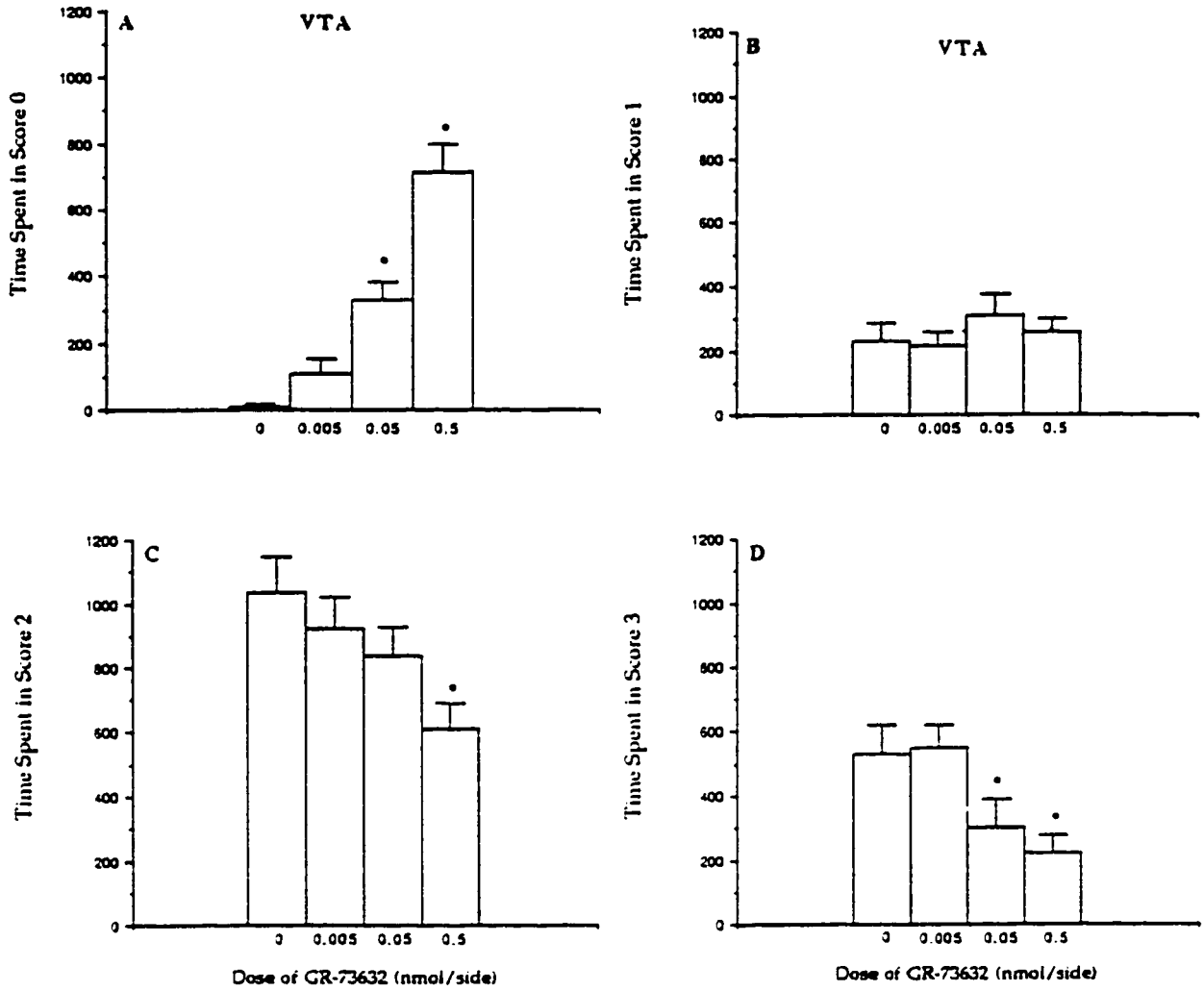
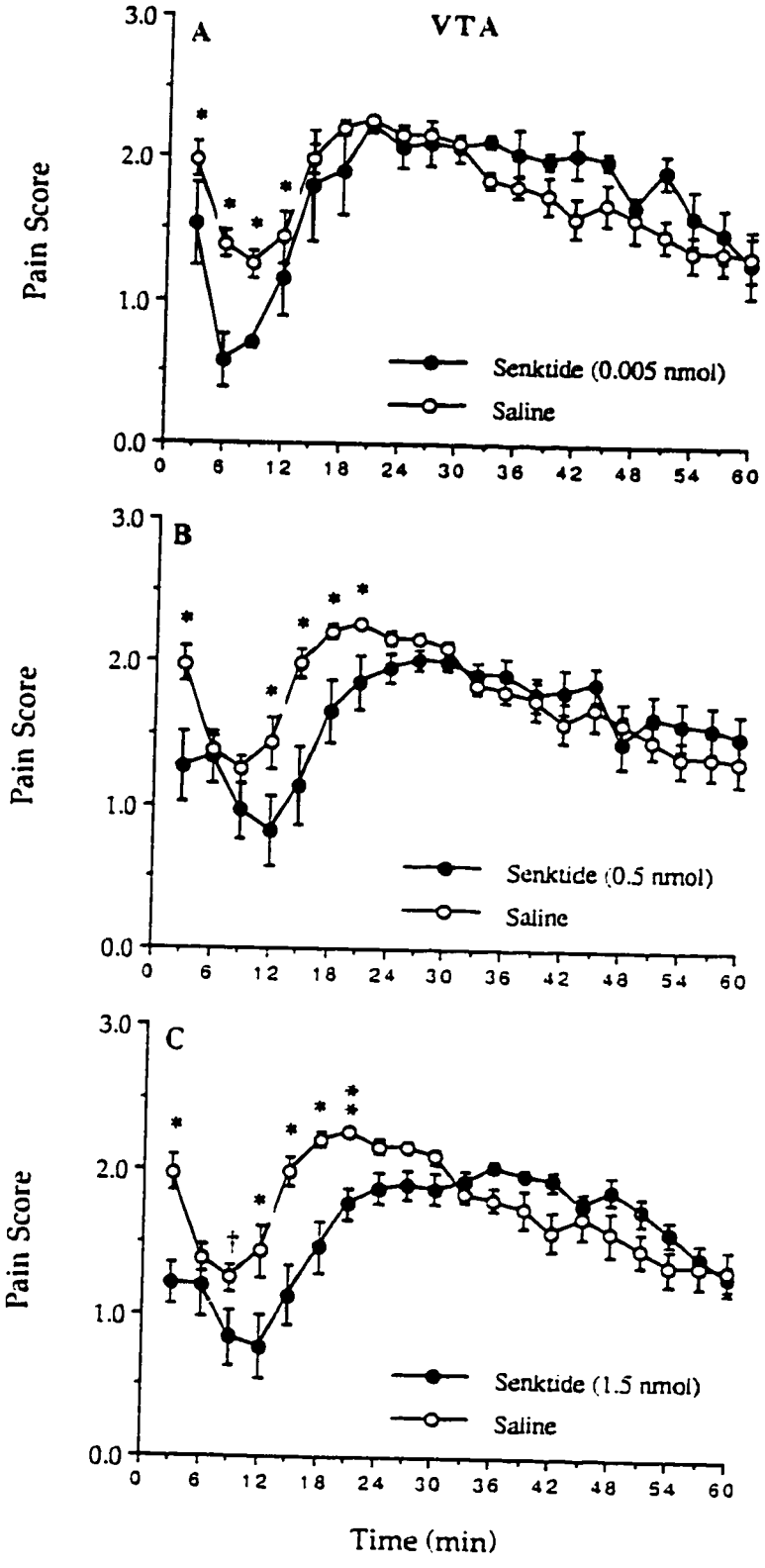


Figure 2. Mean time in seconds (± S.E.M) spent in the different behavioural categories reflecting a weighted pain score of a) 0, b) 1, c) 2, and d) 3 following intra-VTA infusions of the NK-1 agonist, GR-73632, using different doses (0, 0.005, 0.05, or 0.5 nmol/0.5 µl/side. Significantly different from saline (0.0 nmol/0.5 µl/side) : * $p < 0.05$. Rats (n = 5-10) were the same as those whose pain scores are graphed in Figure 1.

Figure 3. Mean formalin pain scores (± S.E.M.) following bilateral intra-VTA infusions of the NK-3 agonist, senktide, using a) 0.005, b) 0.5, or c) 1.5 nmol/0.5 µl/side. Significantly different from saline : * $p < 0.01$; ** $p < 0.005$; † $p < 0.05$. Rats (n = 6-10 per group) were tested in a between-subjects design. For clarity, the three doses were plotted in different graphs, with the same control group in each.


A
VTA
B
C
Pain Score
Pain Score
Pain Score
3.0
2.0
1.0
0.0
0
6
12
18
24
30
36
42
48
54
60
Senktide (0.005 nmol)
Saline
Senktide (0.5 nmol)
Saline
Senktide (1.5 nmol)
Saline
Time (min)

The analgesia induced by infusions of a combined solution containing GR-73632 and senktide into the VTA is shown in Figure 4. The combination induced significant analgesia, $F(1, 5) = 3.15$, $p < 0.0005$.

The location of the injector tips of all rats that received bilateral intra-VTA infusions of either GR-73632, senktide, or a combination of both of these tachykinin agonists is illustrated in Figure 5. As shown, 69 rats had their injector tips within the VTA. Three rats had their injector tips outside the limits of the VTA and thus their data were discarded.

Discussion

The results obtained from the present experiments indicate that agonists selective for the tachykinin NK-1 and NK-3 receptor subtypes induce analgesia in the formalin test for tonic pain following infusions into the VTA. Inhibition of formalin-induced tonic pain responses was observed immediately following intra-VTA infusions of either the NK-1 agonist, GR-73632, the NK-3 agonist, senktide, or a solution containing both compounds. The NK-1 agonist, GR-73632, appeared to be, within the range of doses used, more effective at inducing analgesia in this test than the NK-3 agonist, senktide. These findings suggest that NK-1 receptors in the VTA may play a more important role than NK-3 receptors in the VTA in this response. It is likely that the tachykinin agonists were acting at receptors within the VTA because infusions of the SP analog, DiMe-C7, made 1.0 mm dorsal to the VTA fail to induce the response (Altier and Stewart, 1993).

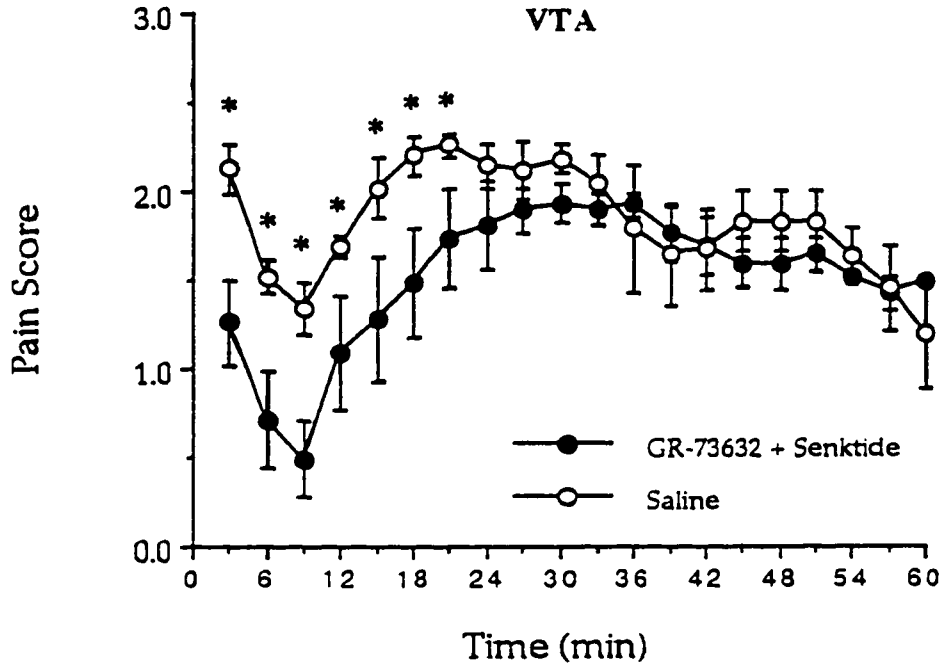


Figure 4. Effect of a combined solution containing the NK-1 agonist, GR-73632 (0.05 nmol/0.5 µl/side) and the NK-3 agonist, senktide (0.5 nmol/0.5 µl/side), or saline, infused bilaterally in the VTA in the formalin test. Mean formalin pain scores (± S.E.M.) were significantly different from saline at all time points during 21 min following the intracranial infusions: * $p < 0.05$. Rats ($n = 6$ per group) were tested in a counterbalanced within-subjects design.

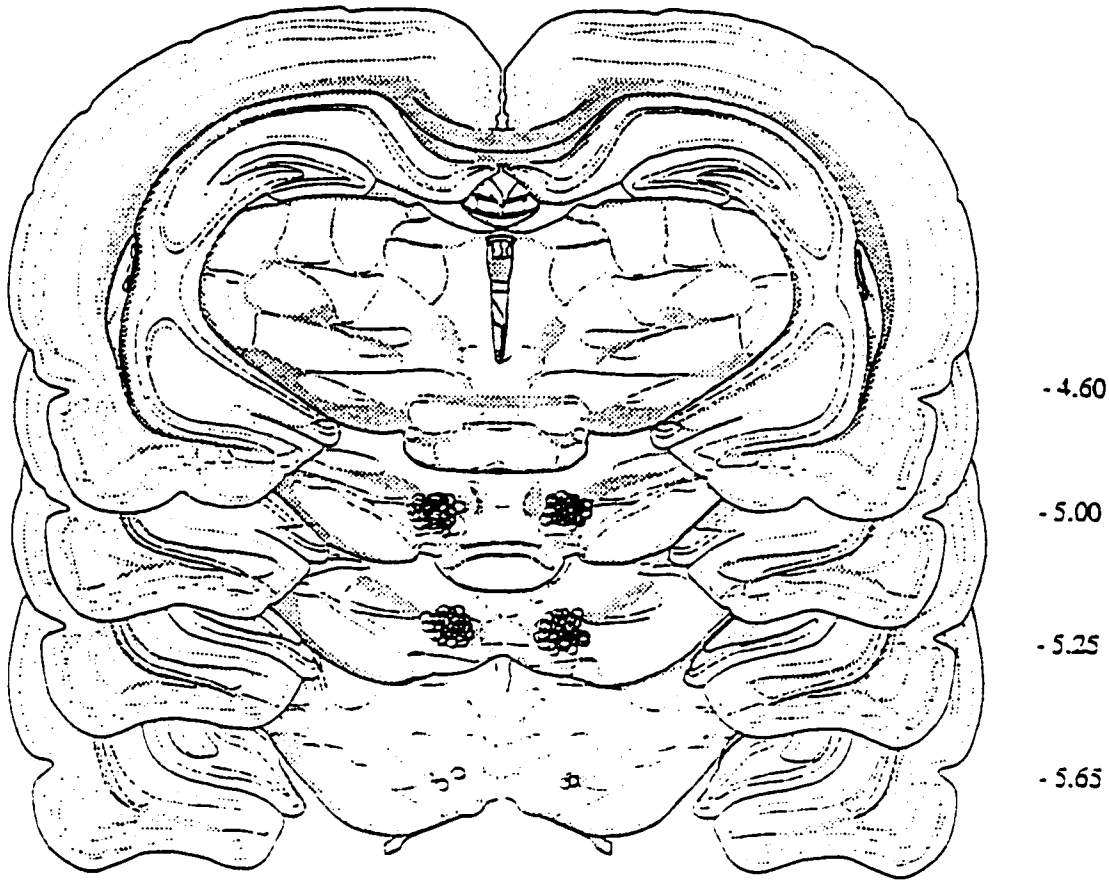


Figure 5. Location of the internal injector cannulae tips of all rats that received intra-VTA infusions of either the NK-1 receptor agonist, GR-73632, the NK-3 receptor agonist, senktide, a solution containing both tachykinin agonists, or saline (n = 69/side). Drawings (adapted from Adobe Illustrator 5.5 for Windows) are from the atlas by Swanson (1992). Values on the right represent distance in mm caudal from bregma.

The present findings that tachykinin NK-1 and NK-3 selective receptor agonists infused into the VTA produce analgesia in the formalin test are consistent with those of the previous study showing that intra-VTA infusions of the SP analog, DiMe-C7, induce analgesia in this test (Altier & Stewart, 1993). They are also in accordance with the results of other studies indicating that midbrain ascending DA neurons play a role in the mediation of analgesia in the formalin test (Anderson & Rompré, 1996; Clarke & Franklin, 1992; Franklin, 1989; Morgan & Franklin, 1990).

SP has been reported previously to increase nociceptive thresholds in phasic pain tests following intraventricular (Frederickson et al., 1978; Kotani et al., 1981; Malick & Goldstein, 1978; Mészaros et al., 1980; Naranjo et al., 1982a; Stewart et al., 1976) and peripheral (Hall & Stewart, 1983; Mohrland & Gebhart, 1979; Oehme et al., 1980; Starr et al., 1978; Szreniawski et al., 1979) administration. More recently, Yeomans and Proudfit (1992) reported that SP microinfused into the spinally-projecting noradrenergic A7 cell group increases nociceptive thresholds in the foot withdrawal test. A7 neurons receive afferent input from SP-containing neurons originating in the ventromedial medulla and appear to participate in the brainstem descending pain-suppression system (Yeomans & Proudfit, 1991, 1992). The present findings agree with those of these previous studies in showing that SP has analgesic properties and extend the results of these previous reports by indicating that SP can act in the VTA of the midbrain to suppress the transmission of tonic pain.

Several lines of indirect evidence suggest that the stimulation of NK-1 and NK-3 receptors in the VTA induces analgesia in the formalin test by activating midbrain ascending DA neurons that innervate the NAS. First, increased locomotor activity, which is thought to reflect enhanced DA activity in the NAS (e.g., Kelley and Iversen,

1976; Pijnenburg et al. 1976), can be induced by intra-VTA infusions of GR-73632 or senktide (Elliott et al. 1991, 1992), SP (Stinus et al. 1978; Kelley et al. 1979, 1985) as well as by DiMe-C7 (Eison et al. 1982 a, b; Naranjo and Del Río, 1984; Elliott and Iversen, 1986). Infusions of GR-73632 (Elliott et al. 1991), SP (Deutch et al. 1985; Cador et al. 1989), and DiMe-C7 (Elliott et al. 1986a) into the VTA have also been reported to enhance DA metabolism in the NAS. In line with these biochemical results, it was found recently, using *in vivo* microdialysis, that intra-VTA infusions of DiMe-C7 increase extracellular levels of DA and its metabolites in the NAS (Altier, 1993). Furthermore, Overton et al. (1992) reported an increase in the firing rate of neurons in the VTA following administration into this site of either GR-73632 or senktide. Finally, there is evidence that infusions into the NAS of amphetamine, which causes synaptic DA release and re-uptake blockade, induce analgesia in the formalin test (Altier & Stewart, 1993). The idea that SP acting in the VTA induces analgesia in the formalin test by enhancing DA transmission in the mesolimbic system will be examined in Experiments 6 and 7.

EXPERIMENT 2

Effects of Intra-NAS Infusions of the Tachykinin NK-1 Selective Agonist, GR-73632, or the Tachykinin NK-3 Selective Agonist, Senktide, in the Formalin test

SP immunoreactivity is also evident in the NAS (Ljungdahl et al. 1978 a,b). SP-containing neurons project from the midbrain periaqueductal gray (Li et al., 1990a) and nucleus of the solitary tract (Li et al., 1990b) to the NAS, where SP-like immunoreactive terminals have been found to form close connections with terminals labeled for tyrosine hydroxylase (Pickel et al., 1988). Autoradiographic studies reveal the presence of all three tachykinin receptor subtypes in the NAS (Saffroy et al., 1988), but results of behavioural studies suggest that only NK-1 receptor stimulation into this area is effective at increasing locomotor activity (Elliott et al., 1992).

Given the findings that SP terminals and receptors are present in the NAS, that SP interacts with DA at this site in an excitatory way (Elliott et al., 1986b, 1992; Kalivas & Miller, 1986), and that amphetamine-induced DA release in the NAS elicits analgesia in the formalin test (Altier & Stewart, 1993), it was of interest to investigate the effect of stimulating different tachykinin receptor subtypes in the NAS on tonic pain. To this end, the following experiment was designed to examine the analgesic effects of intra-NAS infusions of the NK-1 selective receptor agonist, GR-73632, and the NK-3 selective receptor agonist, senktide, in the formalin test.

Method

Surgery

19 mm long, 22 gauge guide cannulae were implanted bilaterally in the NAS at the following coordinates: + 3.0 mm anterior from bregma, + 1.4 mm lateral from midline, and - 6.3 mm ventral from the skull surface (Pellegrino et al. 1967). One group of rats was implanted with cannulae aimed 1.0 mm dorsal to the NAS infusion sites. Cannulae were lowered at an angle of 10 degrees from the perpendicular, and the incisor bar was set 5.0 mm above the interaural line.

Drugs

The doses of the NK-1 agonist, GR-73632 used in these experiments were 0.005, 0.5 and 1.5 nmol/side. The doses of the NK-3 agonist, senktide, used were 0.005, 0.5, or 1.5 nmol/side. These doses of GR-73632 and senktide were reported previously to be effective at stimulating locomotor activity (Elliott et al. 1992). All other information regarding these compounds is described in Experiment 1.

Design and Procedure

On the test day, rats received bilateral intra-NAS infusions of either GR-73632 (0.005, 0.5, or 1.5 nmol/0.5 µl/side), senktide (0.005, 0.5, or 1.5 nmol/0.5 µl/side), or saline, immediately prior to the formalin injection. In order to examine the site specificity of the effects observed following intra-NAS infusions of tachykinin selective agonists, rats received bilateral infusions of either GR-73632 (1.5 nmol/0.5 µl/side), senktide (1.5 nmol/0.5 µl/side), or saline, 1.0 mm dorsal to the NAS, using a counterbalanced within-subjects design. Thus, for this experiment, animals were tested twice (once with an agonist, once with saline) in the formalin test, at a 1-week interval. Either the right or left hind paw was injected on successive tests.

Statistical Analyses

Data were analyzed by two-way ANOVAs with Treatment as either the between-subjects variable (Figures 6 and 7; drug vs saline; 4 levels) or the within-subjects variable (Figure 8; 2 levels) and Time (10 post-infusion time points) as the within-subjects variable; because most of the effects of the drugs occurred within the first 30 minutes of testing, only the first 10 post-infusion time-points were included in the analyses. All analyses were followed, if appropriate, by Tukey's post-hoc test for overall differences between conditions. Tests for simple main effects were used, when appropriate, to analyze the differences at each time point between a control and treatment condition. Data from an animal were included in the analyses only if the injector tips were located within 0.5 mm of the target area.

Results

The effect of intra-NAS infusions of the NK-1 agonist, GR-73632 (0.005, 0.5 or 1.5 nmol/0.5 µl/side), in the formalin test is shown in Figure 6. There was a significant effect of Treatment, $F(3, 18) = 78.3$, $P < 0.0001$, due primarily to the profound inhibition of tonic pain responses induced by the highest dose of 1.5 nmol/0.5 µl/side. The pain scores of this 1.5 nmol/0.5 µl/side group differed significantly from those of each other treatment group ($Ps < 0.05$).

Figure 7 shows the effect on formalin pain responses of intra-NAS infusions of the NK-3 agonist, senktide (0.005, 0.5 or 1.5 nmol/0.5 µl/side), or saline. The ANOVA yielded a significant effect of Treatment, $F(3,17) = 9.50$, $P < 0.001$. Senktide induced significant pain inhibition at the intermediate and highest dose tested, but the pain scores in these groups did not differ significantly from each other.

Figure 6. Mean formalin pain scores (± S.E.M.) following bilateral intra-NAS infusions of the NK-1 agonist, GR-73632 using a) 0.005, b) 0.5, or c) 1.5 nmol/0.5 µl/side. Significantly different from saline : * $p < 0.005$; ÷ $p < 0.05$. Rats (n = 5-6 per group) were tested in a between-subjects design. For clarity, the three doses were plotted in different graphs, with the same control group in each.

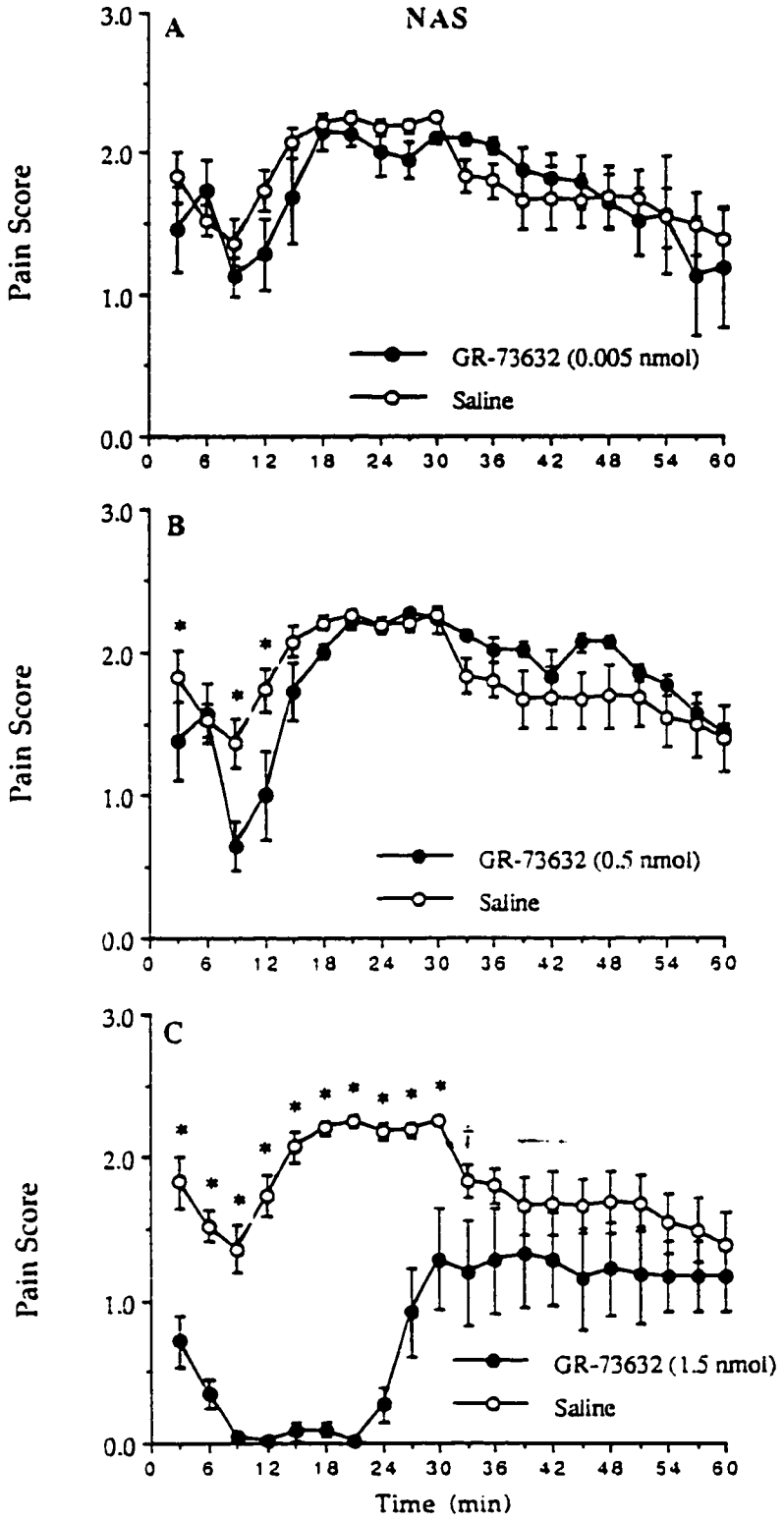

A
NAS
B
C
Pain Score
Pain Score
Pain Score
3.0
2.0
1.0
0.0
0
6
12
18
24
30
36
42
48
54
60
GR-73632 (0.005 nmol)
Saline
GR-73632 (0.5 nmol)
Saline
GR-73632 (1.5 nmol)
Saline
Time (min)

Figure 7. Mean formalin pain scores (± S.E.M) following bilateral intra-NAS infusions of the NK-3 agonist, senktide, using either a) 0.005, b) 0.5, or c) 1.5 nmol/0.5 µl/side. Significantly different from saline : * $p < 0.005$; † $p < 0.05$. Rats (n = 5-6 per group) were tested in a between-subjects design. For clarity, the three doses were plotted in different graphs, with the same control group in each.

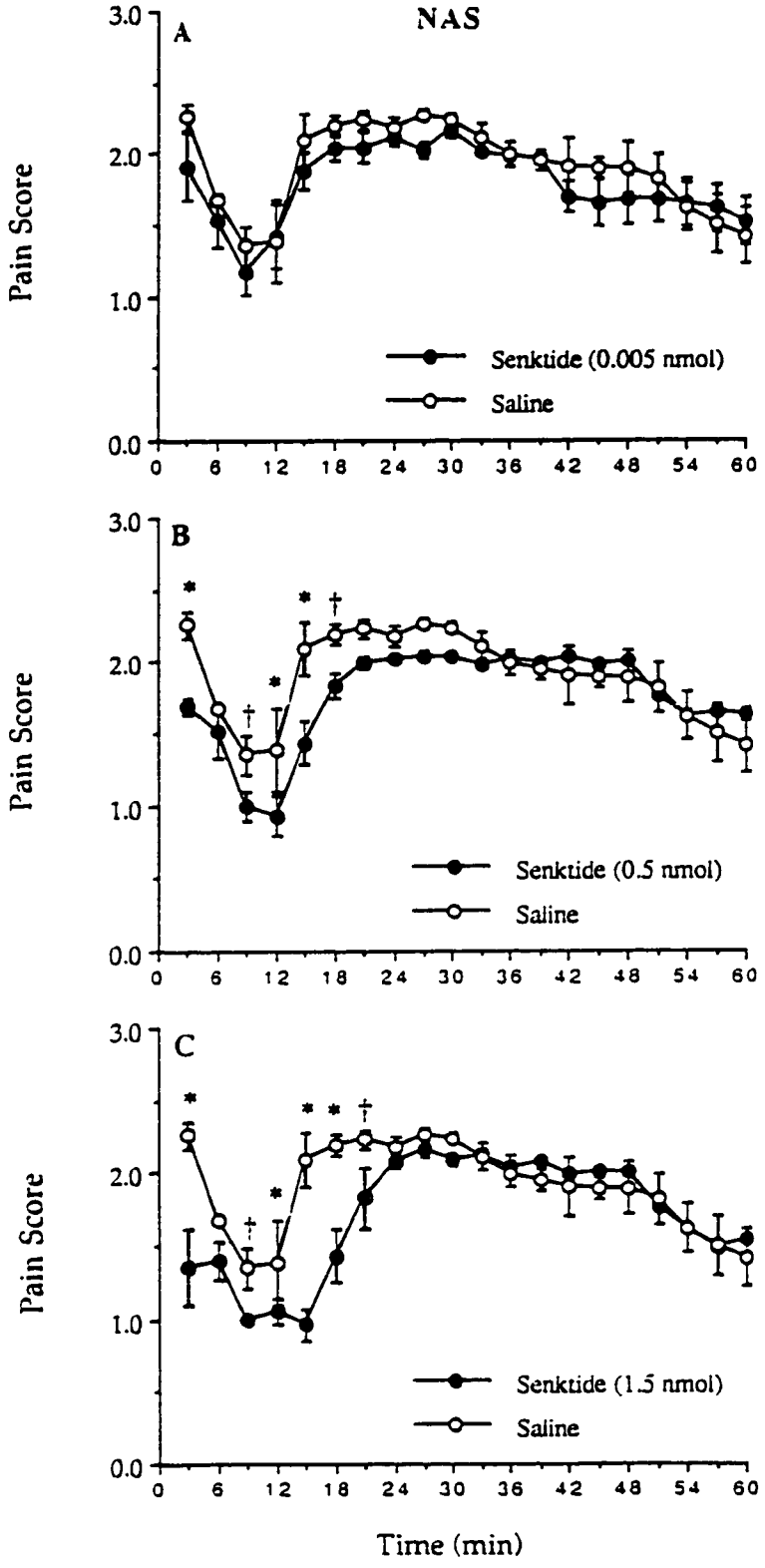

NAS
A
B
C
Pain Score
Pain Score
Pain Score
3.0
2.0
1.0
0.0
0 6 12 18 24 30 36 42 48 54 60
Senktide (0.005 nmol)
Saline
Senktide (0.5 nmol)
Saline
Senktide (1.5 nmol)
Saline
Time (min)

Figure 8 shows the effect of infusions made 1.0 mm above the NAS of the NK-1 agonist, GR-73632 (1.5 nmol/0.5 µl/side), or the NK-3 agonist, senktide (1.5 nmol/0.5 µl/side). As can be seen, these infusions did not reduce pain scores, as compared to saline infusions. There were no significant differences in pain responses between saline and GR-73632 or senktide conditions, $F(1, 3) = 0.06$, $P = 0.825$, and $F(1, 3) = 0.11$, $P = 0.66$, respectively.

Figure 9 illustrates the location of the injector tips of all animals tested in the present experiment. Forty three rats had their injector tips within the NAS. Three rats had their injector tips outside the limits of the NAS and thus their data were discarded. Eight rats in the dorsal control condition had their injector tips outside the limits of the NAS.

Discussion

The results obtained from the present experiments indicate that the NK-1 selective agonist, GR-73632, and the NK-3 selective agonist, senktide, induce analgesia in the formalin test for tonic pain following infusions into the NAS. Because infusions of either GR-73632 or senktide made 1.0 mm dorsal to the NAS were without effects on pain responses in the formalin test, it is likely that the tachykinin agonists induced their analgesic effects by acting at receptors within the NAS. These findings indicate that NK-1 and NK-3 receptors acting in the NAS play a role in mediating the inhibition of tonic pain. As observed with the VTA (Experiment 1), the NK-1 agonist induced more potent analgesia in the formalin test, within the range of doses used, than the NK-

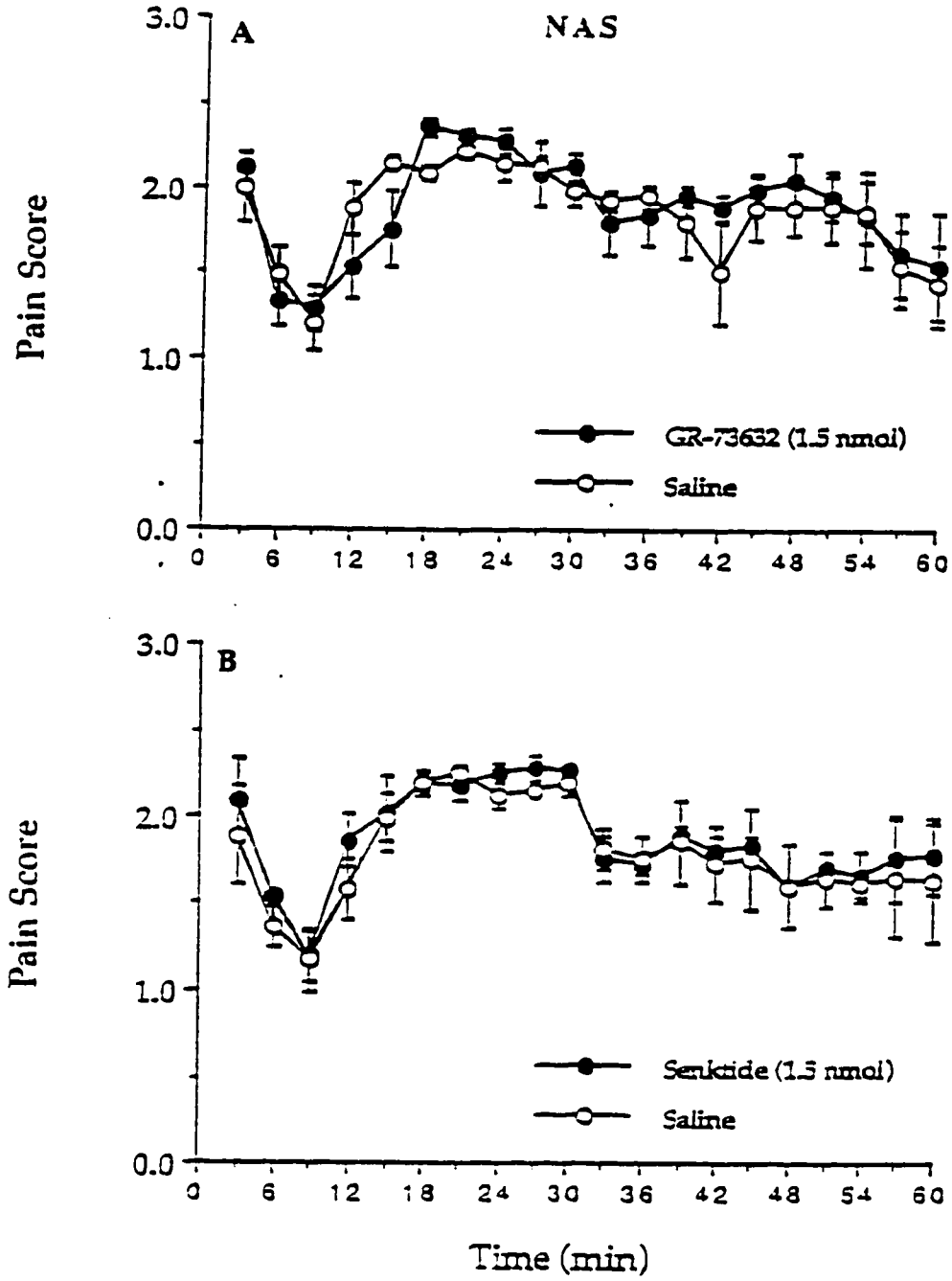


Figure 8. Mean formalin pain scores (± S.E.M) following either a) the NK-1 agonist, GR-73632 (1.5 nmol/0.5 µl/side), or b) the NK-3 agonist, senktide (1.5 nmol/0.5 µl/side) infused bilaterally 1.0 mm dorsal to the NAS infusion sites. Animals (n = 4 per group) were tested in a counterbalanced within-subjects design.

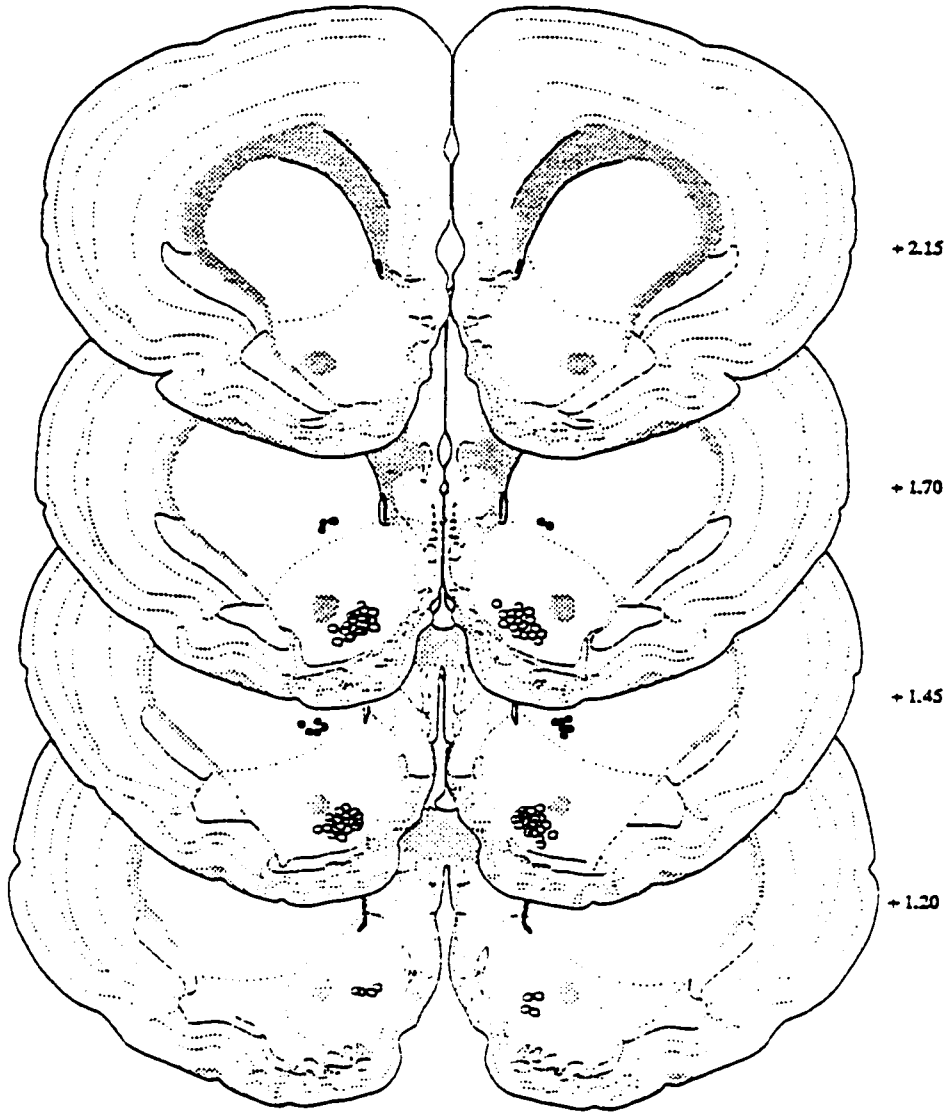


Figure 9. Location of the internal injector cannulae tips of all rats that received intra-NAS infusions of tachykinin agonists and saline. Open circles (n = 43/side) represent effective injection sites whereas closed circles (n = 4/side) represent ineffective injection control sites. Note that effective injection sites were clustered in the shell region of the NAS. Drawings (adapted from Adobe Illustrator 5.5 for Windows) are from the atlas by Swanson (1992). Values on the right represent distance in mm rostral from bregma.

3 agonist, suggesting that NK-1 receptors in the NAS play a more important role than NK-3 receptors in this response.

The mechanisms by which tachykinin receptor agonists infused into the NAS induce analgesia are unclear. There is some evidence to suggest that SP acts in the NAS to enhance DA neurotransmission. Kalivas and Miller (1986), for example, found that SP infused into the NAS increases the levels of DA metabolites at this same site and potentiates the locomotor activating effects of intra-NAS DA administration. It has also been reported that amphetamine-induced locomotor activity is blocked by a SP antibody infused into the NAS (Elliott et al. 1986b). More recently, Elliott et al. (1992) found that the locomotor-stimulant effects of intra-NAS infusions of the NK-1 selective agonist, GR-73632, are blocked by systemic administration of the DA receptor antagonist, haloperidol.

In support of a relationship between SP and DA in the NAS, it has been reported that some SP-immunoreactive terminals form synaptic connections with tyrosine hydroxylase (DA)-containing terminals in the NAS (Pickel et al. 1988). Interestingly, the NAS receives SP-containing terminals originating from the periaqueductal gray in the brainstem (Li et al. 1990), an area that is known to play an important role in pain and its suppression (e.g., Basbaum & Fields, 1984). Although the mechanisms underlying the interaction between SP and DA are unclear, it has been proposed that SP in the NAS may function to regulate presynaptically the release of DA (Elliott et al. 1986b; Kalivas and Miller, 1986; Pickel et al. 1988). The possibility that tachykinin agonists acting in the NAS induce analgesia in the formalin test by enhancing the local release of DA is consistent with the finding that amphetamine-induced DA release in the NAS inhibits tonic pain in the formalin test (Altier & Stewart, 1993) and provides further support for the idea that enhanced DA activity in the NAS is involved in mediating the suppression

of tonic pain.

EXPERIMENT 3

Effects of Pretreatment with the Opioid Antagonist, Naltrexone, on Analgesia Induced by Intra-VTA or Intra-NAS Infusions of Tachykinin Agonists in the Formalin Test

It has been shown previously that SP administered either systemically or into the ventricles induces naloxone-reversible analgesia in phasic pain tests (Frederickson et al. 1978; Malick and Goldstein, 1978; Mohrland and Gebhart, 1979; Oehme et al. 1980; Stewart et al. 1976; Szreniawski et al. 1979). Since SP neither binds to opioid receptors (Terenius, 1975; Onoki et al., 1977; Szreniawski et al. 1979) nor acts like opioids on isolated tissue preparations (Frederickson et al. 1978), it has been proposed that SP produces analgesia indirectly by releasing endogeneous opioid peptides (Frederickson et al. 1978; Malick and Goldstein, 1978). In support of this, subsequent studies indicated that SP-induced analgesia in the vocalization after-discharge test, a test similar to the formalin test in that it induces pain associated with significant negative affect, is blocked by intraventricular infusions of the antibody against met-enkephalin (Naranjo et al. 1982 a,b), and that the amount of met-enkephalin released from the periaqueductal gray correlates with the analgesic potency of SP and its analog, DiMe-C7 (Del Río et al. 1983). Given these findings, the purpose of the following experiments was to explore the involvement of opioids on the analgesic effects induced by DiMe-C7, the NK-1 selective receptor agonist, GR-73632, and the NK-3 selective receptor agonist, senktide, infused into the VTA or NAS (DiMe-C7 only).

DiMe-C7 {(p-Glu^5-MePhe^8-MeGly^9) SP $_{5-11}$} is a carboxyl-terminal fragment of the SP molecule and is resistent to enzymatic degradation in the central nervous system (Sandberg et al., 1981). When infused into the VTA, DiMe-C7 is equipotent with SP in causing locomotor hyperactivity, but the effects of DiMe-C7 are longer-lasting (Eison et al., 1982a, b; Elliott & Iversen, 1986). Furthermore, compared to SP, DiMe-C7 appears to penetrate the blood-brain barrier more readily because of the lack of charged groups and its lipophilic character (Banks & Kastin, 1985; Sandberg et al., 1981). Because of these qualities, DiMe-C7 has been proposed to be better suited for behavioral studies (Eison et al., 1982a; Elliott & Iversen, 1986), hence its use in the present experiments.

Method

Surgery

21 mm long, 22 gauge guide cannulae (Plastics One, Inc.) were implanted, bilaterally, 1.0 mm above the VTA and aimed at the following coordinates : - 5.7 mm posterior to bregma, + 0.6 mm lateral from the midline, and - 7.4 mm ventral from the skull surface (Paxinos and Watson, 1986). The stereotaxic arms were angled at 15 degrees from the perpendicular and the skull was level between lambda and bregma (i.e., flat skull position). For the NAS, 19 mm long, 22 gauge guide cannulae were implanted bilaterally at the following coordinates: + 3.0 mm anterior from bregma, + 1.4 mm lateral from midline, and - 6.3 mm ventral from the skull surface (Pellegrino et al. 1967). Cannulae were lowered at an angle of 10 degrees from the perpendicular, and the incisor bar was set 5.0 mm above the interaural line.

Drugs

The dose of the NK-1 agonist, GR-73632 used in this experiment was 0.5 nmol/0.5 µl/side. The dose of the NK-3 agonist, senktide, was 1.5 nmol/0.5 µl/side. All other information regarding these compounds is described in Experiment 1. DiMe-C7 (Sigma, St. Louis, MO), was infused bilaterally at a dose of 3.0 µg/0.5 µl/side. The compound was dissolved in acid saline (pH = 6.05) in order to inhibit absorption of the peptide to plastic. Stock concentrations (30.0 µg/5.0 µl) of DiMe-C7 and the vehicle, acid saline, were aliquoted into polypropylene vials and frozen at -70 ° C until used. All solutions were thawed within 30 minutes of use. Naltrexone hydrochloride (Endo Laboratories Inc., Garden City, NW) was dissolved in saline and injected subcutaneously using a dose of 2.0 mg/kg.

Design and Procedure

On the test day, rats were tested in the formalin test immediately following infusions of either the SP analog, DiMe-C7 (3.0 µg/0.5 µl/side), the NK-1 agonist, GR-73632 (0.5 nmol/0.5 µl/side), the NK-3 agonist, senktide (1.5 nmol/0.5 µl/side), or the vehicle, into the VTA or NAS (DiMe-C7 only) using a between-subjects design. All animals were pretreated, 10 min prior to the intracranial microinfusions, with either the opioid antagonist, naltrexone (2.0 mg/kg; s.c), or saline.

Statistical Analyses

Data were analyzed by a two-way ANOVAs with Treatment (Naltrexone-Peptide vs Saline-Peptide vs Saline-Vehicle) as the between-subjects variable and Time (10 post-infusion time-points) as the within-subjects variable; because most of the effects of the drugs occurred within the first 30 minutes of testing, only the first 10 post-infusion time-points were included in the analyses. All analyses were followed, if appropriate, by Tukey's post-hoc test for overall differences between conditions. Tests for simple

main effects were used, when appropriate, to analyze the differences at each time point between a control and treatment condition. Data from an animal were included in the analyses only if the injector tips were located within 0.5 mm of the target area.

Results

Figures 10, 11 and 12 show the effect of the opioid antagonist, naltrexone (2.0 mg/kg) or saline pretreatment on the analgesic effects induced by intra-VTA infusions of either the SP analog, DiMe-C7 (3.0 µg/0.5 µl/side), the NK-1 agonist, GR-73632 (0.5 nmol/0.5 µl/side), or the NK-3 agonist, senktide (1.5 nmol/0.5 µl/side), respectively. For each tachykinin agonists, there were significant Treatment effects, $F(2, 21) = 20.76$, $P < 0.0001$, $F(2, 20) = 6.48$, $P < 0.01$, and $F(2, 16) = 14.34$, $P < 0.0005$, respectively. As can be seen from these Figures, each of the agonists induced analgesia ($Ps < 0.05$) and naltrexone pretreatment failed to reverse analgesia induced by either DiMe-C7, GR-73632, or senktide. Post-hoc comparisons indicated that there were no significant differences between naltrexone-peptide and saline-peptide conditions.

Figure 13 illustrates the location of the internal injector cannulae tips of all rats that received intra-VTA infusions of tachykinin agonists or the vehicle. As shown, 61 rats had their internal injector cannulae tips within the VTA. The data from two rats were discarded because the location of their internal injector cannulae tips did not satisfy the criterion of inclusion.

Figure 14 shows the effect of pretreatment with the opioid antagonist, naltrexone (2.0 mg/kg) or saline, on the analgesic effects induced by DiMe-C7 (3.0 µg/0.5 µl/side), or the vehicle, infused into the NAS. There was a significant effect of Treatment, $F(2, 17) = 8.89$, $P < 0.005$. As seen by comparing conditions Saline-

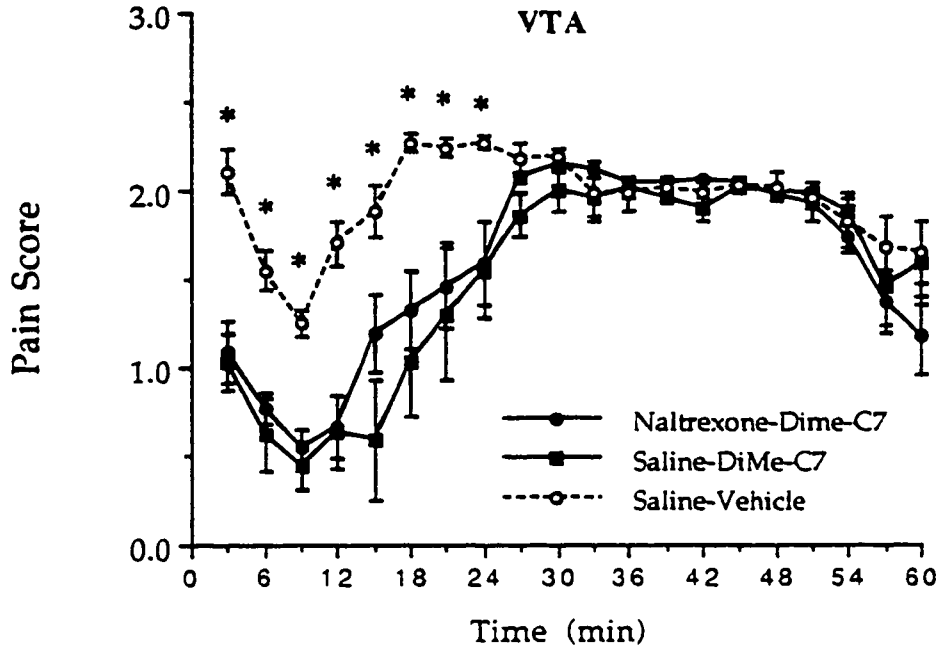


Figure 10. Mean formalin pain scores (± S.E.M.) following bilateral intra-VTA infusions of the SP analog, DiMe-C7 (3.0 µg/0.5 µl/side), or the vehicle in rats pretreated with either the opioid antagonist, naltrexone (2.0 mg/kg) or saline. Significant differences between saline-peptide and saline-vehicle : * $p < 0.001$; ** $p < 0.005$; † $p < 0.05$. Animals ($n = 5$-11 per group) were tested in a between-subjects design. Post hoc comparisons indicated that Naltrexone-DiMe-C7 and Saline-DiMe-C7 conditions differed significantly from the Saline-Vehicle condition ($P < 0.05$) and did not differ significantly from each other.

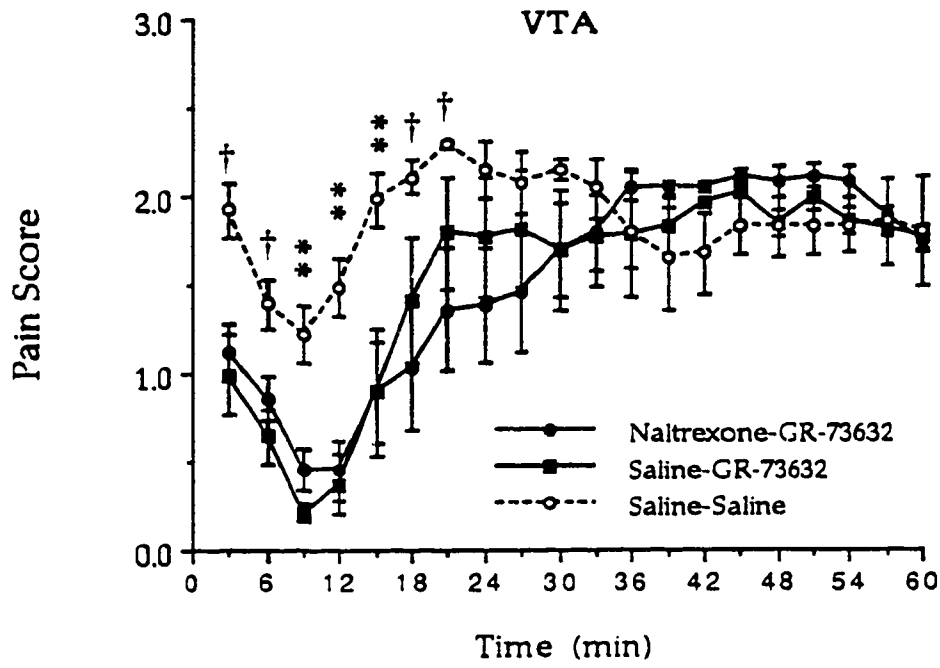


Figure 11. Mean formalin pain scores (± S.E.M.) following bilateral intra-VTA infusions of the NK-1 agonist GR-73632 (0.5 nmol/0.5 µl/side), or saline, in rats pretreated with either the opioid antagonist, naltrexone (2.0 mg/kg) or saline. Significant differences between Saline-GR-73632 and Saline-Saline : * $p < 0.001$; ** $p < 0.005$; † $p < 0.05$. Animals (n = 5-12 per group) were tested in a between-subjects design. Post hoc comparisons indicated that Naltrexone-GR-73632 and Saline-GR-73632 conditions differed significantly from the Saline-Saline condition ($P < 0.05$) and did not differ significantly from each other.

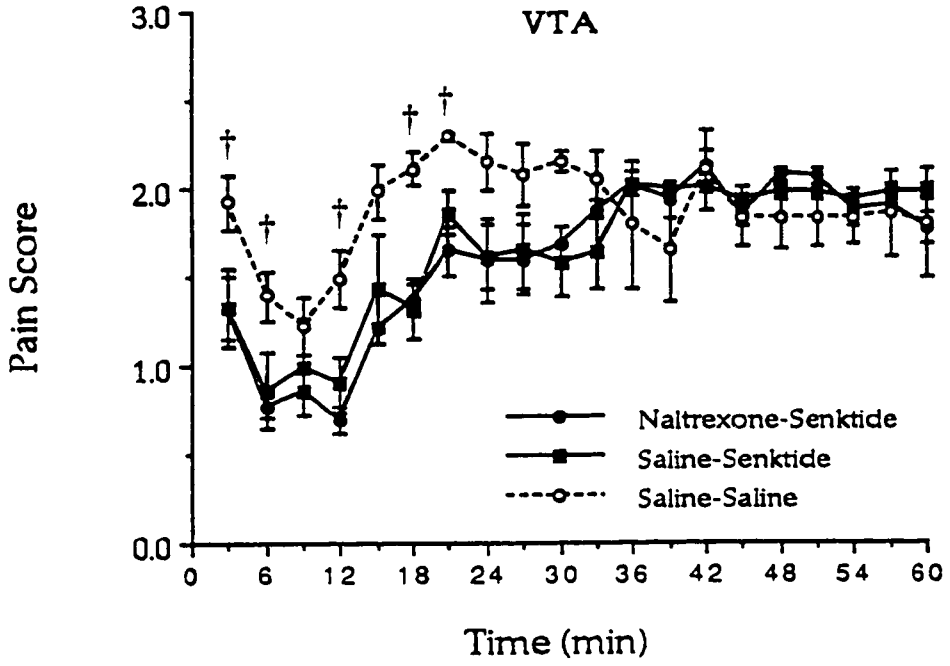


Figure 12. Mean formalin pain scores (± S.E.M.) following bilateral intra-VTA infusions of the NK-3 agonist, senktide (1.5 nmol/0.5 µl/side), or saline, in rats pretreated with either the opioid antagonist, naltrexone (2.0 mg/kg) or saline. Significant differences between Saline-Senktide and Saline-Saline : * $p < 0.001$; ** $p < 0.005$; † $p < 0.05$. Animals (n = 5-9 per group) were tested in a between-subjects design. Animals in the Saline-Saline condition were the same as in Figure 10. Post hoc comparisons indicated that Naltrexone-Senktide and Saline-Senktide conditions differed significantly from the Saline-Saline condition ($P < 0.05$) and did not differ significantly from each other.

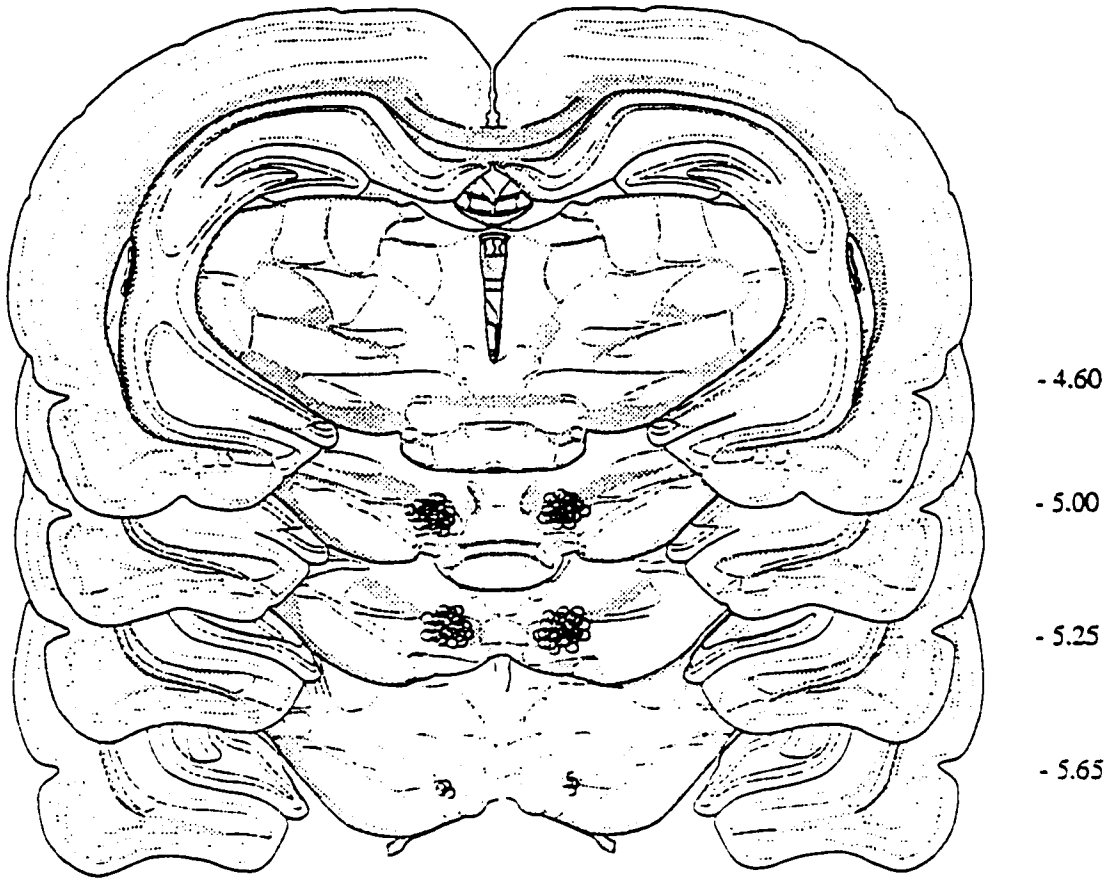


Figure 13. Location of the internal injector cannulae tips of all rats that received intra-VTA infusions of tachykinin agonists and the vehicle (n = 61/side). Drawings (adapted from Adobe Illustrator 5.5 for Windows) are from the atlas by Swanson (1992). Values on the right represent distance in mm caudal from bregma.

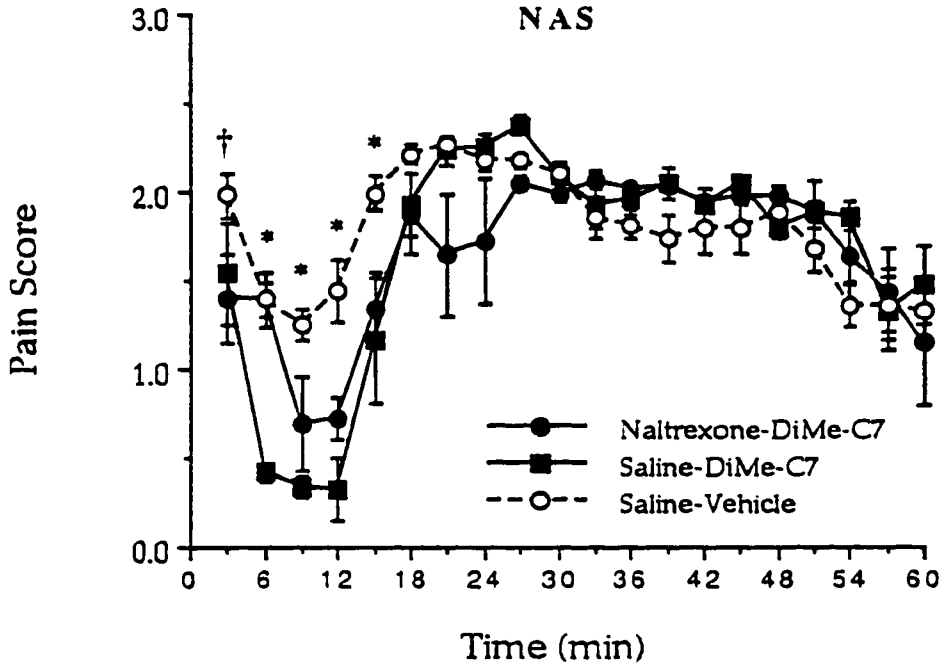


Figure 14. Mean formalin pain scores (± S.E.M.) following bilateral intra-NAS infusions of the SP analog, DiMe-C7 (3.0 µg/0.5 µl/side), or the vehicle, in rats pretreated with either the opioid antagonist, naltrexone (2.0 mg/kg), or saline. Significant differences between Saline-DiMe-C7 and Saline-Vehicle : * $p < 0.001$; † $p < 0.05$. Animals (n = 6-10 per group) were tested in a between-subjects design. Post hoc comparisons indicated that Naltrexone-DiMe-C7 and Saline-DiMe-C7 conditions differed significantly from the Saline-Vehicle condition ($P < 0.05$) and did not differ significantly from each other.

DiMe-C7 and Saline-Vehicle, intra-NAS infusions of DiMe-C7 induced significant analgesia ($P < 0.05$). Naltrexone pretreatment failed to reverse DiMe-C7-induced analgesia, as seen by comparing conditions Naltrexone-DiMe-C7 and Saline-DiMe-C7.

Figure 15 illustrates the location of the internal injector cannulae tips of all rats that received infusions of DiMe-C7 or the vehicle into the NAS. As shown, 20 rats had their internal injector cannulae tips within the NAS. One rat had its tips outside the limits of the NAS and thus its data were discarded.

Discussion

As mentioned previously, SP has been reported previously to induce naloxone-reversible analgesia in phasic pain tests (Stewart et al. 1976; Frederickson et al. 1978; Malick and Goldstein, 1978; Mohrland and Gebhart, 1979; Szreniawski et al. 1979; Oehme et al. 1980). In contrast, the present results indicate that at least those analgesic effects induced by infusions of the SP analog, DiMe-C7, the NK-1 selective agonist, GR-73632, and the NK-3 selective agonist, senktide, into the VTA or NAS (DiMe-C7 only) occur independently from opioid mechanisms. These opposing results could be due to the the fact that, as described previously, the expression and inhibition of tonic versus phasic pain responses are mediated at different levels of the neuraxis and by different neural substrates.

With respect to the VTA effects, these differences could also be due to the fact that, when SP acts at this site, it appears to modulate the activity of midbrain DA systems by interacting directly with tachykinin receptors located on DA cell bodies. Indeed, there is evidence that SP afferents make direct synaptic contacts with DA cell

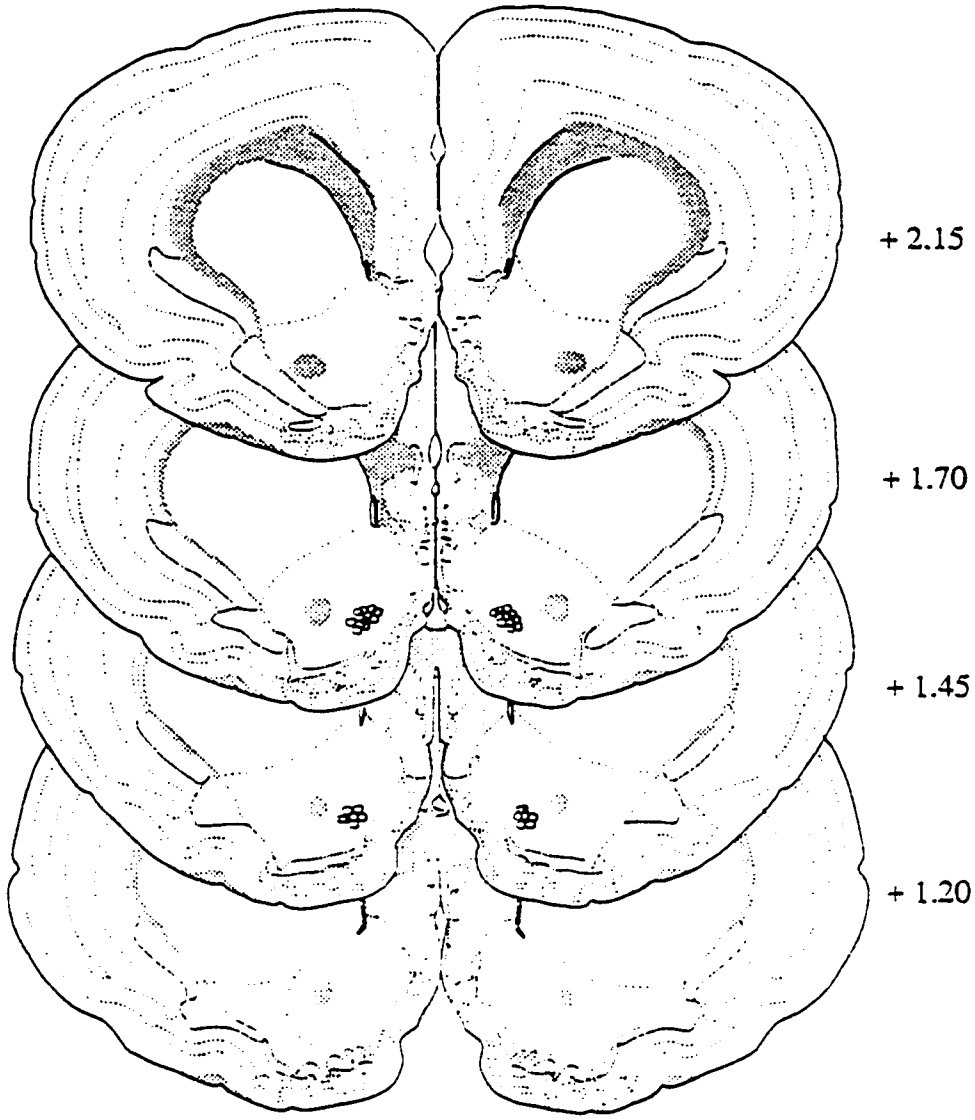


Figure 15. Location of the internal injector cannulae tips of all rats that received intra-NAS infusions of DiMe-C7 or the vehicle (n = 20/side). Drawings (adapted from Adobe Illustrator 5.5 for Windows) are from the atlas by Swanson (1992). Values on the right represent distance in mm rostral from bregma.

bodies in the VTA (Tamiya et al. 1990) and that tachykinin receptors with which SP interacts are located on DA neurons in the midbrain (Stoessl, 1992). It has also been shown that intra-VTA pretreatment with naltrexone methylbromide does not attenuate the capacity of intra-VTA DiMe-C7 to increase DA metabolism in terminal fields (Kalivas and Abhold, 1987), suggesting that the SP analog mediates this biochemical response independently from opioid mechanisms. Similarly, SP acting in the NAS might induce analgesia independently from opioid mechanisms by interacting directly with tachykinin receptors located on DA terminals at this site to enhance DA release through direct presynaptic mechanisms. Support for this idea is provided by the finding that SP terminals in the NAS form close connections with DA-containing terminals at this site (Pickel et al., 1988).

EXPERIMENT 4

Effects of Intra-VTA or Intra-NAS Infusions of the NK-1 Selective Agonist, GR-73632, the NK-3 Selective Agonist, Senktide, and Morphine in the Tail-Flick Test for Phasic Pain

As mentioned previously in the Introduction, it appears that the neural systems mediating analgesia in the formalin test for tonic pain are fundamentally different from those that mediate analgesia in phasic pain tests, such as the spinal reflex withdrawal tail-flick test (e.g. Abbott et al., 1982; Abbott & Melzack, 1982, 1983; Ryan et al., 1985). More specifically, it has been shown that analgesia in tonic and phasic pain tests are dissociable with respect to manipulations of serotonergic, noradrenergic, and opioidergic systems and with respect to the phenomenon of tolerance to morphine's analgesic effects.

Similarly, manipulations of DAergic systems appear to produce different effects on pain sensitivity depending on the nature of the painful stimulus. Thus, DA agonists such as cocaine, amphetamine, apomorphine, and quinpirole produce analgesia in the formalin test (Dennis & Melzack, 1983; Lin et al., 1989; Morgan & Franklin, 1991; Skaburskis, 1980) and in the clinical situation (Battista & Wolff, 1973; Miley et al., 1978; Webb et al., 1978), but are either without effect or elicit hyperalgesia (i.e., increased pain responsiveness) in phasic pain tests (Ben-Sreti et al., 1983; Caroll & Lim, 1960; Dennis & Melzack, 1983; Dunai-Kovács & Székely, 1977; Gonzales et al., 1980; Hernandez et al., 1986; Misra et al., 1987; Nott, 1968; Pertovaara et al., 1988;

Robertson et al., 1981; Tocco & Maickel, 1984; Tocco et al., 1985; Tulunay et al., 1976; Witkin et al., 1961).

As mentioned previously, Morgan and Franklin (1990) were the first to explore more systematically the effects of manipulations of DA on responses to tonic versus phasic pain by examining the effects of DA-depleting 6-hydroxydopamine lesions of the VTA on the analgesic effects of systemic morphine and amphetamine in the formalin and tail-flick tests. To reiterate, it was found in these studies that the lesions attenuated morphine- and amphetamine-induced analgesia in the formalin, but not the tail flick test, suggesting that the DA-containing neurons in the VTA are important for the expression of drug-induced analgesia in the former but not the latter test. In the same vein, it has been reported previously that infusions into mesolimbic sites of compounds known to enhance DA activity produce different effects depending on the type of pain test used (Altier & Stewart, 1993). More specifically, it has been found that infusions of either the SP analog, DiMe-C7, into the VTA or amphetamine into the NAS induce analgesia in the formalin, but not the tail-flick test. Given these findings, it was of interest to compare the effects of intra-VTA (Experiment 1) and intra-NAS (Experiment 2) infusions of the NK-1 selective receptor agonist, GR-73632, and the NK-3 selective receptor agonist, senktide, in the formalin test to those in the tail-flick test. In addition, an experiment was designed to examine the effect of intra-VTA infusions of morphine in the tail-flick test, using a dose that elicits potent analgesia in the formalin test (Manning et al., 1994; Morgan, 1990; also see results of Experiments 6 and 7).

Method

Subjects

In the case of the experiments using the tachykinin selective agonists, animals used had been tested two weeks before in the formalin test in Experiments 1 and 2. In order to control for the possible effects of previous intracranial infusions of the peptides, only those animals that had received saline infusions in Experiments 1 and 2 were used in the present experiments.

Apparatus

The tail-flick test apparatus consisted of a wood platform mounted on the rim of a 43 x 30 x 22 cm clear Plexiglas tank. The tank water was heated at 55 ° C by a Haake E2 Immersion/Open Bath Circulator. Between tests, animals were kept in opaque buckets lined with beta chip.

Drugs

The highest doses that were used in the formalin test were employed in these studies. Thus, the doses of the NK-1 agonist, GR-73632, infused into the VTA and NAS were 0.5 and 1.5 nmol/side, respectively. The doses of the NK-3 agonist, senktide, infused into the VTA and NAS were 1.5 nmol/side in both cases. All other information regarding these compounds is described in Experiment 1. Morphine sulphate (BDH Inc., Québec, Canada) was dissolved in saline and infused bilaterally into the VTA using a dose of 3.0 μg.0.5 μl/side.

Design and Procedure

On each of the 2 days prior to testing, rats were administered 7 habituation trials to the tail-flick test apparatus. On each of these trials, rats were hand-held for approximately 2 minutes on the platform, with their tails hanging over the side of the water tank. The bath circulator was in operation during the habituation trials in order to allow the rats to adapt to its noises. On the test day, each rat was first given 2 habituation trials. Following these, rats received bilateral intra-VTA infusions of either GR-73632 (0.5 nmol/0.5 µl/side), senktide (1.5 nmol/0.5 µl/side), morphine (3.0 µg/0.5 µl/side), or saline, in a counterbalanced within-subjects design, and tail-flick tests were begun immediately and were repeated once every 10 minutes for 1 hour. In the second set of experiments, using the same design and procedure, rats were administered bilateral intra-NAS infusions of GR-73632 (1.5 nmol/0.5 µl/side), senktide (1.5 nmol/0.5 µl/side), or saline followed by tail-flick test trials. In order to examine whether tail-flick latencies are affected by prior formalin testing, tail-flick test trials were administered following intra-VTA infusions of saline in either animals tested two weeks previously in the formalin test following intra-VTA saline, or animals never tested previously in the formalin test.

A test trial consisted of hand-holding a rat on the platform and immersing the distal 5-10 cm of the tail in the 55 ° C water. The latency for the animal to flick its tail out of the water was recorded. Although water heated at 55 ° C yields a relatively low baseline tail-flick latency of approximately 2 seconds, the test is nevertheless sensitive to drug effects; systemic morphine administered under tail-flick test conditions similar to those used here significantly elevates tail-flick latencies (Morgan & Franklin, 1990). Rats were returned to their holding buckets immediately after execution of a tail-flick. A cut-off time of 15 seconds was employed in order to avoid tissue damage. Rats were tested twice with four days between tests.

Statistical Analyses

Data from the tail-flick test were analyzed by two-way ANOVAs with Treatment (Drug vs saline) and Time (7 post-infusion time-points) as within-subjects variables. For the experiment concerning the effect on tail-flick latencies of prior experience in the formalin test, a two-way ANOVA was conducted with Treatment (Formalin-Tail-Flick vs No Formalin-Tail-Flick) as the between-subjects variable and Time (7 post-infusion time-points) as the within-subjects variable. Tests for simple main effects were used, if appropriate, to analyze the differences at each time point between a control and treatment condition. Data from an animal were included in the analyses only if the injector tips were located within 0.5 mm of the target area.

Results

In these experiments, the effects of the tachykinin NK-1 and NK-3 selective agonists were studied in the tail-flick test for phasic pain. The highest doses used in the formalin test were employed in these studies. Figure 16 shows the effect of intra-VTA infusions of a) the NK-1 selective agonist, GR-73632 (0.5 nmol/0.5 µl/side), or saline, or b) the NK-3 selective agonist, senktide (1.5 nmol/0.5 µl/side), or saline. As shown, neither the NK-1 nor the NK-3 selective agonists altered tail-flick latencies following the infusions into the VTA, $F(1, 7) = 1.28$, $P = 0.30$, and $(1, 7) = 1.31$, $P = 0.41$, respectively.

Figure 17 shows the effect of intra-NAS infusions of a) the NK-1 selective agonist, GR-73632 (1.5 nmol/0.5 µl/side), or saline, or b) the NK-3 selective agonist, senktide (1.5 nmol/0.5 µl/side), or saline. There were no significant effects of these

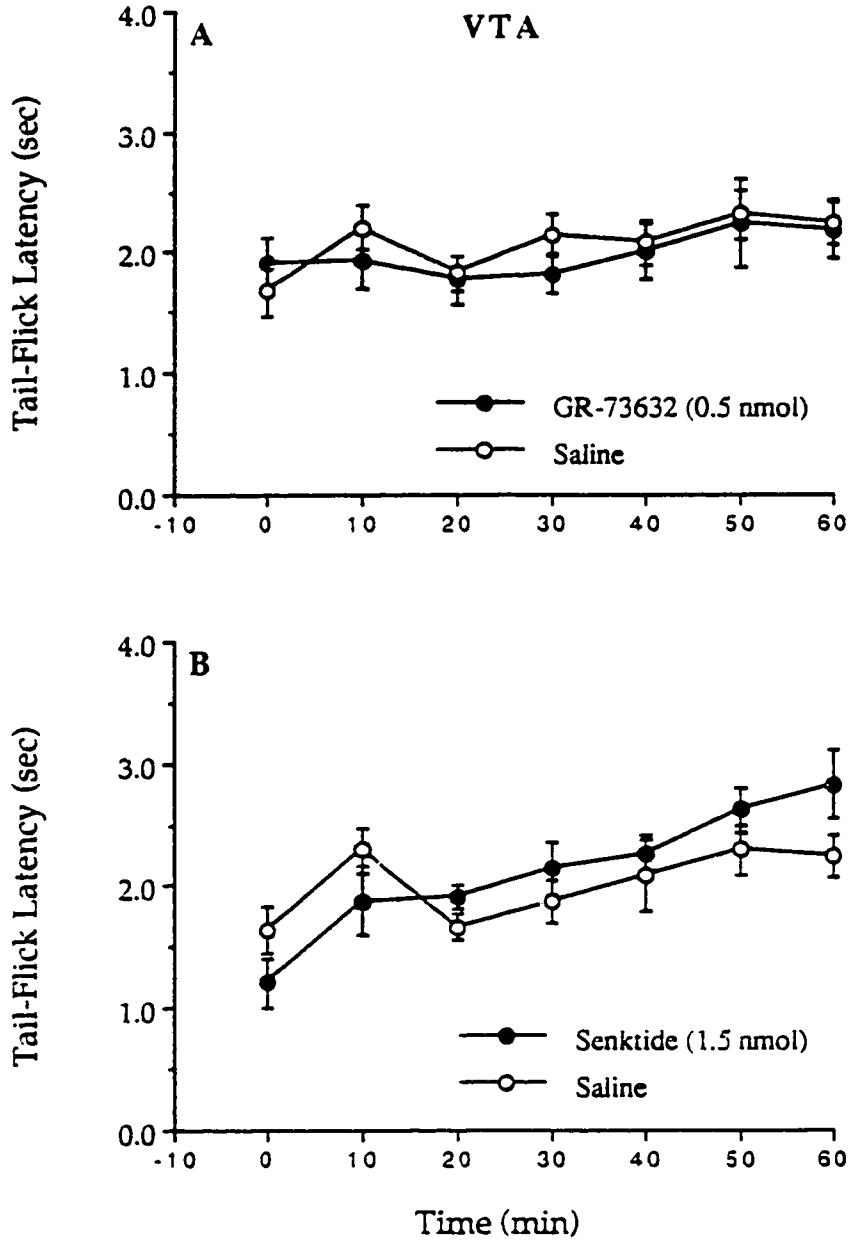


Figure 16. Mean tail-flick latencies (± S.E.M.) following bilateral intra-VTA infusions of either a) the NK-1 agonist, GR-73632 (0.5 nmol/0.5 µl/side), or b) the NK-3 agonist, senktide (1.5 nmol/0.5 µl/side). Rats (n = 8 per graph) were tested in a counterbalanced within-subjects design.

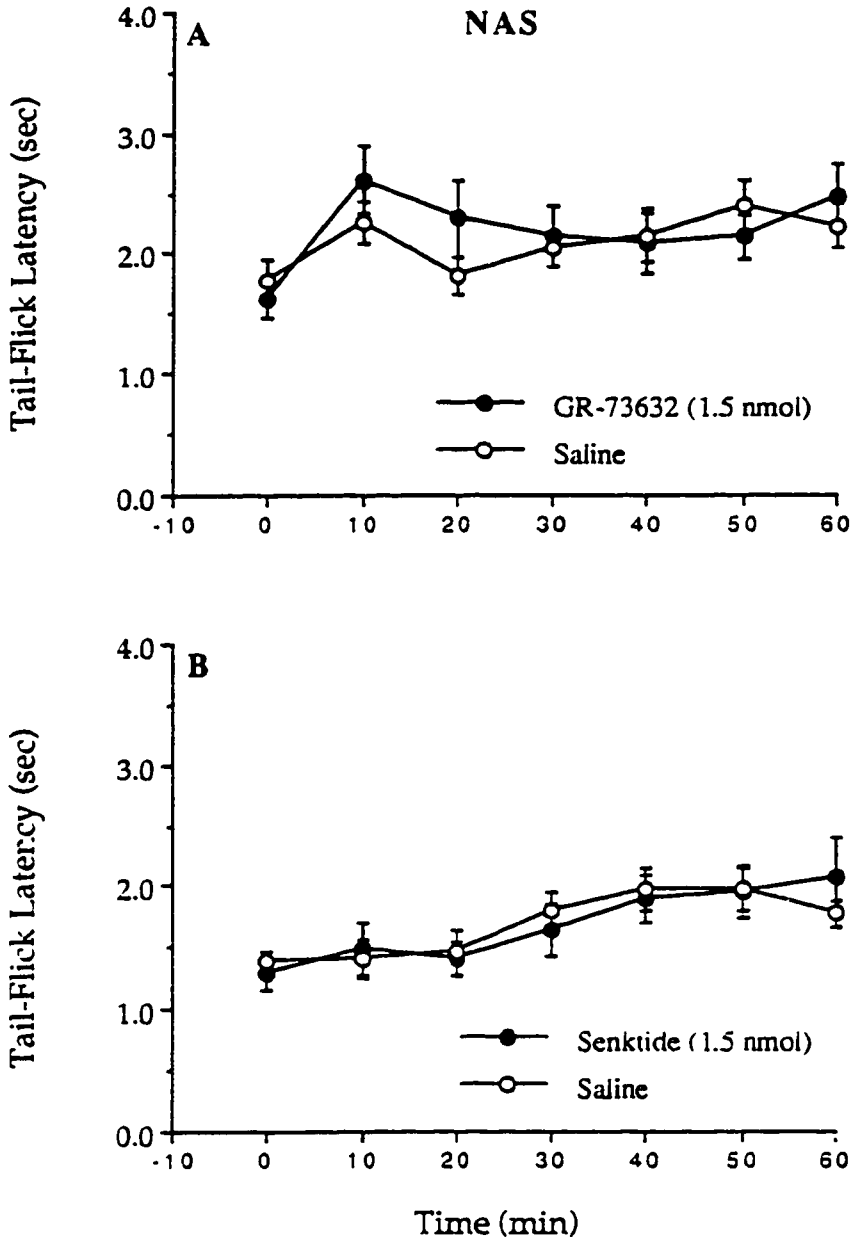


Figure 17. Mean tail-flick latencies (± S.E.M.) following bilateral intra-NAS infusions of either a) the NK-1 agonist, GR-73632 (1.5 nmol/0.5 µl/side), or b) the NK-3 agonist, senktide (1.5 nmol/0.5 µl/side). Rats (n = 8 in a; n = 7 in b) were tested in a counterbalanced within-subjects design.

drugs on tail-flick latencies following the infusions, $F(1, 7) = 0.19$, $P = 0.30$, and $F(1, 6) = 0.38$, $P = 0.56$, respectively.

Figure 18 shows the tail-flick latencies of rats with or without previous experience in the formalin test. There was no significant Treatment effect, $F(1, 9) = 0.133$, $P = 0.724$, suggesting that tail-flick latencies are not significantly affected by prior experience in the formalin test.

The effect of morphine (3.0 μg/0.5 μl/side), or saline, infused into the VTA in the tail-flick test is shown in Figure 19. As seen, intra-VTA morphine had no effect on tail-flick latencies, $F(1, 7) = 1.27$, $P = 0.32$.

Figure 20 illustrates the location of the internal injector cannulae tips of all animals that received intra-VTA infusions. As shown, 27 animals had their tips within the VTA. Figure 21 illustrates the location of the internal injector cannulae tips of all animals that received intra-NAS infusions. As shown, fifteen animals had their tips within the NAS.

Discussion

The present results indicate that the stimulation of NK-1 and NK-3 receptors by selective tachykinin agonists in either the VTA or NAS has no effect on phasic pain, as assessed by the tail-flick test. These results are in contrast with those obtained in Experiments 1 and 2, in which identical manipulations of midbrain DA neurons using the same tachykinin agonists induce potent analgesia in the formalin test. The findings that infusions into either the VTA or NAS of selective tachykinin agonists induce

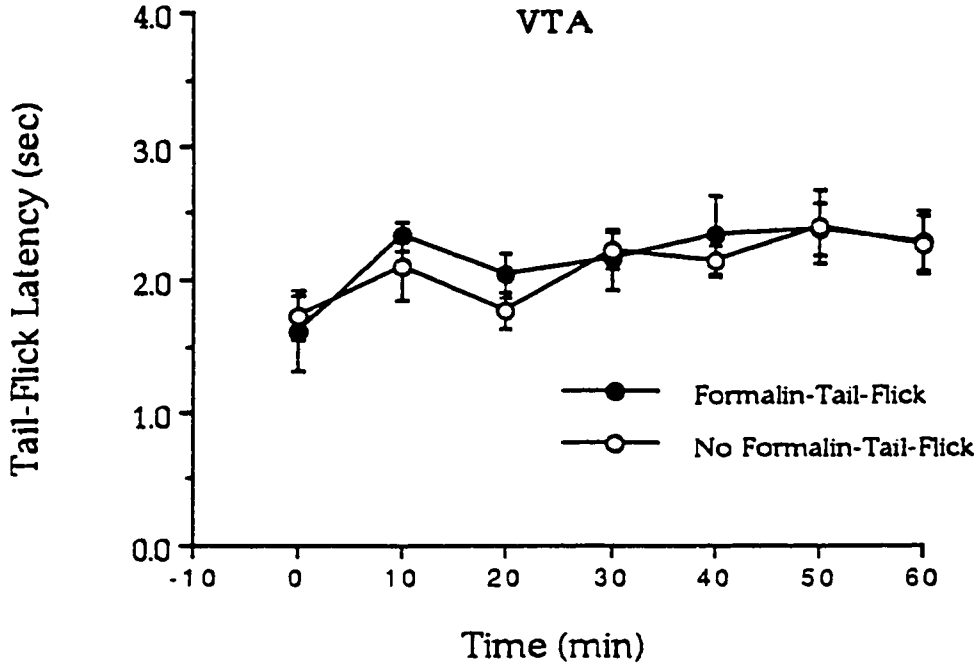


Figure 18. Mean tail-flick latencies (± S.E.M.) following bilateral intra-VTA infusions of saline in animals with (closed circles; n = 6) or without (open circles; n = 5) previous experience with the formalin test. Animals were tested in a between-subjects design.

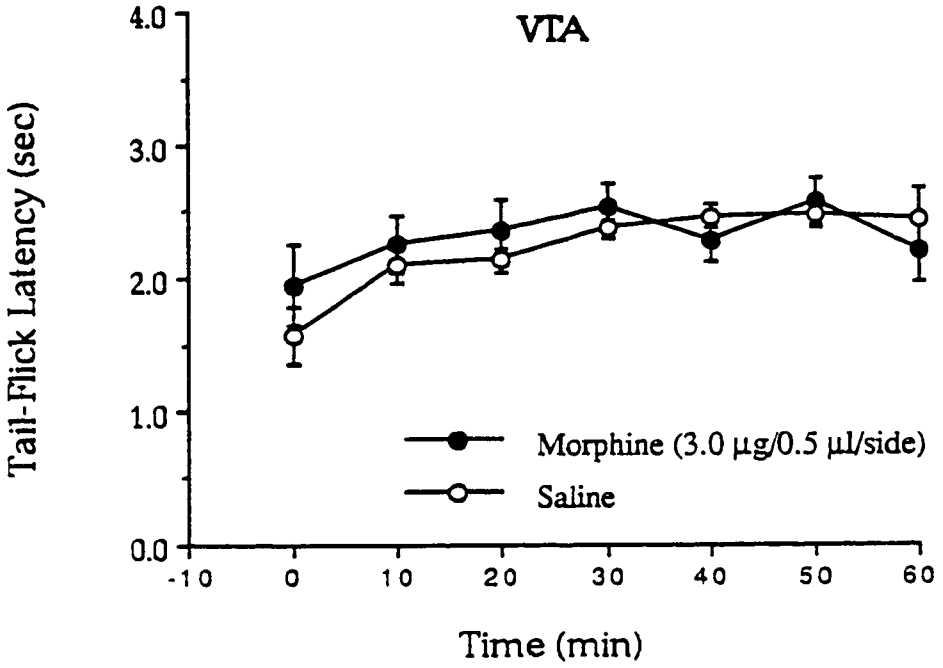


Figure 19. Mean tail-flick latencies (± S.E.M.) following bilateral intra-VTA infusions of morphine (closed circles; 3.0 µg/0.5 µl/side), or saline (open circles). Rats (n = 8) were tested in a counterbalanced within-subjects design.

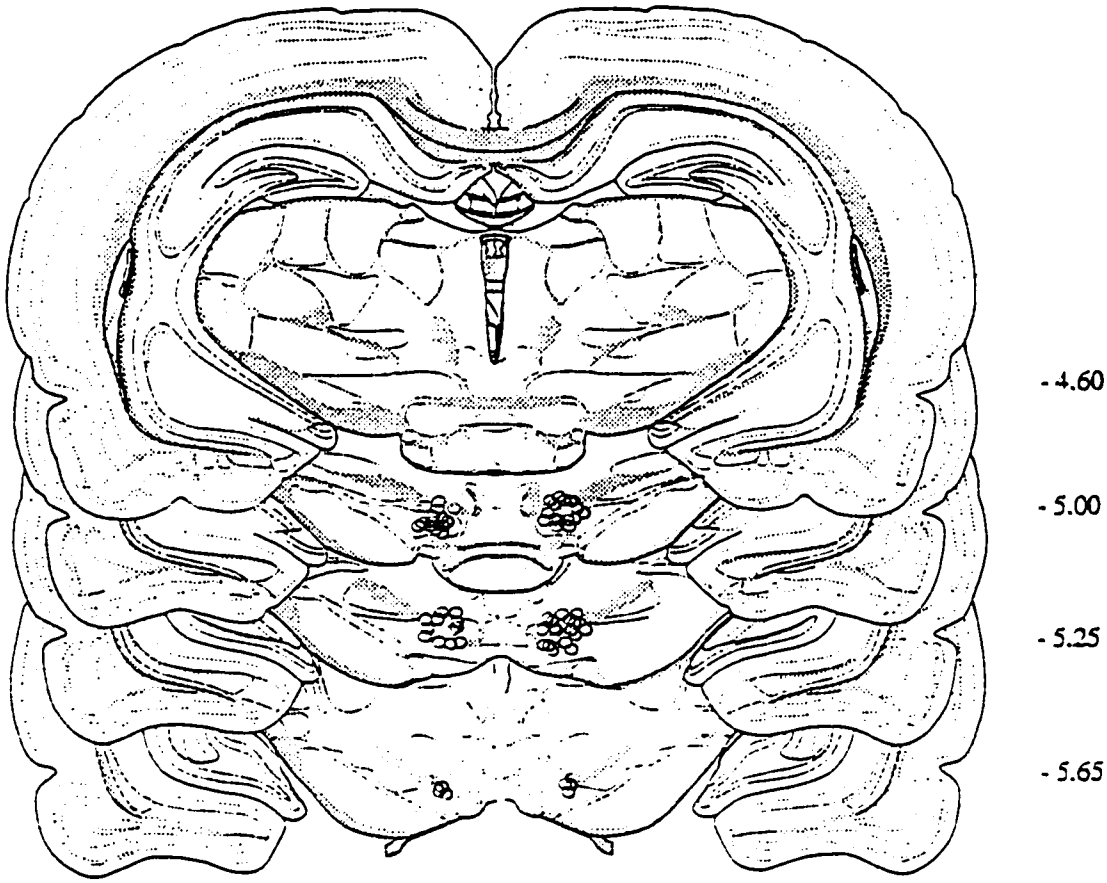


Figure 20. Location of the internal injector cannulae tips of all rats tested in the tail-flick test following intra-VTA infusions of tachykinin agonists or saline (n = 35/side). Drawings (adapted from Adobe Illustrator 5.5 for Windows) are from the atlas by Swanson (1992). Values on the right represent distance in mm caudal from bregma.

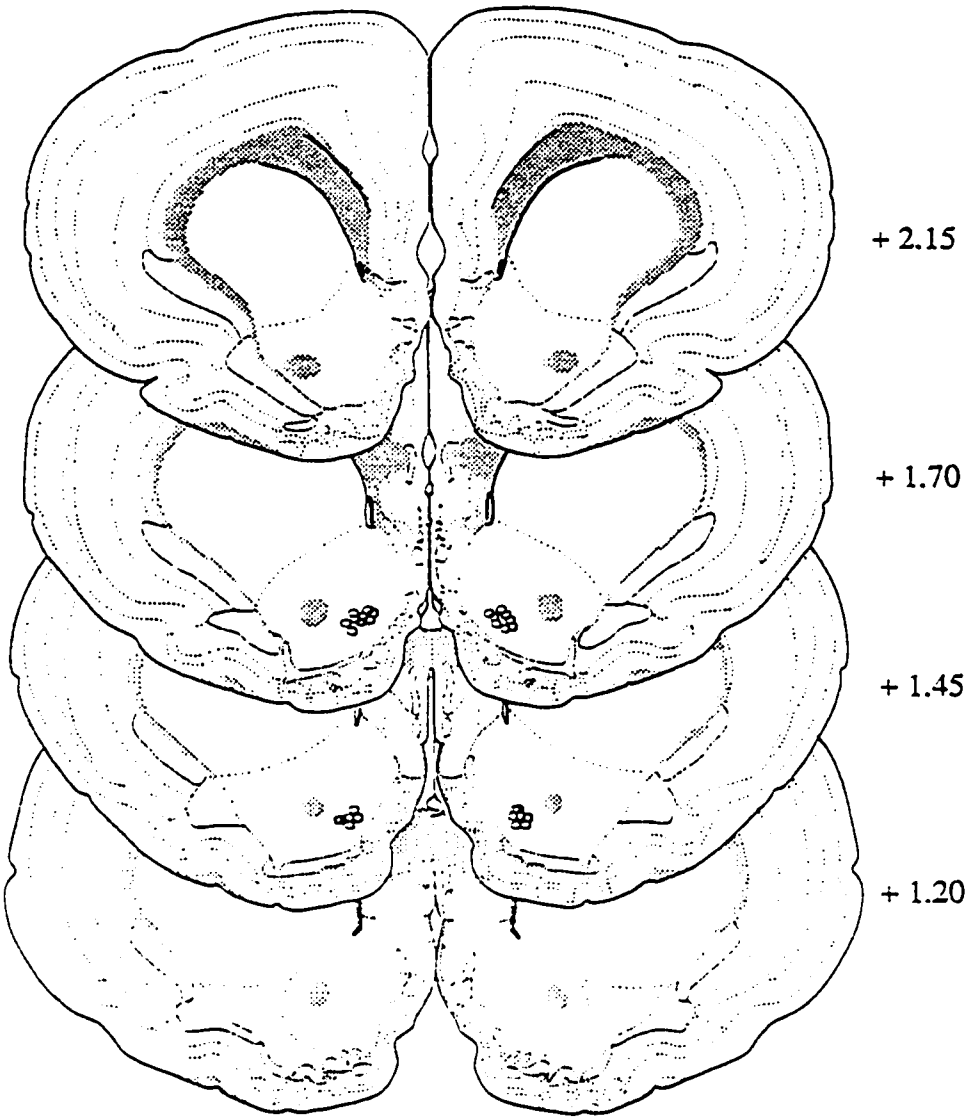


Figure 21. Location of the internal injector cannulae tips of all rats tested in the tail-flick test following intra-NAS infusions of tachykinin agonists or saline (n = 15/side). Drawings (adapted from Adobe Illustrator 5.5 for Windows) are from the atlas by Swanson (1992). Values on the right represent distance in mm rostral from bregma.

analgesia in the formalin, but not the tail-flick test parallel previous observations using intra-VTA DiMe-C7 and intra-NAS amphetamine (Altier and Stewart, 1993). They are also in accordance with the findings of Morgan and Franklin (1990), whose findings indicate that midbrain ascending DA systems mediate the analgesic effects of systemic morphine and amphetamine in the formalin, but not the tail-flick test.

In the course of investigating the effects of identical manipulations of midbrain ascending DA neurons in the formalin and tail-flick pain tests, it was also found in the present study that intra-VTA infusions of morphine, using a dose (3.0 μg/0.5 μl/side) previously reported to produce analgesia in the formalin test (Morgan, 1990; also see results of Experiments 6 and 7), were without effects on tail-flick latencies. Similar findings come from a study by Moreau et al. (1985), who reported that intra-VTA infusions of morphine have no effect on phasic pain, as determined by responses to footshock.

A role of the midbrain in the modulation of phasic pain has been reported previously. Indeed, Baumeister et al. (1988) reported that infusions of the opioid receptor antagonist, naloxone, into the substantia nigra, but not VTA, attenuate morphine analgesia in the tail-flick test. However, the highly lipophilic nature of naloxone and the consequent rapid spread through tissue make the data difficult to interpret. There are also some reports on the effects of opioids infused into the NAS in the tail-flick test. More specifically, it has been reported that intra-NAS infusions of morphine have no effect on tail-flick latencies (Tseng & Wang, 1992), although there is one report of analgesia in the tail-flick test following infusions of a very high dose of morphine (22.5 μg/2μl) bilaterally at this site (Dill & Costa, 1977).

In summary, the findings that infusions of tachykinin agonists or morphine (VTA only) into either the VTA or NAS produce different effects in the formalin and tail-flick tests reinforce the idea that the neural substrates underlying the inhibition of tonic pain are fundamentally different from those underlying the inhibition of phasic pain. A comparison of the neural systems that are involved in mediating the inhibition of tonic versus phasic pain will be presented in the General Discussion.

EXPERIMENT 5

Effect of Intra-VTA Infusions of the Selective NK-1 Receptor Antagonist, RP 67580, on Footshock Stress-Induced Analgesia in the Formalin Test

As described previously, exposure to a wide variety of stressors such as footshock, restraint, conditioned fear, and environmental novelty, has been shown to inhibit the responses to several noxious painful stimuli including application of heat to the tail (e.g., Cannon et al., 1983; Grugan et al., 1985; Lewis et al., 1983) or paws (e.g., Amir & Amit, 1979; Blair et al., 1992), and injections of formalin into a paw (e.g., Abbott et al., 1986; Fanselow, 1984; Fanselow & Baackes, 1982; Vaccarino et al., 1992). Interestingly, as mentioned before, exposure to stressors that induce analgesia has been shown, in different studies, to selectively activate DA transmission in midbrain ascending neurons (see Deutch & Roth, 1990, for review), and there is evidence that this effect is dependant upon opioid mechanisms in the VTA (Kalivas & Abhold, 1987). More specifically, it has been reported that blockade of opioid receptors in the VTA attenuates the stress-induced activation of midbrain DA ascending neurons and that exposure to footshock stress causes the release of met-enkephalin in the VTA (Kalivas & Abhold, 1987). The evidence that opioids in the VTA play a role in the stress-induced activation of midbrain DA neurons is further supported by the findings that intra-VTA infusions of mu opioid agonists stimulate DA-dependant locomotor activity and enhance DA metabolism from the terminals of these neurons (Kalivas et al., 1983; Kalivas & Richardson-Carlson, 1986; Latimer et al., 1987; Stinus et al., 1980). Of interest are the findings that infusions of morphine into the VTA inhibit tonic pain in

the formalin test (Manning et al., 1994; Morgan, 1990) , and that opioid receptors in the VTA mediate stress-induced analgesia in this test (Altier & Stewart, 1996).

There are several reasons to believe that SP acting in the VTA might similarly play an important role in stress-induced analgesia in the formalin test. First, as reviewed previously, there is considerable neuroanatomical, behavioural, and biochemical evidence indicating that SP interacts with midbrain ascending DA neurons in an excitatory way (Cador et al., 1989; Deutch et al., 1985; Eison et al., 1982 a,b; Kelley et al., 1979; Stinus et al., 1978; Tamiya et al., 1990). Second, several findings indicate that SP release in the VTA plays a critical role in the stress-induced activation of midbrain ascending DA neurons. More specifically, it has been reported that pretreatment with a monoclonal SP antibody infused into the VTA completely antagonizes the stress-induced biochemical activation of midbrain DA neurons (Bannon et al., 1983). In addition, the results of several studies indicate that exposure to stress causes the release of SP in the VTA (Bannon et al., 1986; Deutch et al., 1985; Lisoprawski et al., 1981).

Thus, the evidence that exposure to stress both inhibits formalin pain responses and causes SP-mediated activation of midbrain DA neurons and that analgesia in the formalin test is induced by the activation of these neurons suggests that stimulation of SP receptors in the VTA might be a mechanism underlying stress-induced analgesia in the formalin test. Support for this hypothesis is provided by the findings that intra-VTA infusions of DiMe-C7 (Altier & Stewart, 1993) and tachykinin selective NK-1 and NK-3 agonists (Experiment 1) induce analgesia in the formalin test. The purpose of the following experiments was therefore to explore the role of SP in the VTA on stress-induced analgesia in the formalin test. This was accomplished by examining the effect of intra-VTA pretreatment with a SP receptor antagonist on stress-induced analgesia in

this test. Because SP binds preferentially to NK-1 tachykinin receptors and the NK-1 selective agonist, GR-73632, induces more potent analgesa in the formalin test than the NK-3 selective agonist, senktide, a selective NK-1 antagonist was sought to examine the role of midbrain SP in stress-induced analgesia.

Although many SP receptor antagonists are available (Snider et al., 1991), several findings suggest that their use has little heuristic value. For instance, their affinity for the SP (NK-1) receptor is low and they are metabolically unstable (Snider et al., 1991). In recent years, a number of nonpeptide NK-1 receptor antagonists have been developed (Watling, 1991). CP 96345 was the first NK-1 receptor antagonist discovered (Snider et al., 1991) and has been shown to block many physiological and behavioural effects of SP (see Watling, 1991, for review). This nonpeptide NK-1 antagonist, however, displays greater affinity for the NK-1 receptor in bovines, guinea pigs, and humans than in mice and rats (Snider et al., 1991; Beresford et al., 1991; Fardin et al., 1993), which would not be useful to the present study using rats. More recently, a novel nonpeptide NK-1 receptor antagonist, RP 67580, has been synthesized which binds with higher affinity to the rodent than the dog, guinea pig, and human NK-1 receptor (Fardin et al., 1993; Garret et al., 1991). This compound has been reported to effectively antagonize SP-induced physiological and behavioural effects (Culman et al., 1995; Watling et al., 1991). The present experiment therefore employed RP 67580 and its inactive enantiomer, RP 68651, to explore the role of midbrain SP in stress-induced analgesia in the rat. RP 67580 was also used because it is the only NK-1 receptor antagonist, to our knowledge, that can be dissolved in saline for intracranial infusions.

Because footshock was used as a stressor to demonstrate the role of SP in the stress-induced activation of midbrain DA neurons (Bannon et al., 1983), the same

stressor was employed in the present experiments to induce analgesia. The parameters of footshock-stress used here (three 1-sec, 1-mA shocks at 20-sec intervals) were similar to those used by Fanselow (1984; three 0.75-sec, 1-mA shocks at 20-sec intervals), who found that these parameters cause potent analgesia in the formalin test.

Method

Apparatus

Footshock stress was conducted in 42 x 39 x 33.5 cm hexagonal chambers. The contours were constructed with clear Plexiglas, the removable top with wood, and the floor with 22 stainless steel rods set 1.5 cm apart. Each rod was connected to a shock generator (Lafayette Instruments, Lafayette, Indiana). Because these chambers were also used to observe formalin-induced pain responses, a mirror was mounted behind the rear panel to allow a clear view of the rats' paws. From the time between the formalin injection and testing, rats were kept individually in buckets lined with beta chip.

Surgery

21 mm long, 22 gauge guide cannulae were implanted, bilaterally, 1.0 mm above the VTA at the following coordinates : - 5.7 caudal to bregma, + 0.6 mm lateral from midline, and - 7.2 ventral from the dura mater (Paxinos & Watson, 1986). The stereotaxic arms were angled at 15 degrees and the skull was level between lambda and bregma (i.e. flat skull position).

Drugs

The novel nonpeptide NK-1 receptor antagonist, RP 67580, and its inactive enantiomer, RP 68651, were kind gifts from Dr. C. Garret (Rhône-Poulenc Rorer,

France). RP 67580, 2-[1-imino-2-(2 methoxy phenyl) ethyl]-7,7 diphenyl-4 perhydroisoindolone (3aR,7aR), and RP 68651, (3aS,7aS), were dissolved in saline and infused bilaterally into the VTA using a dose of 3.0 μg/0.5 μl/side, in each case.

Design and procedure

On the test day, rats received a formalin injection into one hind paw followed 15 minutes later by intra-VTA infusions of either RP 67580 (3.0 μg/0.5 μl/side), RP 68651 (3.0 μg/0.5 μl/side), or saline. Immediately following the intracranial infusions, rats were placed in the footshock chambers and, after a 1-min adaptation period, were exposed to either footshock (three-1 sec, 1-mA shocks at 20-sec intervals) or no footshock. Pain responses were recorded continuously for 38 minutes immediately following the third shock. Thus, rats were tested in a counterbalanced between-subjects design and were randomly assigned to one of four groups : No Footshock + Saline; Footshock + RP 67580; Footshock + RP 68651; Footshock + Saline. The VTA infusions were timed so that their effects on SIA could occur during the late stable pain phase of the formalin test, starting 20 minutes after the formalin injection.

Statistical Analyses

Data were analyzed by a two-way ANOVA with Treatment (4 levels; see result section for details) as the between-subjects variable and Time (5 post-stress time points) as the within-subjects variable. Because the effects of footshock stress and the antagonist occured within the first 15 minutes, only the first 5 post-stress time-points were included in the analysis. All analyses were followed, if appropriate, by Tukey's post-hoc test for overall differences between conditions. Tests for simple main effects were used, when appropriate, to analyze the differences at each time point between a control and treatment condition. Data from an animal were included in the analyses only if the injector tips were located within 0.5 mm of the target area.

Results

Figure 22 shows the effect of intra-VTA RP 67580 (3.0 μg/0.5 μl/side) or RP 68651 (3.0 μg/0.5 μl/side) pretreatment on footshock stress-induced analgesia. The ANOVA revealed a significant effect of Treatment, $F(3, 17) = 8.19$, $P < 0.005$. Post hoc tests showed that the pain scores of condition No Footshock + Saline (open circles) differed significantly from those of condition Footshock + Saline (closed triangles; $P < 0.05$), indicating that exposure to footshock stress induced significant analgesia. Intra-VTA RP-67580 pretreatment significantly reversed the analgesic effect induced by exposure to footshock stress. There were no significant differences in pain scores between groups No Footshock + Saline (open circles) and Footshock + RP 67580 (closed circles), nor between groups Footshock + RP 68651 (closed squares) and Footshock + Saline (closed triangles), $Ps > 0.05$.

Figure 23 illustrates the location of the internal injector cannulae tips of all rats tested in this experiment. As shown, 21 rats had their tips within the limits of the VTA. The data for one rat were discarded because one of its tip was outside the limits of this site.

Discussion

It was found in the present study that pretreatment with the SP receptor antagonist, RP 67580, infused directly into the VTA completely prevented footshock stress-induced analgesia in the formalin test for tonic pain. In contrast, intra-VTA pretreatment with the inactive enantiomer, RP 68651, had no effect on footshock stress-

induced analgesia in this test. These findings indicate that the stimulation of SP receptors in the VTA plays a critical role in mediating the stress-induced inhibition of tonic pain. These results are in accordance with those reported previously showing that infusions into the VTA of the tachykinin agonists DiMe-C7 (Altier & Stewart, 1993), GR-73632, and senktide (Experiment 1), elicit analgesia in the formalin test. They are also consistent with the findings of previous studies indicating that exposure to stress causes the release of SP in the VTA (Bannon et al., 1986; Deutch et al., 1985; Lisoprawski et al., 1981) and that stimulation of SP receptors at this site mediates the biochemical activation of midbrain ascending DA neurons induced by exposure to footshock stress (Bannon et al., 1983).

Because the SP receptor antagonist RP 67580 binds with high selectivity to the NK-1 tachykinin receptor subtype, the present finding that this compound reverses stress-induced analgesia in the formalin test indicates that this effect is mediated by the blockade of NK-1 tachykinin receptors in the VTA. Using procedures similar to those described in the present experiment, a previous study (Altier, 1993) has examined the effects on stress-induced analgesia of another nonpeptide antagonist which binds with high selectivity to the NK-1 receptor in rat tissue, WIN 51708 (Appell et al., 1992) following infusions into the VTA. Unlike with RP 67580, however, WIN 51708 had no effect on stress-induced analgesia. The different effects on stress-induced analgesia induced by these two nonpeptide NK-1 antagonists probably reflects the fact that WIN 51708, due to its poor solubility in a non-toxic vehicle, was applied to the VTA in crystalline form packed into internal injector cannulae, whereas RP 67580 was dissolved in saline and infused at this site. One potential problem with applying a compound in its crystal form intracranially is that it does not guarantee that it successfully interacted with brain tissue, thereby limiting interpretations of effects observed. The fact that RP 67580 is soluble in non-toxic vehicles and, as found in the

present experiment, prevents stress-induced analgesia following intra-VTA infusions suggests that it is a useful tool to study the physiological and behavioural effects of SP, and more specifically of NK-1 tachykinin receptors, following intracranial infusions.

The finding that the selective NK-3 receptor agonist, senktide, infused into the VTA induces analgesia in the formalin test (Experiment 1) suggests that NK-3 receptors at this site might similarly play a role in stress-induced analgesia, although to a lesser extent than NK-1 receptors given that senktide caused less potent analgesia than the NK-1 selective agonist, GR-73632. A previous study (Altier, 1993) examined the effects of intra-VTA pretreatment with the selective NK-3 receptor antagonist, R-486 (Drapeau et al., 1990; Regoli et al., 1991, 1993 a, b), on stress-induced analgesia in the formalin test, but no effects were found. As with WIN 51708, however, this NK-3 antagonist was applied in crystal form in the VTA because it could not be dissolved for intracranial use. The latter experiment therefore needs to be re-evaluated using a nonpeptide NK-3 selective receptor antagonist that can be dissolved in non-toxic vehicles for intracranial infusions which, to our knowledge, has not yet been developed.

Exposure to stress has been shown to cause the biochemical activation of the DA neurons innervating the prefrontal cortex and, to a lesser extent, the NAS (see Deutch & Roth, 1990, for review). Using footshock parameters that activate mesocortical neurons only, it has also been found that SP receptors in the VTA mediate this stress-induced biochemical response (Bannon et al., 1983). These findings, combined with those of the present study indicating that the stimulation of SP receptors in the VTA plays a role in stress-induced analgesia, suggest that exposure to stress induces analgesia by activating the DA neurons projecting to the prefrontal cortex and/or the NAS. There is some evidence from this laboratory, however, suggesting that DA release from terminals in the NAS, but not the prefrontal cortex, is responsible for the

stress-induced analgesia observed in the present experiment. More specifically, it was found that, as mentioned previously, infusions of amphetamine, which cause DA release and re-uptake blockade, into the NAS, but not the prefrontal cortex, induce analgesia in the formalin test (Altier & Stewart, 1993). Additional evidence in support of this idea is provided by the findings obtained in Experiment 6.

In summary, the results of the present experiment indicate that pretreatment with the novel selective NK-1 receptor antagonist, RP 67580, infused into the VTA completely prevented the analgesic effects induced by exposure to footshock stress in the formalin test for tonic pain. This finding suggests that a critical neurochemical mechanism underlying the stress-induced inhibition of tonic pain is the stimulation of tachykinin NK-1 receptors in the VTA by SP, released from terminals of neurons most likely originating from the habenula (Lisoprawski et al., 1981). In addition, there is some evidence to suggest that the SP-mediated stress-induced inhibition of tonic pain is due to the activation of the DA neurons innervating the NAS.

EXPERIMENTS 6 AND 7

Effects of Blocking DA Receptors in the NAS or Decreasing DA Release in Midbrain Ascending Neurons on SP-, Morphine, and Amphetamine-Induced Analgesia in the Formalin Test

As mentioned previously, morphine and amphetamine administered systemically induce analgesia in the formalin test (Morgan & Franklin, 1990, 1991; Skaburskis, 1980) and appear to exert this effect by interacting with midbrain ascending DA neurons (Morgan & Franklin, 1990). Similarly, the neuropeptide SP induces analgesia in the formalin test for tonic pain when it acts in either the VTA or NAS, as revealed in Experiments 1 and 2 (also see Altier & Stewart, 1993). There are several reasons to believe that SP, morphine, and amphetamine induce analgesia in the formalin test by enhancing the release of DA from terminals in the NAS of midbrain ascending neurons.

In the case of SP, there is considerable evidence to suggest that it interacts in an excitatory way with midbrain ascending DA neurons projecting to the NAS (see Kalivas, 1985, for review). For instance, as reviewed before in more detail, infusions of SP or its analog, DiMe-C7, into the VTA enhance DA metabolism in the NAS (Cador et al., 1989; Deutch et al., 1985a; Elliott et al., 1981) and increase DA-dependent locomotor activity (e.g., Kelley et al., 1979). Likewise, peripherally-administered SP or DiMe-C7 increase extracelluler levels of DA in the NAS, as assessed by *in vivo* microdialysis (Boix et al., 1992 a, b). Similarly, as mentioned before, there is some evidence to suggest that SP acting in the NAS enhances DA transmission at this site.

There is also a substantial amount of evidence indicating that morphine and other mu receptor agonists act to increase DA release in the NAS. For instance, DA metabolism in the NAS is increased following intra-VTA infusions of opioids (Kalivas et al., 1983; Kalivas & Richardson-Carlson, 1986; Latimer et al., 1987), as is extracellular *in vivo* DA following systemic or intraveneous administration of morphine and other mu agonists (Di Chiara & Imperato, 1988; Pontieri et al., 1995; Spanagel et al., 1990). Likewise, intra-VTA infusions of mu agonists stimulate locomotor activity (Joyce et al., 1981; Kalivas et al., 1986; Latimer et al., 1987) and this effect is blocked by either intra-NAS DA antagonists or lesions made to the NAS (Kalivas et al., 1983; Stinus et al., 1980). Numerous studies have also reported that opioids are rewarding (e.g., Devine & Wise, 1994; Phillips & LePiane, 1980, 1982; Stewart, 1984) and that a primary event underlying this effect is the activation of midbrain DA neurons projecting to the NAS. For instance, systemic or intra-VTA administration of mu agonists induce conditioned place preference (Bals-Kubic et al., 1993; Bozarth, 1987; Phillips & LePiane, 1980; Shippenberg et al., 1993), an effect which is abolished by electrolytic or 6-hydroxydopamine lesions of the NAS (Kelsey et al., 1989; Shippenberg et al., 1993).

Finally, numerous studies indicate that amphetamine elevates extracellular levels of DA in the NAS, as revealed by *in vivo* microdialysis (Carboni et al., 1989; Di Chiara & Imperato, 1988; Kuczenski & Segal, 1989; Pontieri et al., 1995; Sharp et al, 1987). Similarly, several studies have shown that amphetamine stimulates locomotor activity (e.g., Kelly & Iversen, 1976) and is rewarding (e.g., Mackey & Van der Kooy, 1985; Kuczenski, 1977; Yokel & Wise, 1975), and that an important mechanism underlying these behavioral responses is enhanced DA release into the NAS. For instance, amphetamine-induced hyperactivity and self-administration are greatly reduced by 6-hydroxydopamine lesions of the NAS (Kelly & Iversen, 1976; Kelly et al., 1975; Lyness et al., 1979) and by microinfusions of DA receptor antagonists directly into the

NAS (Boss et al.; Phillips et al., 1983; Pijnenberg et al., 1975 a, b). It has also been shown that microinfusions of amphetamine directly into the NAS induce conditioned place preference and self-administration (Carr & White, 1983; Hoebel et al., 1983; Phillips et al., 1994) and stimulate locomotor activity (Kelly, 1977; Pijnenburg et al., 1976; Staton & Salomon, 1984; Van Ree et al., 1986).

Thus, the evidence that SP, morphine, and amphetamine act to increase DA transmission in the mesolimbic system, that all are effective at attenuating tonic pain (Experiments 1 and 2; Morgan & Franklin, 1990), and that amphetamine-induced DA release in the NAS elicits analgesia in the formalin test (Altier & Stewart, 1993) suggests that the analgesic effects induced by tachykinins, opioids, and amphetamine are due, at least in part, to enhanced DA release from terminals in the NAS. The following experiments were therefore designed to examine the involvement of DA in the NAS on the analgesic effects induced by these compounds in the formalin test. This was accomplished, in Experiment 6, by examining the effect of pretreatment with the DA receptor antagonist, raclopride, on the analgesic effects of induced by infusions of DiMe-C7 or morphine in the VTA and infusions of amphetamine in the NAS. Raclopride is a highly selective DA D-2 receptor antagonist (Kohler et al., 1985; De Paulis et al., 1986; Hall et al., 1986; Ogren et al., 1986) and was used in the present experiments because there is one report indicating that analgesia in the formalin test is induced by a selective DA D-2, but not DA D-1, receptor agonist (Morgan & Franklin, 1991). In the case of intra-VTA DiMe-C7-induced analgesia, the effect of intra-NAS pretreatments with either the highly selective DA D-1 receptor antagonist, SCH 23390 (Christensen et al., 1984), or the mixed DA D-1/D-2 receptor antagonist, flupenthixol, was examined, for comparison. Experiment 7 was designed to examined the effect of reducing DA release in midbrain ascending neurons, by pretreating animals with a low autoreceptor-specific dose of apomorphine, on the analgesic effects induced by intra-

VTA infusions of either DiMe-C7 or morphine, and intra-NAS infusions of amphetamine.

EXPERIMENT 6

Effects of Intra-NAS Pretreatment with Raclopride, SCH 23390, or Flupenthixol on the Analgesic Effects of Intra-VTA DiMe-C7, Intra-VTA Morphine, and Intra-NAS Amphetamine in the Formalin Test

Method

Surgery

Animals receiving a DA receptor antagonist in the NAS followed by intra-VTA morphine or DiMe-C7 were implanted with four cannulae. First, 21 mm long, 22 gauge guide cannulae (Plastics One, Inc.) were implanted, bilaterally, 1.0 mm above the VTA and aimed at the following coordinates : - 5.7 mm posterior to bregma, + 0.6 mm lateral from the midline, and - 7.4 mm ventral from the dura mater (Paxinos & Watson, 1986). The stereotaxic arms were angled laterally at 15 degrees from the perpendicular and the skull was level between lambda and bregma (i.e., flat skull position). Five stainless steel screws were secured to the skull and the VTA cannulae were anchored to the skull with dental acrylic cement applied around them without blocking bregma from view. The incisor bar was then set 5.0 mm above the interaural line and cannulae were angled at 10 degrees from the perpendicular. Nineteen mm long, 22 gauge guide cannulae were then implanted, bilaterally, 1.0 mm above the NAS at the following coordinates: + 3.0 mm anterior from bregma, + 1.4 mm lateral from midline, and - 6.3 mm ventral from the skull surface (Pellegrino et al, 1979). Cannulae were anchored with dental acrylic cement. Four rats that were implanted with four cannulae died within

two days after surgery. This unusual death toll after surgery in our laboratory suggests that these animals may have died from the physical trauma of being implanted with 4 cannulae at once. Animals receiving infusions into the NAS of raclopride followed by amphetamine were implanted with cannulae in the NAS, using the same coordinates stated above.

Drugs

The SP analog, DiMe-C7 (Sigma, St. Louis, MO) was dissolved in acid saline (pH = 6.05) and infused bilaterally into the VTA using a dose of 3.0 µg/0.5 µl/side. Stock volumes (5.0 µl) of this compound and its vehicle were aliquoted into polypropylene vials and frozen at - 70 ° C. Solutions were thawed within 30 minutes of the infusions. Morphine sulphate (BDH Inc., Québec) was dissolved in saline and infused bilaterally into the VTA using a dose of 3.0 µg.0.5 µl/side. D-amphetamine sulfate (Smith Kline Beecham, Oakville, Ontario) was dissolved in saline and infused bilaterally into the NAS using a dose of 2.5 µg/0.5 µl/side. Raclopride (Astra Pharma, Mississauga, Ontario) was dissolved in saline and infused bilaterally into the NAS using a dose of either 1.0, 3.0, or 5.0 µg/0.5 µl/side. SCH 23390 (RBI Inc) was dissolved in saline and infused into the NAS using a dose of 0.1 µg/0.5 µl/side). The effects of intra-NAS pretreatment with higher doses of SCH 23390 on DiMe-C7-induced analgesia were not examined because they induce pronounced motor side-effects. Cis-flupenthixol (Lundbeck, Kobenhaun) was dissolved in saline and infused bilaterally into the NAS using a dose of either 1.5 or 3.0 µg/0.5 µl/side.

Design and Procedure

On the test day, rats received either bilateral intra-VTA infusions of DiMe-C7 (3.0 µg/0.5 µl/side) or morphine (3.0 µg.0.5 µl/side) ot bilateral intra-NAS infusions of amphetamine (2.5 µg/0.5 µl/side). Ten minutes prior to these intracranial infusions, all

rats were pretreated with bilateral intra-NAS infusions of either raclopride (1.0, 3.0, or 5.0 μg/0.5 μl/side), SCH 23390 (0.1 μg/0.5 μl/side; DiMe-C7 only), flupenthixol (1.5 or 3.0 μg/0.5 μl/side; DiMe-C7 only), or saline. Rats received a s.c. injection of 0.05 ml of 2.5 % formalin into the plantar surface of one hind paw immediately following the last intracranial infusions.

Rats assigned to conditions Raclopride-DiMe-C7 and Saline-DiMe-C7 (Figure 24), Raclopride-Morphine and Saline-Morphine (Figure 25), Raclopride-Amphetamine and Saline-Amphetamine (Figure 26), SCH 23390-DiMe-C7 and Saline-DiMe-C7 (Figure 27), and Flupenthixol-DiMe-C7 and Saline-DiMe-C7 (Figure 28) were tested in the formalin test using a counterbalanced within-subjects design. Thus, animals were tested twice in the formalin test, at a 1-week interval. Either the right or left hind paw was injected on successive tests. Rats assigned to all the remaining conditions were tested in the formalin test once, using a between-subjects design.

Statistical Analyses

To verify whether DiMe-C7, morphine, and amphetamine induced significant analgesia and whether pretreatment with the DA receptor antagonists had any effects on its own, separate two-way ANOVAs were conducted with Treatment Group (Vehicle-Drug vs. Antagonist-Vehicle vs. Vehicle-Vehicle) as the between-subjects variable and Time (10 post-infusion time-points) as the within-subjects variable; because most of the effects of the drugs occurred within the first 30 minutes of testing, only the first 10 post-infusions time-points were included in the analyses. All analyses were followed, when appropriate, by Tukey's post-hoc test for overall differences between groups. Unless otherwise specified, to examine the effects of pretreatment with the DA receptor antagonists on the analgesic effects of DiMe-C7, morphine, and amphetamine, two-way ANOVAs were conducted with Pretreatment Condition (Antagonist-Drug versus

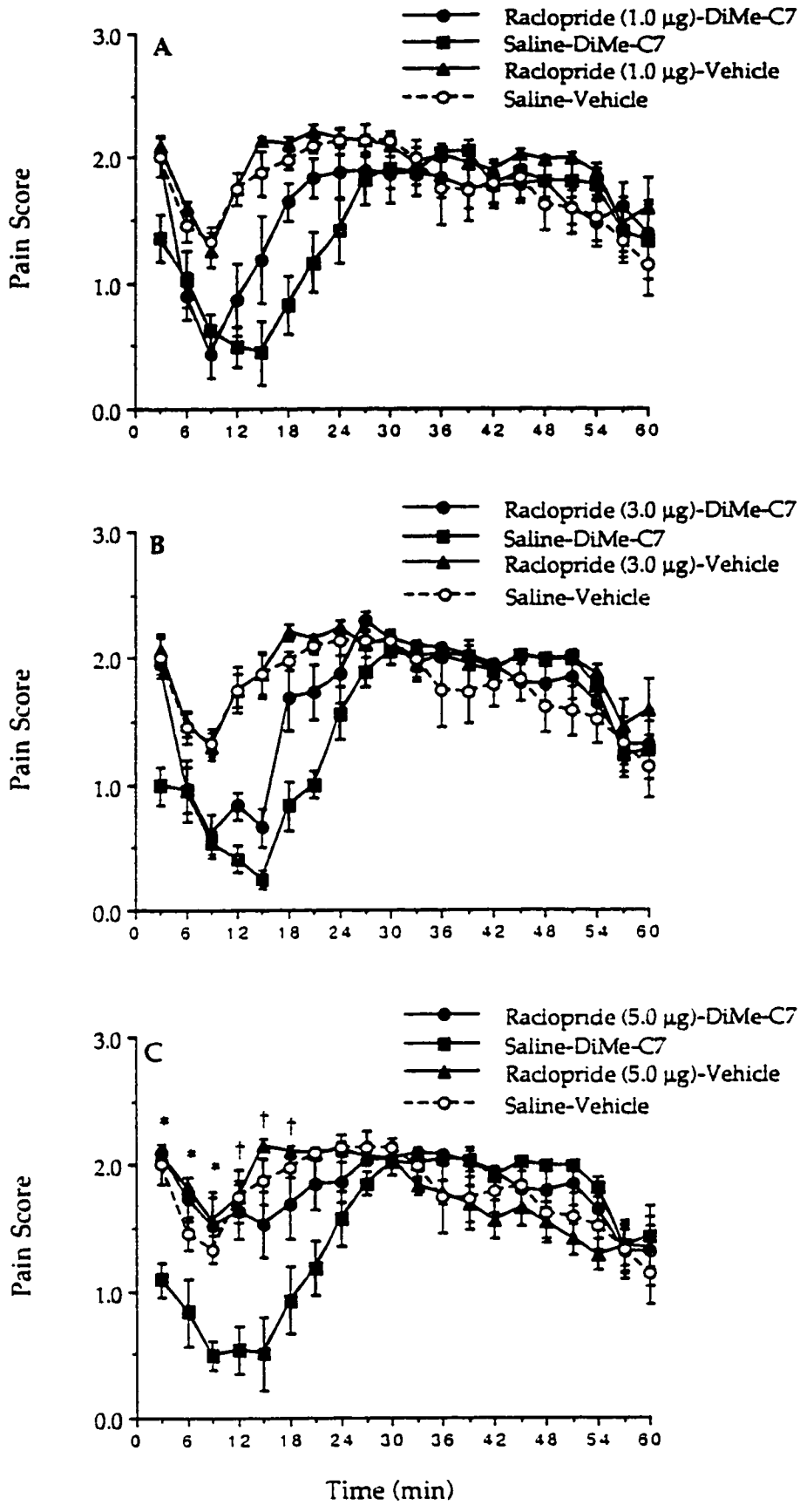
Vehicle-Drug) and Time (10 post-infusion time-points) as within-subjects variables. Tests for simple main effects were used when appropriate to analyze the differences at each time point between the two conditions. Data from an animal were included in the analyses only if the injector tips were located within 0.5 mm of the target area.

Results

Effect of intra-NAS raclopride on DiMe-C7-, morphine-, and amphetamine-induced analgesia

Figures 24 A, B, and C show the effect of intra-NAS pretreatment with escalating doses of raclopride on the analgesic effects of intra-VTA infusions of DiMe-C7 (3.0 μg/0.5 μl/side). The ANOVAs conducted with Treatment Group (Saline-DiMe-C7 vs. Raclopride-Vehicle vs. Saline-Vehicle) and Time variables for each dose of raclopride (see A,B, and C) revealed significant overall Treatment Group effects, $F(2, 18) = 6.05$, $P < 0.01$, $F(2, 17) = 67.72$, $P < 0.0001$, and $F(2, 16) = 37.3$, $P < 0.0001$, respectively. In each case, DiMe-C7 significantly reduced pain scores ($P < 0.05$). A three-way ANOVA with Dose (1.0, 3.0 or 5.0 μg/0.5 μl/side) as the between subjects variable, and both Pretreatment Condition (Raclopride-DiMe-C7 or Saline-DiMe-C7) and Time (10 post-infusion time-points) as within subjects variables revealed a significant main effect of Pretreatment Condition, $F(1, 16) = 17.033$, $P < 0.001$. As shown by comparing conditions Raclopride-DiMe-C7 (closed circles) and Saline-DiMe-C7 (closed squares) in A, B, and C, the analgesic effect induced by DiMe-C7 was significantly blocked by intra-NAS pretreatment with the highest ($P < 0.05$), but not the lowest nor the intermediate dose of raclopride. At the highest dose of raclopride tested, DiMe-C7 analgesia was blocked at all time points during 18 minutes after the formalin injection. As seen by comparing groups Raclopride-Vehicle (closed triangles) and

Figure 24. Mean formalin pain scores (± S.E.M.) following bilateral intra-VTA infusions of the SP analog, DiMe-C7 (3.0 µg/0.5 µl/side), or the vehicle, in animals pretreated with the DA D-2 receptor antagonist, raclopride, infused bilaterally into the NAS using a dose of either A) 1.0 µg/0.5 µl/side, B) 3.0 µg/0.5 µl/side, or C) 5.0 µg/0.5 µl/side. Significant differences between Raclopride-DiMe-C7 and Saline-DiMe-C7 conditions : * $p < 0.005$; † $p < 0.05$. Animals assigned to groups Raclopride-DiMe-C7 and Saline-DiMe-C7 in A) (n = 7), B) (n = 6), and C) (n = 6) were tested in a counterbalanced within-subjects design. Animals assigned to the Raclopride-Vehicle condition in A) (n = 6), B) (n = 6), and C) (n = 5) and the Saline-Vehicle (n = 8) condition were tested in a between-subjects design. Animals in the Saline-Vehicle condition were the same in both A, B, and C.


A
Raclopride (1.0 µg)-DiMe-C7
Saline-DiMe-C7
Raclopride (1.0 µg)-Vehicle
Saline-Vehicle
B
Raclopride (3.0 µg)-DiMe-C7
Saline-DiMe-C7
Raclopride (3.0 µg)-Vehicle
Saline-Vehicle
C
Raclopride (5.0 µg)-DiMe-C7
Saline-DiMe-C7
Raclopride (5.0 µg)-Vehicle
Saline-Vehicle
Pain Score
3.0
2.0
1.0
0.0
0
6
12
18
24
30
36
42
48
54
60
Time (min)

Saline-Vehicle (open circles), raclopride pretreatment alone was without effect on pain scores at any of the doses tested.

The effect of intra-NAS raclopride (5.0 μg/0.5 μl/side) pretreatment on the analgesic effect of intra-VTA morphine is shown in Figure 25. The ANOVA conducted with Treatment Group (Saline-Morphine vs.Saline-Saline vs. Raclopride-Saline) and Time variables revealed a significant overall Treatment Group effect, $F(2, 22) = 46.38$, $P < 0.0001$. As shown by comparing groups Saline-Morphine (closed squares) and Saline-Saline (open circles), intra-VTA infusions of morphine significantly reduced pain scores for approximately 30 minutes after the formalin injection ($P < 0.05$). The ANOVA conducted with Pretreatment Condition (Raclopride-Morphine vs. Saline-Morphine) and Time variables revealed a significant effect of Pretreatment Condition, $F(1, 7) = 41.65$, $P < 0.0005$, indicating that the analgesic effect of morphine was significantly blocked by raclopride pretreatment. Simple main effects tests indicated that the pain scores between Raclopride-Morphine and Saline-Morphine Pretreatment Conditions were significantly different at all time points during 27 minutes following the formalin injection, except at time point 6. As shown by comparing groups Raclopride-Saline (closed triangles) and Saline-Saline (open circles), raclopride pretreatment appeared to induce mild hyperalgesia during the first few minutes following the formalin injection. This effect, however, was not statistically significant.

Figure 26 shows the effect of pretreatment with raclopride (5.0 μg/0.5 μl/side) infused into the NAS on the analgesic effects induced by amphetamine (2.5 μg/0.5 μl/side) infused at the same site. The ANOVA yielded a significant overall effect of Treatment Group, $F(2, 14) = 29.15$, $P < 0.0001$. As seen by comparing Saline-Amphetamine (closed squares) and Saline-Saline (open circles) groups, amphetamine infused into the NAS significantly reduced formalin pain scores ($P < 0.05$). The

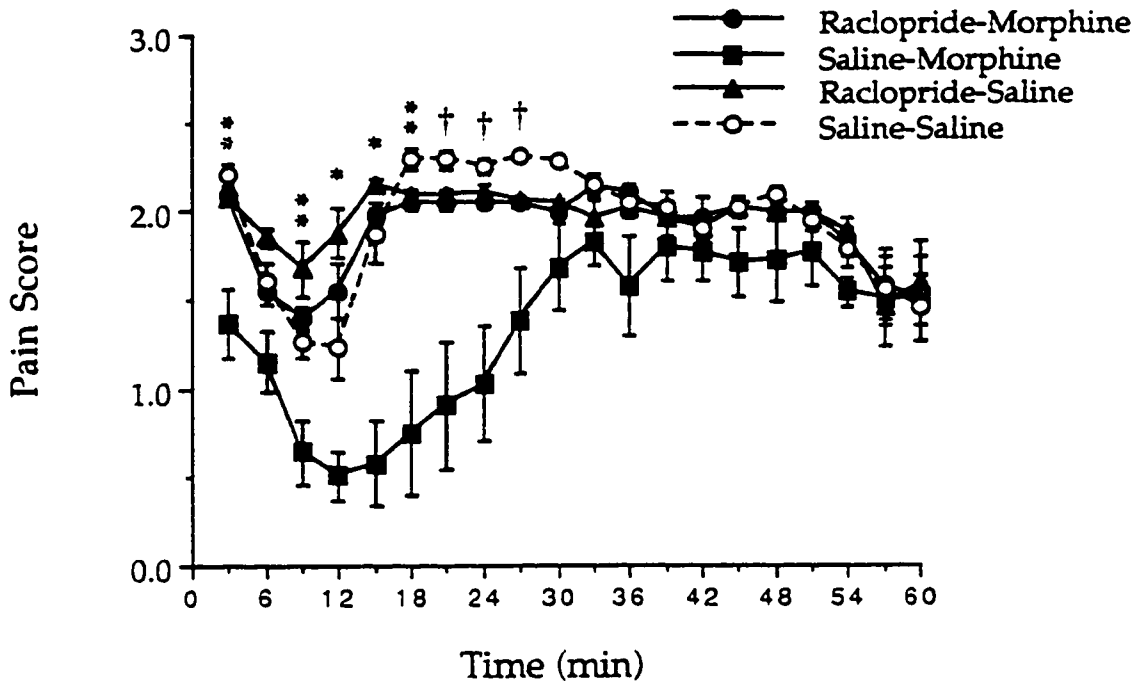


Figure 25. Mean formalin pain scores (± S.E.M.) following bilateral intra-VTA infusions of morphine (3.0 µg/0.5 µl/side), or saline, in animals pretreated with raclopride (5.0 µg/0.5 µl/side), or saline, infused bilaterally into the NAS. Significant differences between Raclopride-Morphine and Saline-Morphine conditions: * $p < 0.001$; ** $p < 0.01$; † $p < 0.05$. Animals ($n = 8$) assigned to the Raclopride-Morphine and Saline-Morphine conditions were tested in a counterbalanced within-subjects design. Separate groups of animals were assigned to conditions Raclopride-Saline ($n = 8$) and Saline-Saline ($n = 9$).

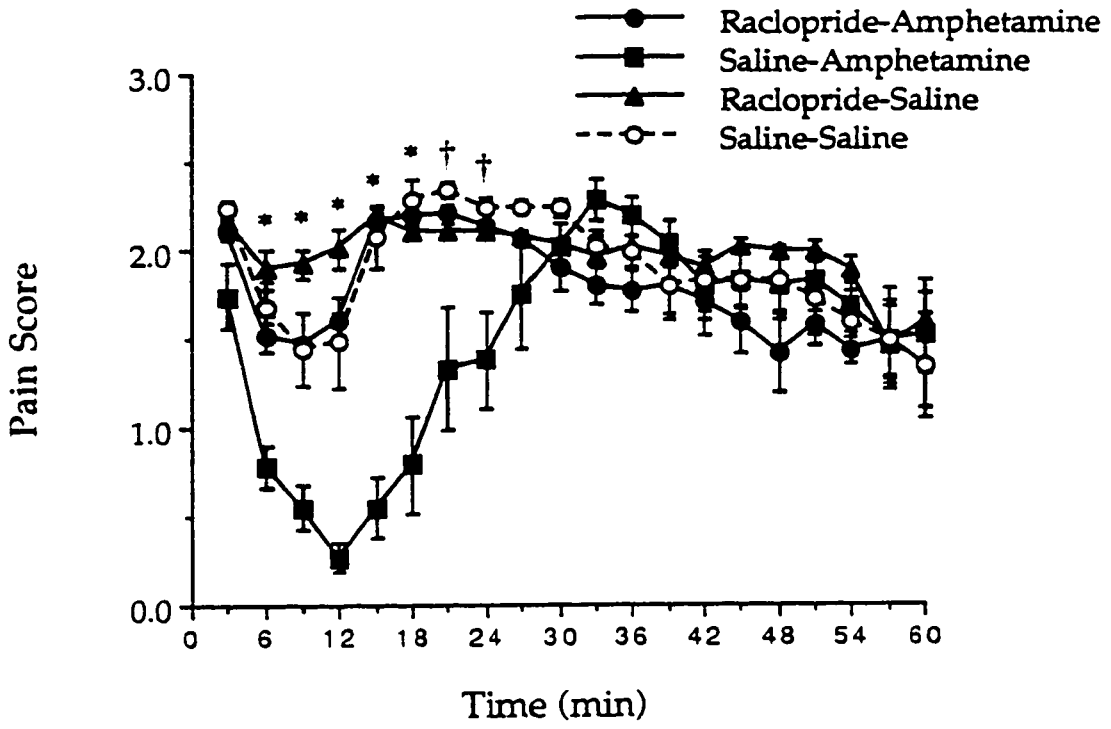


Figure 26. Mean formalin pain scores (± S.E.M.) following bilateral infusions of amphetamine (2.5 µg/0.5 µl/side), or saline, into the NAS in animals pretreated with raclopride (5.0 µg/0.5 µl/side), or saline, infused bilaterally at the same site. Significant differences in pain scores between Raclopride-Amphetamine and Saline-Amphetamine conditions : * $p < 0.001$; † $p < 0.05$. Animals (n = 6) assigned to groups Raclopride-Amphetamine and Saline-Amphetamine were tested in a counterbalanced within-subjects design. Animals assigned to groups Raclopride-Saline (n = 5) and Saline-Saline (n = 6) were tested in a between-subjects design.

ANOVA conducted with Pretreatment Condition (Raclopride-Amphetamine and Saline-Amphetamine) and Time variables revealed a signicant overall effect of Pretreatment Condition, $F(1,5) = 35.56$, $P < 0.005$, indicating that this amphetamine-induced analgesic effect was prevented by intra-NAS raclopride pretreatment. Simple main effects tests indicated that the differences in pain scores between Conditions Raclopride-Amphetamine and Saline-Amphetamine were significant from 6 to 24 minutes following the formalin injection. Raclopride pretreatment followed by intra-NAS saline (closed triangles) appeared to elevate formalin pain scores relative to the Saline-Saline group (open circles), from approximately 9 to 12 minutes following the formalin injection. This hyperalgesic trend, however, was not significant.

Effect of intra-NAS SCH 23390 on DiMe-C7-induced analgesia

Figure 27 shows the effect of intra-NAS pretreatment with the DA D-1 receptor antagonist, SCH 23390 (0.1 µg/0.5 µl/side), on the analgesic effects of intra-VTA DiMe-C7 (3.0 µg/0.5 µl/side). The ANOVA conducted with Treatment Group (Saline-DiMe-C7 vs. SCH 23390-Saline vs. Saline-Vehicle) and Time variables revealed a significant overall effect of Treatment Group, $F(2, 12) = 55.04$, $P < 0.0001$. As shown by comparing groups Saline-DiMe-C7 (closed squares) and Saline-Vehicle (open circles), intra-VTA infusions of DiMe-C7 induced significant analgesia during approximately 30 minutes after the formalin injection ($P < 0.05$). The ANOVA conducted with Pretreatment Condition (SCH 23390-DiMe-C7 and Saline-DiMe-C7) and Time variables revealed a significant effect of Pretreatment Condition, $F(1, 5) = 10.85$, $P < 0.05$. As seen by comparing conditions SCH 23390-DiMe-C7 and Saline-DiMe-C7, the analgesic effect of intra-VTA DiMe-C7 was significantly attenuated, but not completely prevented, by pretreatment with SCH 23390 in the NAS ($P < 0.05$). It can also be seen, by comparing groups SCH 23390-Vehicle (closed triangles) and Saline-Vehicle (open circles), that SCH 23390 pretreatment alone had no effect on pain

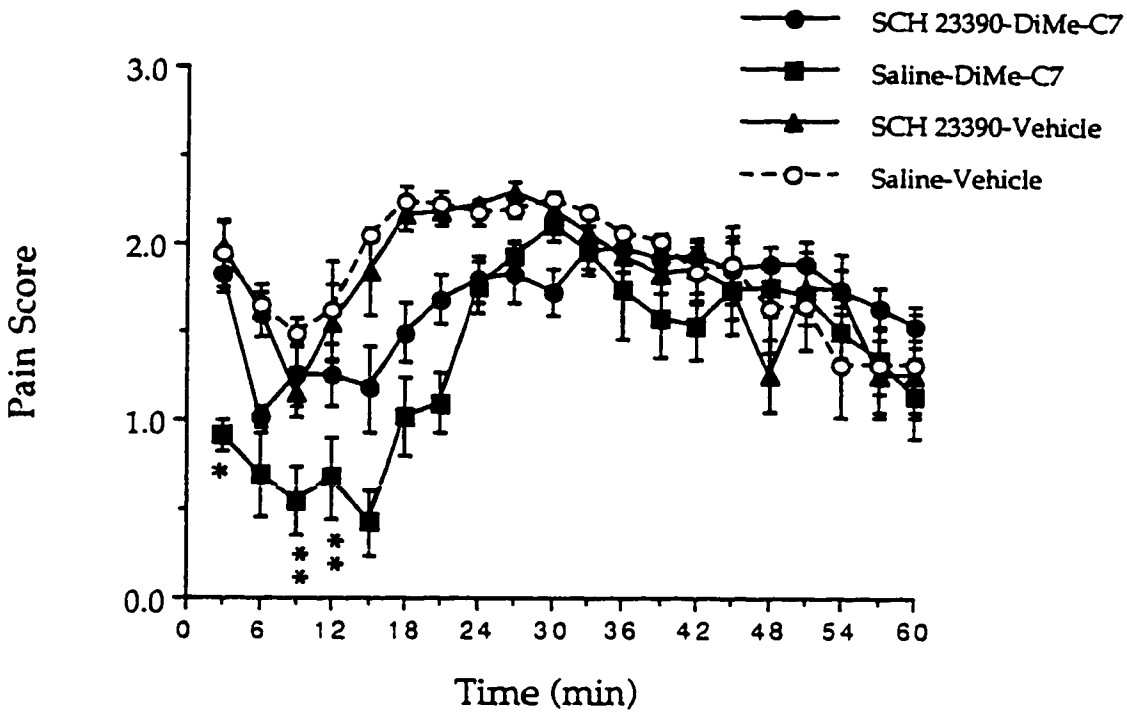


Figure 27. Mean formalin pain scores (± S.E.M.) following bilateral intra-VTA infusions of DiMe-C7 (3.0 µg/0.5 µl/side), or the vehicle, in rats pretreated with the D-1 receptor antagonist, SCH 23390 (1.0 µg/0.5 µl/side), or saline, infused bilaterally into the NAS. Significant differences in pain scores between SCH23390-DiMe-C7 and Saline-DiMe-C7 conditions : * $p < 0.005$; ** $p < 0.05$. Animals (n = 6) assigned to groups SCH 23390-DiMe-C7 and Saline-DiMe-C7 were tested in a counterbalanced within-subjects design. Animals assigned to groups SCH-Vehicle (n = 5) and Saline-Vehicle (n = 5) were tested in a between-subjects design.

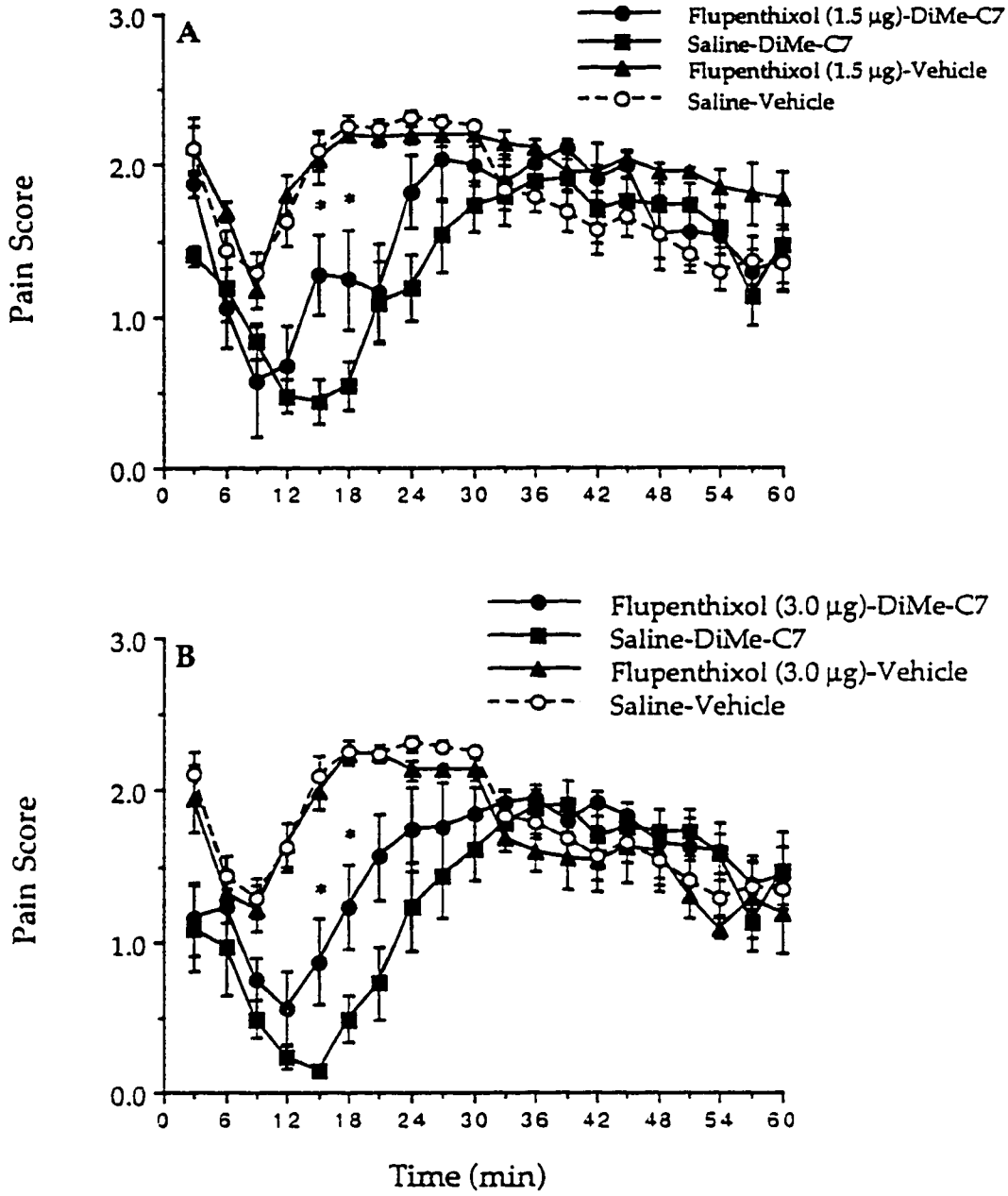
scores. The data for one rat pretreated with SCH 23390 were discarded because the animal was unusually inactive following these infusions of this drug in the NAS.

Effect of intra-NAS flupenthixol on DiMe-C7-induced analgesia

Figure 28 shows the effect of intra-NAS pretreatment with the mixed DA D-1 and D-2 receptor antagonist, flupenthixol (A: 1.5 µg/0.5 µl/side or B: 3.0 µg/0.5 µl/side) on the analgesic effects of DiMe-C7 (3.0 µg/0.5 µl/side) infused into the VTA. Seperate ANOVAs conducted with Treatment Group (Saline-DiMe-C7 vs. Saline-Vehicle vs. Flupenthixol-Vehicle) and Time variables for each dose of flupenthixol (see A and B) revealed significant Treatment Group effects, $F(2, 15) = 60.954$, $P < 0.0001$ and $F(2, 16) = 43.194$, $P < 0.0001$, respectively. As seen by comparing groups Saline-DiMe-C7 (closed squares) and Saline-Vehicle (open circles) in both A and B, intra-VTA DiMe-C7 significantly attenuated pain scores ($Ps < 0.05$) during approximately 30 minutes.

Intra-NAS flupenthixol pretreatment using a dose of either A) 1.5 µg/0.5 µl/side or B) 3.0 µg/0.5 µl/side attenuated the analgesic effect of intra-VTA DiMe-C7 from time points 12 to 21, as seen by comparing conditions Flupenthixol-DiMe-C7 and Saline-DiMe-C7 in both A and B. A three-way ANOVA conducted with Dose (1.5 or 3.0 µg/0.5 µl/side) as the between-subjects variable and both Pretreatment Condition (Flupenthixol-DiMe-C7 vs. Saline-DiMe-C7) and Time (10 post-infusion time points) as within-subjects variables revealed a significant main effect of Pretreatment Condition, $F(1, 9) = 5.64$, $P < 0.05$. Simple main effects tests indicated that pretreatment with either dose of flupenthixol significantly attenuated DiMe-C7-induced analgesia from 15 to 18 minutes following the formalin injection. Finally, as seen by comparing groups Flupenthixol-Vehicle versus Saline-Vehicle in A and B, flupenthixol pretreatment alone had no effect on pain scores.

Figure 28. Mean formalin pain scores (± S.E.M.) following bilateral intra-VTA infusions of DiMe-C7 (3.0 µg/0.5 µl/side), or the vehicle, in rats pretreated with the mixed DA D-1 and D-2 receptor antagonist, flupenthixol, or saline, infused bilaterally into the NAS using a dose of either A) 1.5 µg/0.5 µl/side) or B) 3.0 µg/0.5 µl/side). Significant differences in pain scores between Flupenthixol-DiMe-C7 and Saline-DiMe-C7 : * $p < 0.05$. Animals assigned to conditions Flupenthixol-DiMe-C7 and Saline-DiMe-C7 in A) (n = 5) and B) (n = 6) were tested in a counterbalanced within-subjects design. Separate groups of animals were assigned to conditions Flupenthixol (1.5 µg)-Vehicle (n = 5), Flupenthixol (3.0 µg)-Vehicle (n = 5) and Saline-Vehicle (n = 7). Animals in the Saline-Vehicle condition were the same in both A and B.


A
Flupenthixol (1.5 µg)-DiMe-C7
Saline-DiMe-C7
Flupenthixol (1.5 µg)-Vehicle
Saline-Vehicle
Pain Score
3.0
2.0
1.0
0.0
0
6
12
18
24
30
36
42
48
54
60
B
Flupenthixol (3.0 µg)-DiMe-C7
Saline-DiMe-C7
Flupenthixol (3.0 µg)-Vehicle
Saline-Vehicle
Pain Score
Time (min)

The histological planes presented in Figure 29 illustrate the location of the internal injector cannulae tips within the VTA of all rats that received infusions of either DiMe-C7, morphine, or their respective vehicle. One hundred and fifty rats had their internal injector cannulae tips within the VTA. The data from 11 rats were discarded because their cannulae tips were not within the limits of the VTA.

Figure 30 illustrates the location of the internal injector cannulae tips within the NAS of all rats that were pretreated with a DA antagonist, or saline, at this site as well as rats that received infusions of amphetamine at this site. One hundred and forty seven rats had injector tips in the NAS. The data for seven rats were discarded because the location of their injector tips did not satisfy the criterion for inclusion.

Discussion

The present findings indicate that blockade of DA D-2 receptors in the NAS prevents the analgesic effects induced by SP, morphine, and amphetamine in the formalin test for tonic pain. More specifically, it was found that pretreatment with the DA D-2 selective receptor antagonist, raclopride, infused directly into the NAS blocks the analgesic effects of intra-VTA infusions of either the SP analog, DiMe-C7, or morphine, and of intra-NAS infusions of amphetamine. In the case of DiMe-C7, animals were pretreated with three different doses of raclopride and a dose-dependent attenuation of DiMe-C7 was observed, with the highest dose (i.e., 5.0 μg/0.5 μl/side) resulting in a complete blockade of the effects of DiMe-C7. Likewise, this highest dose of raclopride caused a complete blockade of intra-VTA morphine- and intra-NAS amphetamine-induced analgesia. These findings are in accordance with those of

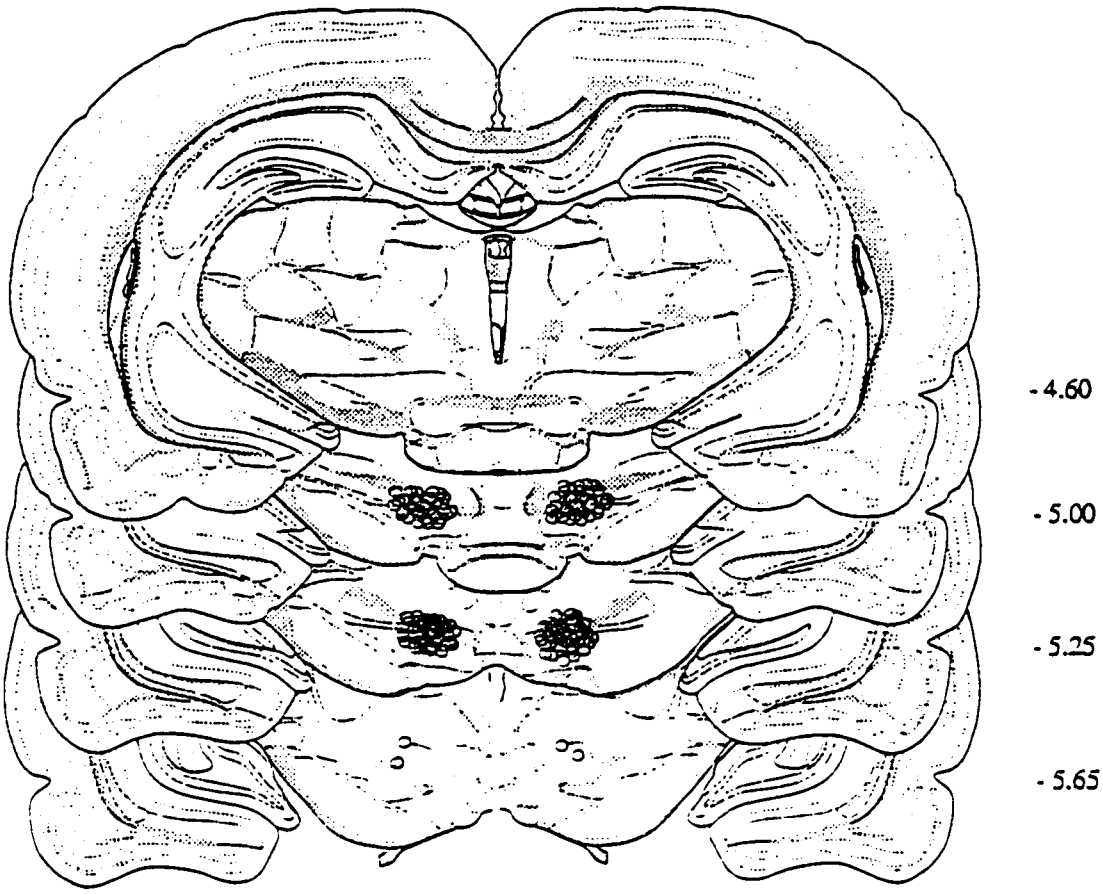


Figure 29. Location of the internal injector cannulae tips in the VTA (n = 150/side) of all rats tested in Experiment 7. Drawings (adapted from Adobe Illustrator 5.5 for Windows) are from the atlas by Swanson (1992). Values on the right represent distance in mm caudal from bregma.

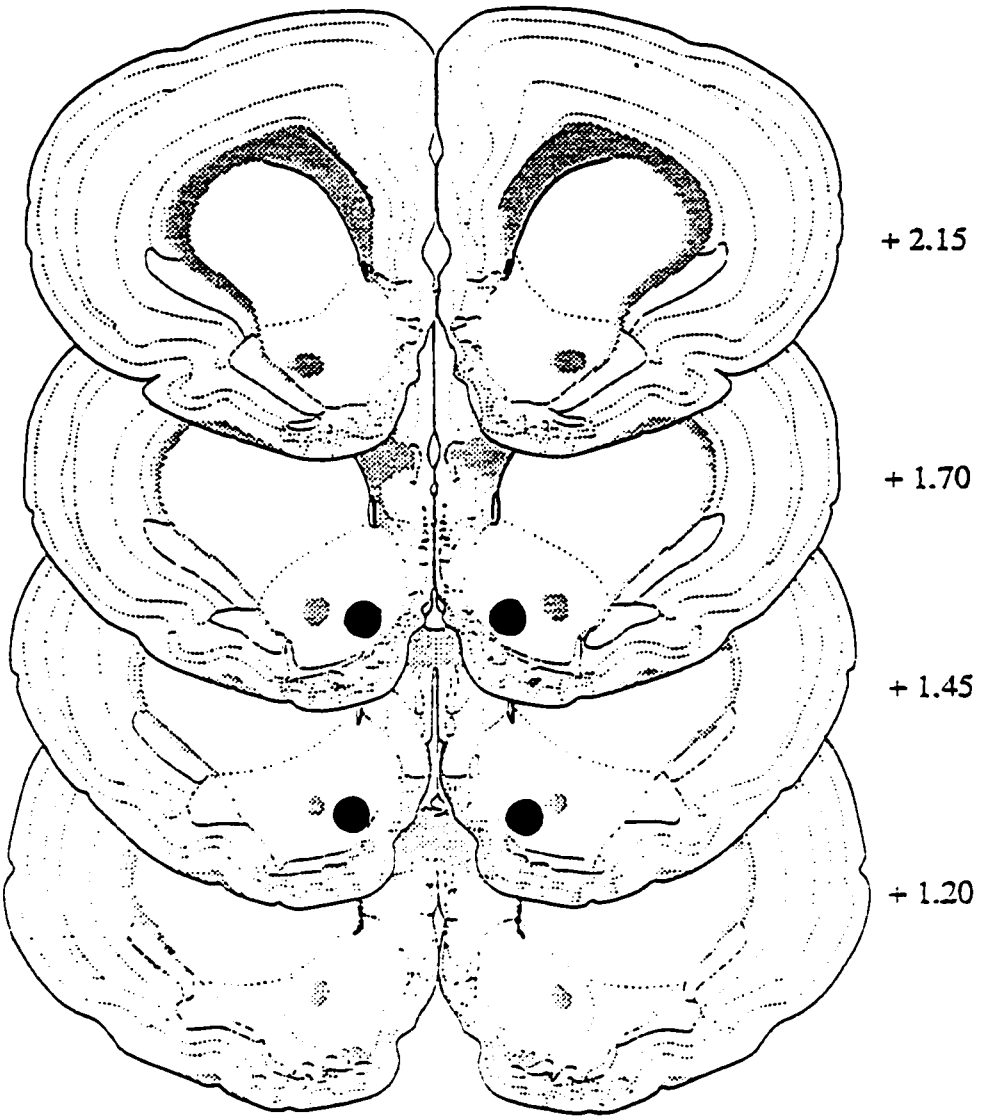


Figure 30. Location of the internal injector cannulae tips in the NAS (n = 147/side) of all rats tested in Experiment 6. Note that the infusion sites were clustered in the shell region of the NAS. Drawings (adapted from Adobe Illustrator 5.5 for Windows) are from the atlas by Swanson (1992). Values on the right represent distance in mm rostral from bregma.

previous studies implicating midbrain ascending DA neurons in the inhibition of tonic pain (Anderson & Rompré, 1996; Altier & Stewart, 1993, 1996; Franklin, 1989; Manning et al., 1994; Morgan & Franklin, 1990) and, more specifically, implicating enhanced DA activity in the NAS in this effect (Clarke & Franklin, 1992).

Previous studies have reported that selective DA D-2 agonists administered systemically induce analgesia in the formalin test (Morgan & Franklin, 1991) and the vocalization after discharge test (Carr, 1984), the latter of which induces pain associated with significant negative affect (Morgan, 1990). It has also been reported that selective DA D-2 receptor antagonists administered systemically attenuate the analgesic effects of systemic amphetamine, morphine, and cocaine in the formalin test (Lin et al., 1989; Morgan & Franklin, 1991; Skaburskis, 1980) and in the vocalization-after discharge test (Carr, 1984; Paalzow & Paalzow, 1975). The present findings are consistent with those of these previous studies implicating DA D-2 receptors in the inhibition of tonic pain and extend their results by showing that the NAS is the neuroanatomical site where these DA receptor subtypes mediate this response. The present findings also extend those of previous studies by showing that DA D-2 receptors in the NAS mediate the analgesic effects of morphine and amphetamine when they are infused directly at sites within the mesolimbic system, namely in the VTA and NAS, respectively. Finally, the present results indicate that, as is the case for morphine and amphetamine, DA D-2 receptors in the NAS also mediate the analgesic effects induced by SP acting in the VTA.

The present findings suggest that a mechanism underlying the inhibition of tonic pain is the stimulation of DA D-2 receptors in the NAS by DA released from terminals of midbrain neurons arising from the VTA. These findings corroborate those of Harris and Aston-Jones (1994), who showed that the DA D-2 receptors in the NAS are

involved in the modulation of aversive somatic withdrawal symptoms which, like tonic pain, are presumably associated with significant negative affect. More specifically, they found that intra-NAS infusions of a selective DA D-2 agonist inhibit naloxone-precipitated somatic withdrawal symptoms, whereas those of a selective DA D-2 receptor antagonist elicit somatic withdrawal symptoms in morphine-dependent rats. The evidence that DA D-2 receptors in the NAS mediate the inhibition of both tonic pain and aversive withdrawal symptoms suggest that analgesia in the formalin test may be due to a greater extent to the alleviation of negative affect rather than to that of the sensory dimension of tonic pain. A discussion of this issue will be presented in the General Discussion.

In the present study, it was also found that pretreatment with the selective DA D-1 receptor antagonist, SCH 23390, infused into the NAS attenuates the analgesic effects induced by the SP analog, DiMe-C7, infused into the VTA. Together with the findings on the effects of raclopride, this finding suggests that SP acting in the VTA inhibits tonic pain by causing the release of DA in the NAS from mesolimbic terminals which in turn stimulates both DA D-1 and DA D-2 receptors at this site. The finding that DA D-1, in addition to DA D-2, receptors in the NAS mediate the analgesic effects induced by SP acting in the VTA agrees with the results of other reports indicating that SCH 23390 administered systemically attenuates the analgesic effects of amphetamine, morphine, and cocaine in the formalin test (Lin et al., 1989; Morgan & Franklin, 1991). It is likely that the NAS is the site where DA D-1 receptors also mediate the analgesic effects of morphine and amphetamine administered either systemically or into the VTA or NAS, respectively, given the similarities reported in the present studies between the effects of DiMe-C7, morphine, and amphetamine on tonic pain, and on the involvement of DA D-2 receptors in the NAS in these effects. This remains, however, to be tested in the future.

Although the antagonism of either DA D-1 or D-2 receptors in the NAS attenuated the analgesic effect induced by intra-VTA DiMe-C7, the DA D-2 receptor antagonist, raclopride, was more effective at attenuating analgesia than the DA D-1 receptor antagonist, SCH 23390. These findings suggest that D-2 receptors may play a more important role than D-1 receptors in the NAS in the mediation of analgesia induced by SP, and perhaps, amphetamine and morphine. This difference between the effects of raclopride and SCH 23390 on analgesia, however, may reflect the fact that the dose of the DA D-1 receptor antagonist, SCH 23390, was not high enough to induce a more potent attenuation of analgesia. Only one dose of SCH 23390 was used to examine its effects on SP analgesia, whereas three doses of the DA D-2 receptor antagonist, raclopride, were employed to explore the same effect. Alternatively, the finding that pretreatment with SCH 23990 induces a less potent attenuation of SP analgesia than that with raclopride could be due to the anti-serotonergic actions of SCH 23390. Indeed, as Morgan and Franklin (1991) pointed out for the purposes of explaining an unexpected potentiation of morphine analgesia by pretreatment with SCH 23390, the latter compound has been documented to bind to 5-HT-2 receptors (Bisehoff et al., 1986), in addition to DA D-1 receptors, and to depress serotonergic activity through an interaction with these receptors (Bijak & Smialowski, 1989; Hicks et al., 1984). As mentioned previously, drugs or lesions that deplete serotonin have been reported to potentiate morphine analgesia (Abbott & Melzack, 1983; Dennis & Melzack, 1979). Thus, although intra-NAS SCH 23390 prevented SP-induced analgesia through the antagonism of DA D-1 receptors, it is possible that this effect was incomplete because the compound also synergised with SP to attenuate tonic pain, through a blockade of 5-HT-2 receptors. Finally, the incomplete attenuation of DiMe-C7-induced analgesia by intra-NAS SCH 23390 may have also been due to the apparent DA-enhancing properties of the DA D-1 antagonist (Imperato & Di Chiara, 1989).

It might be interesting in the future to compare the effects of intra-NAS pretreatment with the DA D-1 receptor antagonist, SCH 23390, on the analgesic effects of VTA DiMe-C7 in the formalin test to those of intra-NAS infusions of a selective DA D-1 agonist in this test. In a previous study, Morgan and Franklin (1991) found that, whereas pretreatment with SCH 23390 administered systemically attenuates the analgesic effects of systemic amphetamine and morphine in the formalin test, the systemic administration of the selective DA D-1 agonist, SKF 38393, was without effect in this test. They suggested that these contradictory findings could be explained by the 'enabling' action of D-1 receptors on D-2 receptors. More specifically, given the evidence that D-1 and D-2 receptors interact with each other in opposing or synergistic ways (Clark & White, 1987), they proposed that , in the case where morphine or amphetamine are administered and induce analgesia by stimulating D-2 receptors, blockade of D-1 receptors inhibits the 'enabling' effect that these receptors have on D-2 receptors, thereby accounting for the attenuation of morphine and amphetamine analgesia. However, in the absence of analgesia induced by the stimulation of D-2 receptors by morphine or amphetamine, administration of a D-1 agonist has no effect on tonic pain. Based on this account, Morgan and Franklin's findings suggest that D-1 and D-2 receptors are involved in morphine and amphetamine analgesia in the formalin test, but that D-1 receptors are involved only in the case where analgesia is induced by D-2 receptor stimulation. Thus, to further explore this idea, it might be worthwhile to examine whether a DA D-1 agonist infused directly into the NAS might similarly be without effects on pain responses in the formalin test.

In the present studies, it was also found that pretreatment with the mixed DA D-1 and D-2 receptor antagonist, flupenthixol, infused into the NAS attenuates the analgesic effects of intra-VTA DiMe-C7. This observation agrees with the findings

reported here indicating that intra-NAS infusions of the selective D-1 receptor antagonist, SCH 23390, or the selective D-2 receptor antagonist, raclopride, attenuate the analgesic effects of intra-VTA DiMe-C7. In addition, this finding is consistent with the results of previous reports indicating that the mixed DA D-1 and D-2 receptor antagonists flupenthixol or chlorpromazine administered systemically attenuate the analgesic effects of systemic amphetamine, morphine, and cocaine in the formalin test (Lin et al., 1989; Morgan & Franklin, 1991) and that mixed DA D-1 and D-2 agonists administered systemically induce analgesia in this test (e.g., Dennis & Melzack, 1983; Lin et al., 1989; Morgan & Franklin, 1991).

In a previous study by Clarke and Franklin (1992), it was found that 6-hydroxydopamine lesions made bilaterally into the NAS attenuate the analgesic effects induced by systemic amphetamine in the formalin test. The present findings concerning the role of DA in the NAS on amphetamine-induced analgesia in the formalin test are consistent with those of Clarke and Franklin and extend their findings by showing that the attenuation of amphetamine-induced analgesia resulting from blockade of DA transmission in the NAS occurs when the psychostimulant is infused directly into the NAS. In contrast to the present results, however, Clarke and Franklin found that 6-hydroxydopamine lesions in the NAS did not reduce the analgesic effects of systemically administered morphine in the formalin test whereas, in the present study, blockade of DA neurotransmission in the NAS prevented the analgesic effects induced by morphine infused into the VTA. The discrepancy between Clarke and Franklin's results and those of the present study regarding the role of DA in the NAS in morphine analgesia probably relates to differences in the effectiveness of the manipulations aimed at blocking DA transmission in the NAS. Indeed, it is likely that the manipulations carried out in Clarke and Franklin's study did not affect morphine-induced analgesia, whereas they did in the present study, because blockade of DA transmission was not

substantial enough in their study, as opposed to the present study. In support of this idea, Clarke and Franklin report that the 6-hydroxydopamine lesions made in the NAS caused a 78.9 % DA depletion at this site. Although this small amount of DA depletion in the NAS was sufficient to attenuate the analgesic effect of systemic amphetamine, that of systemic morphine was not sensitive to this effect. In fact, it is suprising that a 21.1 % DA depletion in the NAS was effective at attenuating amphetamine-induced analgesia, given that at least a 90 % DA depletion in midbrain ascending DA terminal fields is required to observe any behavioural change (e.g., motor deficits). Perhaps this low amount of DA depletion in the NAS was effective at disrupting amphetamine-induced analgesia because amphetamine, although it acts at several sites in the brain, modulates tonic pain by acting to a large extent in the NAS.

Differences in the routes of morphine administration might also account for the discrepancy between Clarke and Franklin's results and those of the present study on the role of DA in the NAS on morphine analgesia. Indeed, it is possible that the manipulations carried out in Clarke and Franklin's study aimed at decreasing DA transmission in the NAS did not block morphine analgesia because the opioid given systemically elicited analgesia by acting at several sites involved in modulating tonic pain. There is evidence that morphine infused at several supraspinal sites other than the VTA, such as the posterior hypothalamic area, periaqueductal gray, and habenula, produces analgesia in the formalin test (Cohen & Melzack, 1995; Manning et al., 1994; Vaccarino & Chorney, 1994). In contrast, in the present study, morphine analgesia may have been prevented successfully by DA receptor antagonism in the NAS because the opioid was infused directly into the VTA, thereby restricting the effect of the opioid on tonic pain to midbrain ascending DA systems.

In summary, the results derived from the present studies indicate that blockade of DA D-2 receptors in the NAS by raclopride prevents the analgesic effects induced by either infusions into the VTA of the SP analog, DiMe-C7, or morphine, or infusions of amphetamine into the NAS. It was also found that intra-NAS infusions of either the DA D-1 receptor antagonist, SCH 23390, or the mixed DA D-1 and D-2 receptor antagonist, flupenthixol, attenuate the analgesic effects of intra-VTA DiMe-C7. Several lines of evidence suggest that DA D-1 receptors in the NAS might similarly be involved in mediating the analgesic effects of intra-VTA morphine and intra-NAS amphetamine. Together, all these findings suggest that a mechanism underlying the inhibition of tonic pain is the stimulation of DA D-1 and D-2 receptors in the NAS by DA released from terminals of mesolimbic neurons.

As a follow-up, Experiment 7 was designed to examine the effect of reducing DA release in midbrain ascending neurons, via pretreatment with a low autoreceptor-specific dose of apomorphine, on the analgesic effects induced by intra-VTA SP, intra-VTA morphine, and intra-NAS amphetamine.

EXPERIMENT 7

Effects of Decreased DA Release in Midbrain Ascending DA Neurons on the Analgesic Effects of Intra-VTA DiMe-C7, Intra-VTA Morphine, and Intra-NAS Amphetamine in the Formalin Test

Method

Surgery

21 mm long, 22 gauge guide cannulae (Plastics One, Inc.) were implanted, bilaterally, 1.0 mm above the VTA and aimed at the following coordinates : - 5.7 mm posterior to bregma, + 0.6 mm lateral from the midline, and - 7.4 mm ventral from the skull surface (Paxinos and Watson, 1986). The stereotaxic arms were angled at 15 degrees from the perpendicular and the skull was level between lambda and bregma (i.e., flat skull position).

Drugs

DiMe-C7 (Sigma, St. Louis, MO), morphine sulphate (BDH Inc., Québec), and D-amphetamine sulfate (Smith Kline Beecham, Oakville, Ontario) were used exactly as described in Experiment 6. Apomorphine hydrochloride (Sigma, St. Louis, MO) was dissolved in 0.001 N hydrochloric acid and injected s.c. using a dose of 0.05 mg/kg. This dose of apomorphine, which acts specifically at autoreceptors, has been shown previously to prevent sensitization to the behavioural effects of amphetamine (Riffee & Wilcox, 1985)

Design and Procedure

Rats were pretreated with apomorphine (0.05 mg/kg; s.c.) or the vehicle, 10 minutes prior to receiving either bilateral infusions of DiMe-C7 (3.0 μg/0.5 μl/side) or morphine (3.0 μg.0.5 μl/side) into the VTA, or bilateral infusions of amphetamine (2.5 μg/0.5 μl/side) into the NAS. Rats received a s.c. injection of 0.05 ml of 2.5 % formalin into the plantar surface of one hind paw immediately following the last intracranial infusions.

Rats assigned to conditions Apomorphine-DiMe-C7 and Vehicle-DiMe-C7 (Figure 31), Apomorphine-Morphine and Vehicle-Morphine (Figure 32), and Apomorphine-Amphetamine and Vehicle-Amphetamine (Figure 33) were tested in the formalin test using a counterbalanced within-subjects design. Thus, animals were tested twice in the formalin test, at a 1-week interval. Either the right or left hind paw was injected on successive tests. Rats assigned to all the remaining conditions were tested in the formalin test once, using a between-subjects design.

Statistical Analyses

To verify whether DiMe-C7, morphine, and amphetamine induced significant analgesia and whether pretreatment with the antagonists or apomorphine had any effects on its own, separate two-way ANOVAs were conducted with Treatment Group (Vehicle-Drug vs. Apomorphine-Vehicle vs. Vehicle-Vehicle) as the between-subjects variable and Time (10 post-infusion time-points) as the within-subjects variable; because most of the effects of the drugs occurred within the first 30 minutes of testing, only the first 10 post-infusions time-points were included in the analyses. All analyses were followed, when appropriate, by Tukey's post-hoc test for overall differences between groups. To examine the effects of pretreatment with apomorphine on the analgesic effects of DiMe-C7, morphine, and amphetamine, two-way ANOVAs were conducted

with Pretreatment Condition (Apomorphine-Drug vs. Vehicle-Drug) and Time (10 post-infusion time-points) as within-subjects variables. Tests for simple main effects were used when appropriate to analyze the differences at each time point between the two conditions. Data from an animal were included in the analyses only if the injector tips were located within 0.5 mm of the target area.

Results

The effect on formalin pain responses of DiMe-C7 (3.0 µg/0.5 µl/side) infused into the VTA in rats pretreated with apomorphine (0.05 mg/kg) is shown in Figure 31. The ANOVA conducted with Treatment Group (Vehicle-DiMe-C7 vs. Apomorphine-Vehicle vs. Vehicle-Vehicle) and Time variables yielded a significant overall Treatment Group effect, $F(2, 17) = 16.022$, $P < 0.001$. As shown by comparing groups Vehicle-DiMe-C7 and Vehicle-Vehicle, DiMe-C7 infused into the VTA significantly attenuated pain scores ($P < 0.05$). The ANOVA conducted with Pretreatment Condition (Apomorphine-DiMe-C7 vs. Vehicle-DiMe-C7) and Time variables indicated that there was no significant overall main effect, $F(1, 5) = 0.071$, $P = 0.79$. As seen by comparing conditions Apomorphine-DiMe-C7 and Vehicle-DiMe-C7, apomorphine pretreatment did not reverse the analgesic effects induced by intra-VTA DiMe-C7. Pretreament with apomorphine alone appeared to elevate pain scores, as shown by comparing conditions Apomorphine-Vehicle (closed triangles) and Vehicle-Vehicle (open circles). This trend, however, was not statistically significant.

The effect on formalin pain responses of morphine (3.0 µg/0.5 µl/side) infused into the VTA in rats pretreated with apomorphine (0.05 mg/kg) is shown in Figure 32. The ANOVA conducted with Treatment Groups (Vehicle-Morphine vs.Apomorphine-

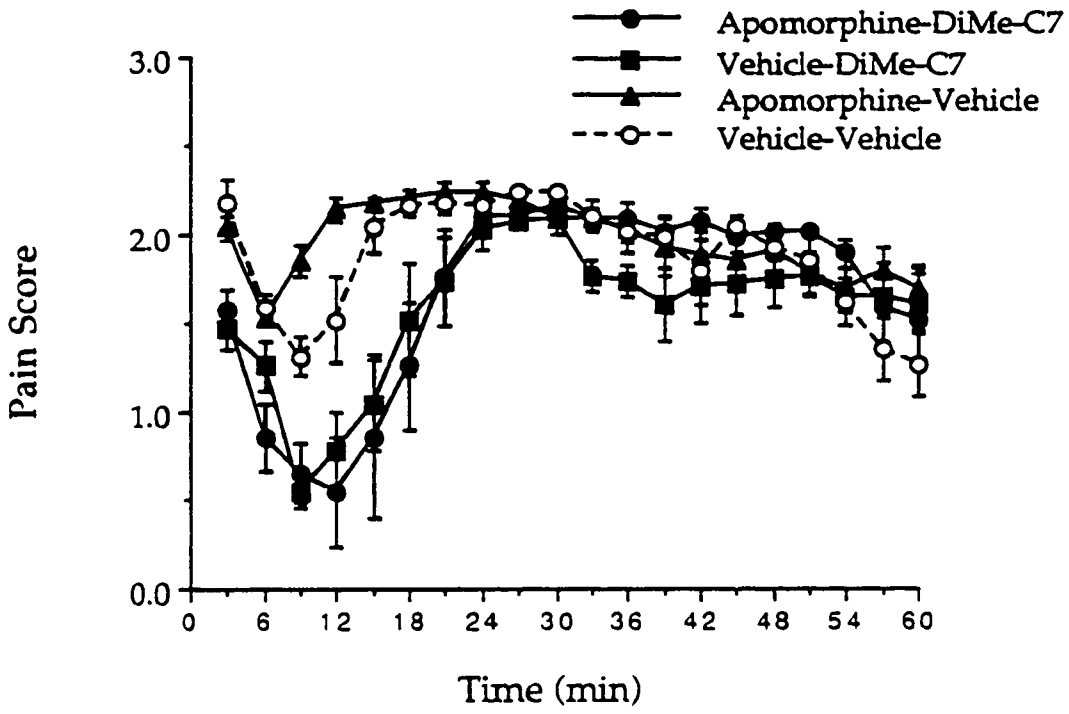


Figure 31. Mean formalin pain scores (± S.E.M.) following bilateral intra-VTA infusions of DiMe-C7 (3.0 µg/0.5 µl/side), or the vehicle, in rats pretreated with apomorphine (0.05 mg/kg) or the vehicle. Animals (n = 6) assigned to conditions Apomorphine-DiMe-C7 and Vehicle-DiMe-C7 were tested in a counterbalanced within-subjects design. Separate groups of animals were assigned to conditions Apomorphine-Vehicle (n = 6) and Vehicle-Vehicle (n = 8).

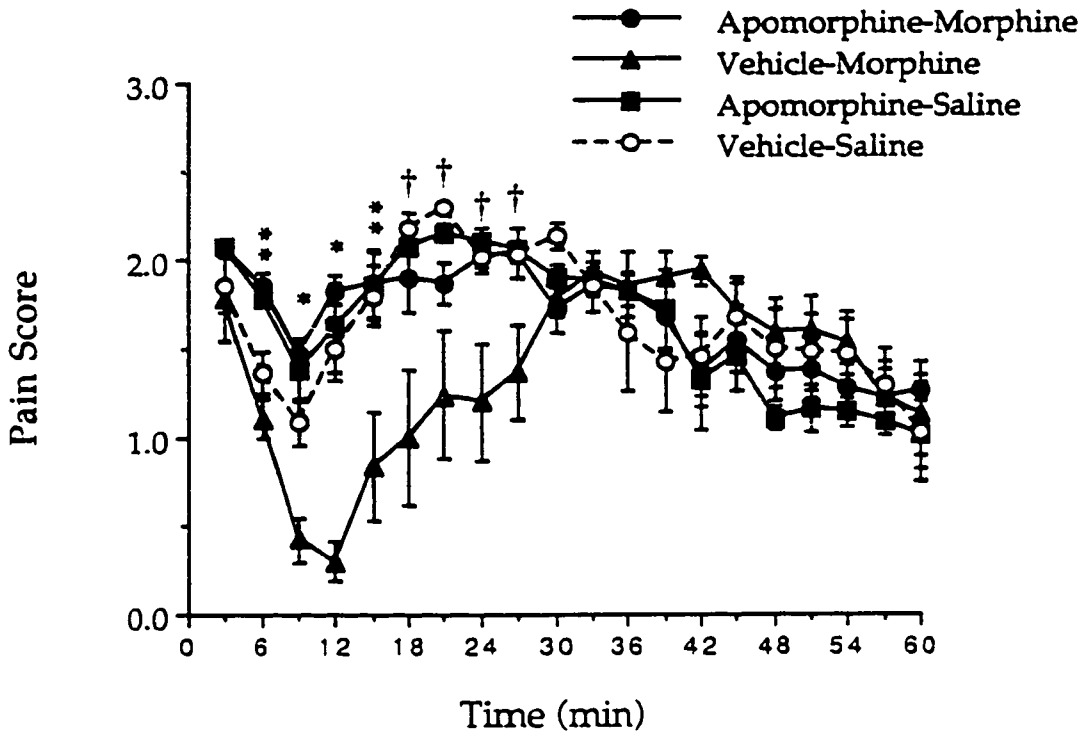


Figure 32. Mean formalin pain scores (± S.E.M.) following bilateral intra-VTA infusions of morphine (3.0 µg/0.5 µl/side), or saline, in rats pretreated with apomorphine (0.05 mg/kg) or the vehicle. Significant differences between Apomorphine-Morphine and Vehicle-Morphine conditions : * $p < 0.001$; ** $p < 0.005$; † $p < 0.05$. Animals (n = 6) assigned to groups Apomorphine-Morphine and Vehicle -Morphine were tested in a counterbalanced within-subjects design. Animals assigned to groups Apomorphine-Saline (n = 4) and Vehicle-Saline (n = 6) were tested in a between-subjects design.

Saline vs. Vehicle-Saline) and Time as variables revealed a significant overall main effect of Treatment Groups, $F(2, 14) = 8.517$, $P < 0.005$. It can be seen by comparing groups Vehicle-Saline and Vehicle-Morphine that intra-VTA morphine induced significant analgesia ($P < 0.05$). The ANOVA conducted with Pretreatment Condition (Apomorphine-Morphine and Vehicle-Morphine) and Time as variables yielded a significant main effect of Pretreatment Condition, $F(1, 5) = 39.93$, $P < 0.005$. As shown by comparing conditions Apomorphine-Morphine and Vehicle-Morphine, apomorphine pretreatment completely prevented the analgesic effect induced by intra-VTA morphine. Apomorphine pretreatment alone did not have a significant effect on pain scores.

The effect on formalin pain responses of amphetamine (2.5 μg/0.5 μl/side) infused into the NAS in rats pretreated with apomorphine (0.05 mg/kg) is shown in Figure 33. The ANOVA conducted with Treatment Group (Vehicle-Amphetamine vs.Vehicle-Saline vs. Apomorphine-Saline) and Time variables revealed a significant overall main effect of Treatment Group, $F(2, 13) = 13.455$, $P < 0.001$. As seen by comparing Vehicle-Amphetamine and Vehicle-Saline conditions, amphetamine infused into the NAS elicited significant analgesia ($P < 0.05$). The ANOVA conducted with Pretreatment Condition (Apomorphine-Amphetamine vs.Vehicle-Amphetamine) and Time as variables revealed that there was no significant main effect of Pretreatment Condition, $F(1, 4) = 0.02$, $P = 0.89$. Thus, apomorphine pretreatment did not attenuate the analgesic effect induced by intra-NAS amphetamine. Also, as seen in Figure 33, apomorphine pretreatment alone appeared to elevate pain scores, but this effect was not statistically significant.

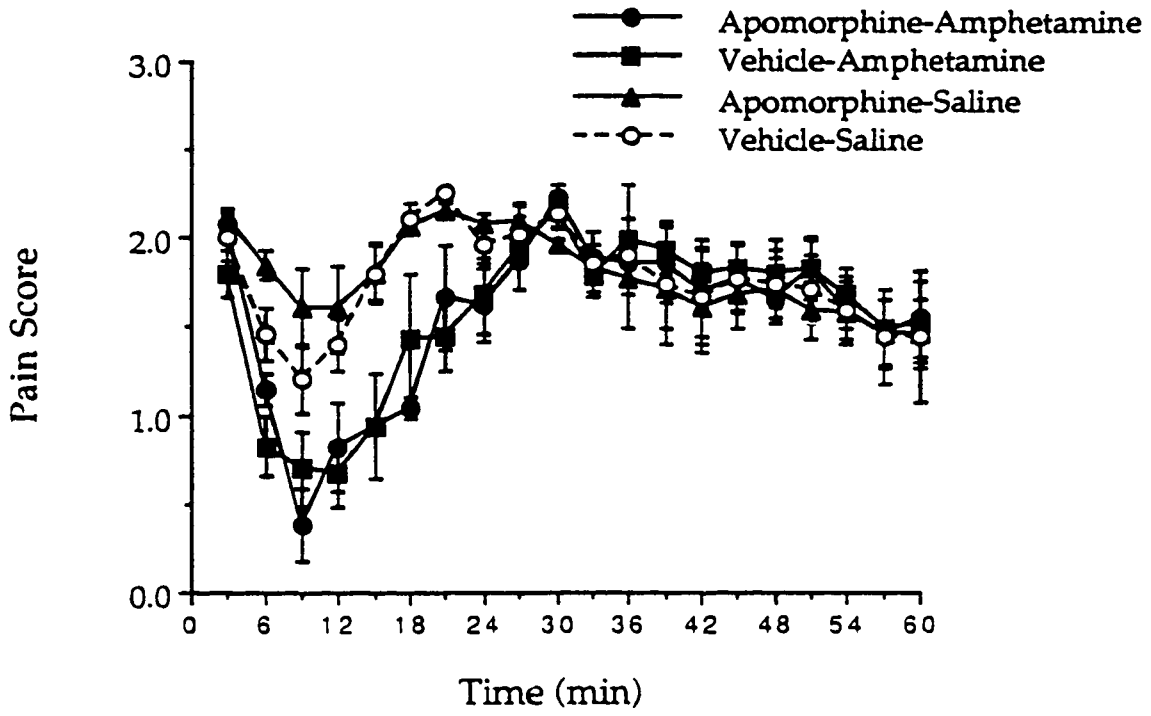


Figure 33. Mean formalin pain scores (± S.E.M.) following bilateral intra-NAS infusions of amphetamine (2.5 µg/0.5 µl/side), or saline, in rats pretreated with apomorphine (0.05 mg/kg) or its vehicle. Animals (n = 5) assigned to Apomorphine-Amphetamine and Vehicle-Amphetamine conditions were tested in a counterbalanced within-subjects design. Separate groups of animals were assigned to Apomorphine-Saline (n = 5) and Vehicle-Saline (n = 6) conditions.

Figure 34 illustrates the location of the internal injector cannulae tips in the VTA. As shown, 69 rats had their tips within the limits of the VTA. The data for three rats were discarded because their tips were not within the limits of the VTA.

Discussion

The results obtained from these experiments indicate that decreased DA release in midbrain ascending neurons by pretreatment with a low autorector-specific dose of apomorphine blocks the analgesic effects of intra-VTA morphine, but not of intra-NAS amphetamine or intra-VTA DiMe-C7 in the formalin test. These findings suggest that enhanced DA transmission in midbrain ascending neurons plays an important role in mediating the inhibition of tonic pain induced by morphine, but not amphetamine nor SP. At first glance, these findings might appear to be inconsistent with those of Experiment 6 showing that pretreatment with the DA receptor antagonist, raclopride, prevents the analgesic effects of intra-VTA SP, intra-VTA morphine, and intra-NAS amphetamine.

The differential effects of pretreatment with a low dose of apomorphine on SP-, morphine-, and amphetamine-induced analgesia can be explained, however, by the different neurochemical actions of these drugs on midbrain DA ascending neurons. Morphine and other mu opioids are thought to activate DA transmission in the mesolimbic system indirectly by stimulating mu receptors located on GABA interneurons in the VTA, the effects of which in turn inhibit the inhibitory effects of GABA on midbrain DA ascending neurons (e.g., Johnson & North, 1992; Matthews & German, 1984). Thus, because morphine increases firing in DA neurons indirectly by

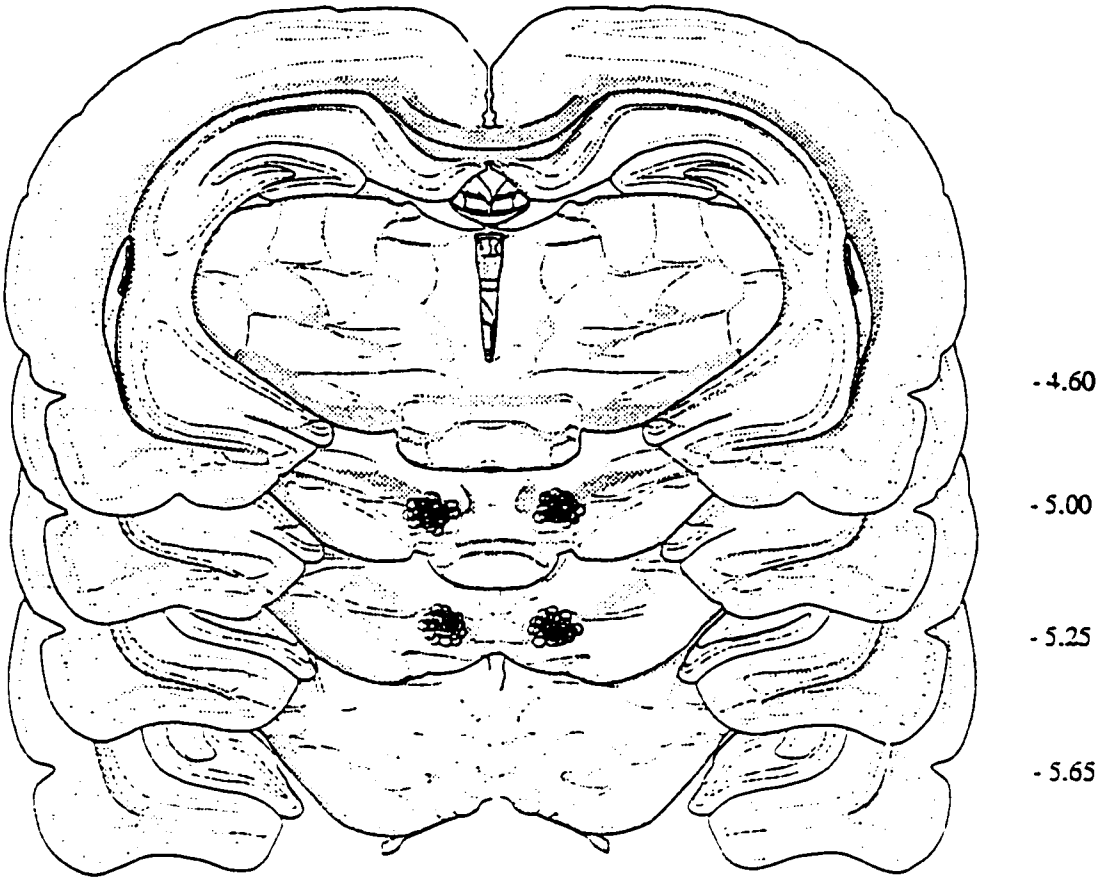


Figure 34. Location of the internal injector cannulae tips in the VTA (n = 69/side) of all rats tested in Experiment 7. Drawings (adapted from Adobe Illustrator 5.5 for Windows) are from the atlas by Swanson (1992). Values on the right represent distance in mm caudal from bregma.

the inhibition of GABA interneurons, any effect of the opioid is blocked by a low dose of apomorphine because its depressant effects on DA impulse flow cannot be counteracted by the opioid. In contrast, SP increases firing in midbrain DA neurons by stimulating tachykinin receptors located directly on DA cell bodies in the VTA (Stoessl, 1992). Amphetamine, on the other hand, promotes synaptic DA release independently from cell firing and blocks DA reuptake from terminals (Kuzcenski, 1983; Reith et al., 1986). Thus, intra-VTA SP and intra-NAS amphetamine elicit analgesia despite pretreatment with a low dose of apomorphine because its effects on DA release are counteracted by the direct actions of SP and amphetamine on midbrain DA neurons.

The present results are, therefore, in accordance with those of Experiment 6 in showing that enhanced DA transmission in midbrain ascending neurons plays an important role in the inhibition of tonic pain.

General Discussion

In the first series of experiments presented in this thesis, the analgesic effects of the SP analog, DiMe-C7, infused into the cell body region of the VTA (Altier & Stewart, 1993) were further explored by examining the role of tachykinin NK-1 and NK-3 receptors in both the VTA and terminal projection site in the NAS in the modulation of tonic pain. It was found that agonists selective for the tachykinin NK-1 and NK-3 receptor subtypes induce analgesia in the formalin test for tonic pain when they are infused into either the VTA or the NAS (Experiments 1 and 2). At both sites of infusions, the NK-1 agonist, GR-73632, appeared to be, within the range of doses used, more effective at inducing analgesia in the formalin test than the NK-3 agonist, senktide. Because neither infusions of the SP analog, DiMe-C7, made 1.0 mm dorsal to the VTA (Altier & Stewart, 1993) nor infusions of either GR-73632 or senktide made 1.0 mm dorsal to the NAS induce analgesia in the formalin test (Experiment 2), it is likely that the tachykinin agonists were acting at receptors in the VTA and NAS. These findings are consistent with the previous report that intra-VTA infusions of the SP analog, DiMe-C7, induce analgesia in the formalin test (Altier & Stewart, 1993) and extend it by showing that SP also acts in the NAS to modulate tonic pain. These findings also lend support to previous studies implicating midbrain ascending DA neurons in the modulation of tonic pain (Anderson & Rompré, 1996; Clarke & Franklin, 1992; Franklin, 1990; Manning et al., 1994; Morgan, 1990; Morgan & Franklin, 1990, 1991).

The analgesic effects induced by tachykinin NK-1 and NK-3 selective agonists infused into either the VTA or NAS were dose-dependent, especially in the case of the NK-1 selective agonist, as revealed by the weighted-pain scores method described by

Dubuisson and Dennis (1977). More specifically, it was found that, as the doses of these agonists escalated, pain scores decreased progressively. It is likely that these agonists induced analgesia, using the weighted-scores method, by affecting all behavioural responses reflecting various pain intensities. Evidence in support of this idea comes from the results described in Experiment 1 on the effects of escalating doses of the NK-1 agonist, GR-73632, infused in the VTA on the time spent in each behavioural category. It was found that, as the doses of GR-73632 increased, animals spent progressively less time in behavioural categories reflecting more intense pain and more time in those reflecting less intense pain (see Figure 2). Similar findings were reported by Coderre et al. (1993) using various escalating doses of morphine administered systemically. The findings that GR-73632 and morphine (Coderre et al., 1993) induce analgesia dose-dependently, as revealed by the weighted-pain scores method, and affect dose-dependently the time spent in all behavioural categories suggest that it is a valid method for studying the effects of manipulations in the formalin test for tonic pain. In addition, these findings support the idea that the weighted-pain scores method accurately describes the overall experience of tonic pain by measuring several pain-related behaviours reflecting varying intensities of pain.

The inhibition of formalin-induced tonic pain by intra-VTA or intra-NAS infusions of tachykinin agonists does not appear to be mediated by endogeneous opioids, for this inhibition was not prevented by pretreatment with the opioid receptor antagonist, naltrexone (Experiment 3). These findings are different from those of other studies showing that the analgesic effects of SP administered either systemically or into the ventricles are mediated indirectly via the release of endogeneous opioids (Del Río et al. 1983; Frederickson et al. 1978; Malick and Goldstein, 1978; Naranjo et al. 1982 a,b; Yashpal et al., 1995). The finding that tachykinin agonists infused into the VTA or NAS elicit analgesia independently from opioid mechanisms probably reflects the fact

that SP terminals make direct contacts with cell bodies in the VTA (Tamiya et al., 1990) and terminals in the NAS (Pickel et al., 1988), thereby allowing for the direct interaction of SP with the mesolimbic DA system (see Discussion of Experiment 3).

Effects of Manipulations in the Mesolimbic DA System on Tonic and Phasic Pain

One of the most interesting findings revealed from the present investigation is that manipulations of the mesolimbic DA system produce different effects depending on the type of pain test employed. More specifically, it was found that stimulation of tachykinin NK-1 and NK-3 receptors in either the VTA or NAS induces analgesia in the formalin test for tonic pain (Experiments 1 and 2), but not in the tail-flick test for phasic pain (Experiment 4). Similarly, whereas infusions of morphine into the VTA inhibit tonic pain in the formalin test (Manning et al., 1994; Morgan, 1990; also see Experiments 6 and 7), they are without effects in the tail-flick test (Experiment 4). These findings parallel those of previous studies using intra-VTA DiMe-C7 and intra-NAS amphetamine (Altier & Stewart, 1993), as well as those of Morgan and Franklin (1990), who showed that 6-hydroxydopamine lesions of midbrain DA neurons attenuate morphine- and amphetamine-induced analgesia in the formalin, but not the tail-flick test. The present findings are also in accordance with previous findings indicating that DA agonists produce analgesia in the formalin test (Dennis & Melzack, 1983; Lin et al., 1989; Morgan & Franklin, 1991; Skaburskis, 1980), but are either without effect or elicit hyperalgesia in phasic pain tests (Ben-Sreti et al., 1983; Caroll & Lim, 1960; Dennis & Melzack, 1983; Dunai-Kovács & Székely, 1977; Gonzales et al., 1980; Hernandez et al., 1986; Misra et al., 1987; Nott, 1968; Pertovaara et al., 1988; Robertson et al., 1981; Tocco & Maickel, 1984; Tocco et al., 1985; Tulunay et al., 1976; Witkin et al., 1961). The present findings provide additional evidence in support

of the idea that the neural substrates underlying the inhibition of tonic pain are fundamentally different from those underlying the inhibition of phasic pain. As described previously, there is considerable evidence indicating that analgesia in tonic and phasic pain tests are also dissociable with respect to manipulations of serotonergic (e.g., Abbott & Melzack, 1982a, b; Abbott & Young, 1988), noradrenergic (Dennis & Melzack, 1980), opioidergic (e.g.Cohen & Melzack, 1985) systems and with respect to the phenomenon of tolerance to morphine analgesia (Abbott et al., 1981, 1982).

The biological significance of this dissociation in the effects of drugs (e.g., tachykinin agonists, opioids) on tonic versus phasic pain is unclear. One possibility is that this dissociation evolved to promote survival under conditions of stress. As described previously, exposure to stress causes the release of opioids and SP in the VTA which, in turn, augment DA transmission in terminals fields of midbrain ascending neurons. The evidence provided here and in past studies that enhanced activity of these peptides and transmitter (Altier & Stewart, 1993), at sites where they are endogeneously released by stress, inhibits tonic pain in the formalin test, but is without effect in the tail-flick test for phasic pain suggests that, under naturally stressful conditions, these mechanisms may function to minimize the behavioural recuperative responses to pain when it is tonic and inescapable, but preserve reflexive withdrawal and active avoidance when pain is phasic and escapable. Thus, by inhibiting tonic pain without affecting phasic pain, these neural mechanisms may serve to help organisms cope with stress and pain more effectively while preventing or minimizing further tissue damage. This possibility may also help to explain why exogeneous opioids relieve clinical post-operative pain but fail, except at high doses, to suppress the sensations caused by the surgical wound (Dennis & Melzack, 1979; Jaffe & Martin, 1980).

Similarities and differences in the neural substrates underlying the inhibition of tonic versus phasic pain

Although there are reports that structures rostral to the periaqueductal gray mediate analgesia in phasic pain tests (e.g., Baumeister et al., 1987, 1988, 1993, Hardy, 1985; Terenzi et al., 1990; Terenzi & Prado, 1990), there is considerable evidence to suggest that the inhibition of phasic pain depends to a greater extent on the activation of a brainstem-descending pain-suppression system that originates in the periaqueductal gray. The latter brainstem site also plays a role in the modulation of tonic pain. For instance, it has been shown that infusions of morphine or electrical stimulation into the periaqueductal gray induce analgesia in the formalin test (Manning et al., 1994; Vaccarino & Chorney, 1994). Thus, the neural systems that underlie the inhibition of phasic and tonic pain appear to be the same at the level of the periaqueductal gray. In contrast to phasic pain, however, tonic pain appears to be additionally modulated by a pain-suppression system located at sites more rostral to the periaqueductal gray, one component of which appears to depend upon the activation of the mesolimbic DA system, through the release of various peptides, such as endogeneous opioids and SP. The habenula is another site that has been shown to play a role in the modulation of tonic pain (Cohen & Melzack, 1985, 1986, 1993; Fuchs & Cox, 1993). Interestingly, exposure to stress causes the release of SP in the VTA from terminals thought to originate in the habenula (Lisoprawski et al., 1981). Given the evidence presented in this thesis implicating midbrain SP in the inhibition of tonic pain, it is possible that the habenula, VTA, and NAS are interconnected in the same circuitry that serves to modulate tonic pain. Future studies should be conducted to explore this idea.

The Role of Midbrain SP in Stress-Induced Analgesia

It was hypothesized that SP release in the VTA might mediate stress-induced analgesia in the formalin test. This idea was based on the evidence that the stimulation of SP receptors in the VTA mediates the stress-induced activation of midbrain ascending DA neurons (Bannon et al., 1983), that exposure to stress causes the release of SP in the VTA (Bannon et al., 1983, Deutch et al., 1985; Lisoprawski et al., 1981) and inhibits tonic pain (Fanselow, 1982), and that stimulation of SP receptors in the VTA by infusions at this site of the SP analog, DiMe-C7, and selective tachykinin NK-1 and NK-3 agonists induce analgesia in the formalin test (Experiment 1). Experiment 5 was designed therefore to examine the effect of blocking SP receptors in the VTA on stress-induced analgesia in the formalin test. It was found that intra-VTA pretreatment with the selective NK-1 receptor antagonist, RP 67580, prevents footshock stress-induced analgesia in the formalin test, suggesting that the stimulation of SP receptors in the VTA is a mechanism underlying the stress-induced inhibition of tonic pain. This finding, together with the evidence that the stimulation of SP receptors in the VTA mediates the stress-induced activation of midbrain DA systems, also suggests that exposure to stress induces analgesia in the formalin test by causing, at least in part, the release of SP in the VTA which in turn activates midbrain ascending DA neurons. Support for this idea is provided by the results of Experiment 7 in which it was found that the analgesic effects induced by intra-VTA infusions of the SP analog, DiMe-C7, are abolished by intra-NAS pretreatment with the DA D-2 receptor antagonist, raclopride.

It was mentioned in the Introduction that exposure to stress induces analgesia that is either opioid- or non-opioid-mediated and that the neurochemical nature of stress-induced analgesia is determined by several variables, the most important being stress severity (intensity x duration; e.g., Terman et al., 1984). In a previous study, it was

found that exposure to footshock stress, using the same parameters as those used here, causes analgesia that is mediated, at least in part, by the stimulation of opioid receptors in the VTA (Altier & Stewart, 1996). More specifically, it was found that infusions into the VTA of the opioid receptor antagonist, naltrexone methylbromide, at a dose (0.2 μg/rat) reported previously to prevent the stress-induced activation of midbrain DA systems (Kalivas & Abhold, 1987), attenuate footshock stress-induced analgesia in the formalin test. This finding suggests that the stimulation of opioid receptors in the VTA contributes significantly to the stress-induced analgesia reported in the present study. Furthermore, this finding, together with the present finding that the stimulation of NK-1 receptors in the VTA also contributes significantly to stress-induced analgesia in the formalin test, suggests that both opioid and non-opioid mechanisms in the VTA are involved in mediating the stress-induced inhibition of tonic pain.

Given the findings that exposure to stress inhibits tonic pain by causing the stimulation of SP and opioid receptors in the VTA, it was surprising to find that the blockade of either receptors at this site prevented stress-induced analgesia in the formalin test. It may be that intra-VTA blockade of SP receptors was effective at preventing stress-induced analgesia, despite concurrent stimulation of opioid receptors in the VTA, because SP directly activates midbrain ascending DA neurons by stimulating tachykinin receptors located on the DA neurons (Stoessl, 1992), whereas the effect of opioids acting at this site on the DA neurons is indirect (Johnson & North, 1992). On the other hand, the antagonism of opioid receptors in the VTA may have similarly been effective at preventing stress-induced analgesia, despite concurrent SP receptor stimulation at this site, because the inhibitory effect of intra-VTA opioid receptor blockade on the DA neurons can counteract the direct excitatory effect of SP receptor stimulation on the same neurons.

The findings reported in the present thesis and elsewhere (Del Río & Naranjo, 1983; Frederickson et al., 1978; Kotani et al., 1981; Malick & Goldstein, 1978; Mészaros et al., 1980; Naranjo & Del Río, 1982; Naranjo et al., 1981; Starr et al., 1978; Stewart et al., 1976; Szreniawski et al., 1979; Yeomans & Proudfit, 1992) implicating SP in analgesia may appear counterintuitive in light of the role that SP is considered to play in the transmission of nociceptive information from the periphery to higher levels of the central nervous system (e.g., Brodin et al., 1987; Go et al., 1987; Henry, 1976; Kuraishi et al., 1985). Indeed, SP is present in the dorsal horn of the spinal cord (Cuello et al., 1977; Cuello & Kanazawa, 1978; Hokfelt et al., 1977), where small diameter primary afferent fibers coming from the periphery terminate (Light & Perl, 1979; Sugiura et al., 1986) and where high concentrations of SP receptors are present (Quirion et al., 1983; Yashpal et al., 1990). Furthermore, it has been shown that SP in the dorsal horn preferentially excites nociceptive-sensitive neurons (De Koninck & Henry, 1991; Henry, 1976; Radhakrishnan & Henry, 1991; Salter & Henry, 1991) and that this effect is blocked by an NK-1 receptor antagonist (De Koninck & Henry, 1991; Radhakrishnan & Henry, 1991). It has also been reported that intrathecal (i.e., spinal) administration of SP elicits hyperalgesia in the tail-flick test (e.g., Cridland & Henry, 1986; Rochford & Henry, 1988; Yashpal et al., 1993) and that pretreatment with an NK-1 receptor antagonist blocks this effect (Yashpal et al., 1993). Similarly, NK-1 receptor antagonism has been shown to reduce the behavioural responses to noxious thermal and chemical stimulation (Yashpal et al., 1993). Finally, there is evidence from the clinical literature that humans afflicted with a congenital depletion of SP-containing afferents display diminished pain sensitivity (Pearson et al., 1982).

These opposing roles of SP in the transmission and modulation of pain may be understood, however, when it is considered that aversive stimuli can, in themselves, be

the source of pain inhibition. Indeed, there is ample evidence that, as mentioned previously, exposure to a wide variety of stressors induces analgesia (e.g., Fanselow, 1984; Fanselow & Sigmundi, 1986; Lewis et al., 1983; Maier et al., 1984). Moreover, there is evidence that exposure to noxious painful stimuli suppresses the response to another noxious painful stimulus delivered to a different part of the body (Le Bars et al., 1979 a,b; Yashpal et al., 1995) and it appears that this effect is mediated by SP (Yashpal et al., 1995). In fact, many remedies used in folk medecine to combat pain involve pain infliction (see Melzack & Wall, 1988, and Melzack, 1989, for review). For instance, cupping, which involves placing an inverted glass cup heated by flaming alcohol or coals over a a painful body part, was used historically to alleviate pain resulting from a number of conditions such as headaches, backaches, and arthritis. Other strategies that make use of pain to inhibit pain include scarification, cauterization, counterirritation, acupuncture, and transcutaneous electrical nerve stimulation (see Melzack & Wall, 1988, for review).

Interestingly, SP is released in the VTA in response to stress (Bannon et al., 1986; Deutch et al., 1983, 1985; Lisoprawski et al., 1981) and, recently, it has been found that formalin-induced pain increases the number of DA neurons in the VTA expressing Fos-positive immunoreactivity (Ma et al., 1993). Thus, the evidence that exposure to stress or pain inhibits pain and activates midbrain DA systems, that SP in the VTA plays a role in mediating stress-induced analgesia, and that pain-induced analgesia is mediated by SP suggests that SP release in the VTA is part of an endogenous pain-suppression system that is naturally triggered by exposure to aversive stimuli such as stress and pain.

The Analgesic Effects of Tachykinin Agonists, Morphine, and Amphetamine

In the present investigation, it was found that infusions of either the SP analog, DiMe-C7, tachykinin NK-1 or NK-3 selective agonists, or morphine into the VTA, and infusions of amphetamine into the NAS induce potent analgesia during approximately 30 minutes in the formalin test for tonic pain. Intra-VTA infusions of either DiMe-C7, NK-1 or NK-3 agonists, or mu opioid agonists, and intra-NAS infusions amphetamine have been shown previously to induce biochemical and behavioural effects lasting for at least one hour (e.g., Boss et al., 1988; Eison et al., 1982a,b; Elliott & Iversen, 1986; Joyce et al., 1981; Kalivas & Richardson-Carlson, 1986; Kalivas et al., 1983; Latimer et al., 1987; Stinus et al., 1980). It was, therefore, somewhat surprising to find that these compounds induced analgesia in the formalin test for no longer than 30 minutes. These differences in time course suggest that the effects of DiMe-C7, NK-1 and NK-3 selective agonists, morphine, and amphetamine infused at sites within the mesolimbic DA system on tonic pain are short-lasting. Previous studies also found that intra-VTA morphine (Manning et al., 1994) and intra-NAS amphetamine (Morgan, 1990) induce analgesia during 30 minutes following the intracranial infusions. Unfortunately, for comparison purposes, it is not known from these previous studies whether the analgesic effects were also short-lasting, as in the present studies, because pain responses were not recorded beyond 30 minutes following the intracranial infusions.

It is unlikely that the short-lasting analgesic effects induced by intra-VTA tachykinin agonists, intra-VTA morphine, and intra-NAS amphetamine were due to a selectivity of the effects on the early pain phase because analgesia was observed during both the early pain phase and during the first part of the late pain phase of the formalin test. In support of this, it was found, in a previous study, that intra-VTA infusions of

DiMe-C7 made 25 minutes following a formalin injection induce analgesia during approximately 30 minutes (Altier & Stewart, 1993). Similarly, morphine and amphetamine infused into the VTA and NAS, respectively, 20 minutes following a formalin injection induce analgesia during the late pain phase (Morgan, 1990; Manning et al., 1994).

It is unclear why the analgesic effects induced by intra-VTA tachykinin agonists, intra-VTA morphine, and intra-NAS amphetamine are short-lasting. One possibility is that the short-lasting analgesia and the rapid reinstatement of tonic pain responses serve an adaptive function under conditions of stress. More specifically, it is possible that the short-lasting analgesic effects induced by the activation of the mesolimbic DA system by SP, morphine, and amphetamine serve to reduce pain temporarily, allowing the animal to engage in defensive behaviours (i.e., fight or flight) while the threat may still be present. When an organism successfully escapes from the stressor or when the threat poses no more danger, analgesia may no longer serve a purpose and thus gradually dissipates, perhaps through the activation of an anti-analgesic system involving neuropeptide FF (Altier & Stewart, 1997), thereby allowing the organism to engage in recuperative behaviours (i.e., limping) which promote healing of the injured body part. The evidence that SP, opioids, and DA are all naturally release by exposure to stress at sites within the mesolimbic DA system lends support to this idea.

The Role of DA in the NAS in Analgesia

Based on several lines of evidence described previously, it was hypothesized that enhanced DA transmission in the NAS mediates the analgesic effects of tachykinin agonists, morphine, and amphetamine in the formalin test. Experiments were designed, therefore, to examine the effects of DA receptor antagonism into the NAS on analgesia

induced by intra-VTA infusions of either the SP analog, DiMe-C7, or morphine, and intra-NAS infusions of amphetamine. The results indicated that pretreatment with the DA D-2 receptor antagonist, raclopride, infused into the NAS prevents the analgesic effects induced by intra-VTA DiMe-C7, intra-VTA morphine, and intra-NAS amphetamine. It was also found that intra-NAS pretreatment with either the selective DA D-1 receptor antagonist, SCH 23390, or the mixed DA D-1 and D-2 receptor antagonist, flupenthixol, attenuates the analgesic effects induced by DiMe-C7 infused into the VTA. These findings suggest that a mechanism underlying the inhibition of tonic pain is the stimulation of DA D-1 and D-2 receptors in the NAS by DA released from terminals of mesolimbic neurons. In support of this idea, it was found, in Experiment 7, that reduced DA release in midbrain ascending systems, by the administration of a low autoreceptor-specific dose of apomorphine, prevents the analgesic effect of morphine infused into the VTA.

With regards to the effects of blocking DA receptors in the NAS or reducing DA release in midbrain systems on tonic pain, it was found that intra-NAS infusions of either raclopride, SCH 23390, or flupenthixol, and systemic apomorphine had no effect on their own on pain responses in the formalin test. Although these manipulations appeared to induce mild hyperalgesia (i.e., increased pain responsiveness), this effect was expected to be more pronounced given the evidence presented in this thesis that DA plays a role in modulating tonic pain. It is possible, however, that these manipulations were not effective at inducing the expected hyperalgesic effects because pain scores, recorded following an injection of 2.5 % formalin , show a ceiling effect. In support of this idea, it has been reported in a previous study that pain scores recorded following the injection of 5.0 % formalin do not differ from those recorded following the injection of 2.5 % formalin (Coderre et al., 1993). These findings suggest that it might be worthwhile re-examining the effects of blocking DA receptors in the NAS or reducing

DA release in midbrain systems on tonic pain, using a concentration of formalin lower than that used in the present studies.

Analgesia and Reward : A Common Neural Substrate ?

There is considerable evidence to suggest that psychostimulant drugs such as amphetamine, cocaine and mu agonists such as heroin or morphine are rewarding and that a common primary event underlying this effect is enhanced DA transmission in the NAS (e.g., Fibiger & Phillips, 1988; Koob & Bloom, 1988; Wise, 1987, 1988, 1989; Wise & Rompré, 1989). For instance, these psychostimulants have all been reported to be self-administered and to induce conditioned place preference (e.g., Bals-Kubic et al., 1993; Bozarth, 1987; Phillips & LePiane, 1980; Shippenberg et al., 1993) and there is considerable evidence indicating that these effects are abolished by 6-hydroxydopamine lesions or infusions of DA antagonists directly into the NAS (Boss et al., Kelly & Iversen, 1976; Kelly et al., 1975; Kelsey et al., 1989; Lyness et al., 1979; Phillips et al., 1983; Pijnenberg et al., 1975 a, b; Shippenberg et al., 1993). In addition, it has been shown that drugs that are rewarding in these paradigms all share the ability to enhance DA release in the NAS (Di Chiara et al., 1987). There is also evidence indicating that SP is rewarding in the conditioned place preference paradigm (Hasenöhrl et al., 1990; Holzhäuer-Oitzl et al., 1987; Huston & Oitzl, 1989; Oitzl et al., 1990; Rüdiger et al., 1991; Stäubli & Huston, 1985). Although the role of DA in the NAS was not examined in these studies, it is interesting to note that, as in the case of psychostimulant drugs, SP enhances DA transmission in the NAS (Boix et al., 1992 a, b; Cador et al., 1985a; Elliott et al., 1986). Given the latter evidence and that implicating DA transmission in the NAS in reward, it likely that DA in the NAS plays a role in the rewarding effects of SP, although this needs to be verified experimentally.

Interestingly, as mentioned throughout this thesis, the psychostimulant drugs amphetamine, cocaine, morphine, and the SP analog, DiMe-C7, are effective at inhibiting tonic pain and it appears that enhanced DA transmission in the NAS is an important mechanism underlying this effect (Experiments 6 and 7). The evidence that amphetamine, morphine, and DiMe-C7 are both rewarding and analgesic in the formalin test and appear to induce these behavioural effects primarily by causing the release of DA from terminals of mesolimbic DA neurons suggests that the neural substrates that mediate reward and analgesia (more specifically, the inhibition of tonic pain) are the same. In fact, as Franklin (1989) also pointed out, the findings that all drugs that are rewarding are also analgesic in the clinical situation supports this idea. There is some recent evidence, however, indicating that these substrates are dissociated at the level of the pedunculopontine tegmental nucleus, the latter of which is part of the brain circuitry mediating the behavioural effects of psychostimulant drugs (e.g., Klitenick & Kalivas, 1994). Indeed, Olmstead and Franklin (1993) have shown that lesions of the pedunculopontine tegmental nucleus abolish the rewarding effects of morphine in the conditioned place preference paradigm, but are without effects on morphine analgesia in the formalin test.

Analgesia and Affect

The findings that drugs that are rewarding are also effective against clinical pain, and that both reward and analgesia in the formalin test appear to be mediated by a common neural substrate involving the activation of the mesolimbic DA system provide clues as to the nature of the inhibition of tonic pain. Psychostimulant drugs are thought to be rewarding because they induce positive affect (Gunne et al., 1972; Jaffe & Martin, 1985). This finding, combined with the evidence suggesting that reward and analgesia in the formalin test are mediated by a common neural substrate, and that drugs that are

rewarding are also potent analgesics in the clinical situation, suggest that psychostimulant drugs inhibit clinical pain and tonic pain in the formalin test by alleviating negative affect, the latter of which is strongly associated with these types of pain. Reports from the clinical literature support this idea. For instance, it has been observed that relief from post-operative pain following opioid administration is associated with a change in mood and that patients treated with opioids often report that they still feel the pain but are no longer bothered by it (Franklin, 1989; Jaffe & Martin, 1975). Thus it appears that opioids cause ' dissociative analgesia ' by alleviating the affective-motivational ' suffering ' dimension of clinical pain without modulating the sensory-discriminative dimension of clinical pain.

The idea that reward and analgesia in the formalin test are associated behavioural phenomena, in that they are the result of the positive affect-enhancing effects of psychostimulant drugs, may be better understood by viewing them on a hedonic continuum (Morgan, 1990; Young, 1961), with extreme negative and positive affect located at opposite ends, and normal affect located in the middle of the continuum. Thus under conditions of tonic pain and, hence, strong negative affect, psychostimulant drugs may induce ' dissociative ' analgesia by shifting affect from one extreme closer towards normal levels at the middle of the continuum. In contrast, under pain-free conditions, these drugs may be rewarding because they shift affect from normal levels closer towards extreme positive affect at one end of the continuum. Viewing reward and analgesia on this continuum might help to explain why opioids neither induce tolerance nor result in drug dependence when taken on a long-term basis solely for the relief of clinical pain, whereas they induce these effects in humans not experiencing clinical pain. More specifically, it is possible that these problems are not encountered when using opioids for the management of clinical pain because opioids modulate the affective dimension of clinical pain by helping patients to achieve levels of affect that are normally

experienced in a pain-free state. In contrast, when opioids are taken during a pain-free state and, therefore, in the absence of negative affect, they induce levels of positive affect that are rewarding and hence lead to habit-formation and tolerance.

Several other lines of evidence support the idea that analgesia in the clinical situation and in the formalin test is due to the alleviation of negative affect. For instance, there are reports indicating that manipulations carried out in midbrain ascending neurons are effective at suppressing the responses to aversive stimuli, the latter of which, like tonic pain, are presumably associated with significant negative affect. Thus, it has been reported that rewarding electrical stimulation of the VTA, which induces analgesia in the formalin test (Anderson & Rompré, 1996), prevents the escape responses elicited by concomitant aversive electrical stimulation of the nucleus reticularis gigantocellularis (Anderson et al., 1995), a site involved in aversion (e.g., Casey, 1971; Guilbaud et al., 1973). It has also been shown that enhanced DA transmission in the NAS plays an important role in mediating the suppression of aversive somatic withdrawal symptoms (Harris & Aston-Jones, 1994), as it does in mediating that of tonic pain (Experiment 6 and 7). Finally, it is interesting to note that the analgesia induced by escalating doses of systemic morphine (Coderre et al., 1993) or infusions of the NK-1 agonist, GR 73632, into the VTA (Experiment 1) is characterized by progressive decreases in the time spent in behavioural categories reflecting more intense pain (i.e., scores 2 and especially 3) which are presumably associated with more intense negative affect, and by little or no effect in the time spent in those reflecting less intense pain (i.e., score 1).

DA in the NAS in Relation to the Pain-Suppression System that Serves to Inhibit Tonic Pain

Given the evidence presented in this thesis that enhanced DA transmission in the NAS is part of a pain-suppression system that serves to inhibit tonic pain, what is the broader neuroanatomical circuitry underlying this effect ? In other words, how does DA release in the NAS modulate information about tonic noxious stimulation coming from the periphery ? A survey of the literature on ascending pathways carrying pain information from the periphery to supraspinal sites and on projections from the NAS to sites involved in the modulation of pain suggests at least three possibilities. One possibility involves the inhibition of tonic pain directly at the level of the NAS. It has been found using retrograde labeling techniques that hundreds of neurons in the spinal cord project directly to the NAS (Burnstein and Giesler, 1989; Cliffer et al. 1991). Although the importance of this pathway in the transmission of pain has yet to be explored, it is possible that DA release in the NAS induces analgesia in the formalin test by inhibiting, directly at this site, information about tonic noxious stimulation transmitted by neurons originating in the spinal cord. Another way in which DA release in the NAS could affect pain information is via a projection from the NAS to the medial thalamus (Koikegami et al. 1967; Powell and Leman, 1976; Williams et al. 1977; Smeets and Medina, 1995). Indeed, it is possible that DA release in the NAS stimulates the neurons projecting from the latter to the medial thalamus which, in turn, inhibit activity of medial thalamic cells known to be driven by spinal dorsal horn neurons sensitive to noxious stimuli (Casey and Jones, 1978; Giesler et al. 1979; Craig and Burton, 1981; Kevetter and Willis, 1982; Albe-Fessard et al. 1985). This area of the brain would seem well suited for a role in the modulation of tonic pain in that it is considered to be involved in mediating the affective-motivational and not the sensory-discriminative dimension of pain (Carr and Bak, 1988). A third possibility involves a

projection from the NAS to the amygdala (Yao and Zhou, 1983; Yu and Han, 1990; Ma and Han, 1991) and from the latter to the spinal cord (Mizuno et al., 1985; Sandrew et al., 1986; Follet, 1989). In this case, DA release in the NAS could act, via the NAS-amygdala projection, to inhibit cells in the amygdala involved in aversive reactions and autonomic responding. Activation by DA of the NAS-amygdala pathway could, in turn, activate the neurons projecting from the amygdala to the spinal cord, where it could inhibit cells that transmit tonic pain.

Alternative Mechanisms

Although the findings revealed from the present studies strongly suggest that enhanced DA transmission in the NAS mediates the inhibition of tonic pain, the possibility that different neural mechanisms are involved in mediating this effect should not be overlooked. In fact, the evidence that infusions of either tachykinin agonists or morphine into the VTA, and infusions of amphetamine into the NAS induce effects on DA that outlast those on tonic pain suggest that other mechanisms should be considered. Several findings from previous reports suggest that non-DAergic mechanisms might mediate the analgesic effects induced by tachykinin agonists and psychostimulants. For instance, one such mechanism might involve decreased activity of the neurotransmitter acetylcholine in the NAS. There is evidence that the systemic administration of either morphine (Rada et al., 1991a,b,1996) or SP (Boix et al., 1994) decreases extracellular levels of acetylcholine in the NAS, in addition to increasing those of DA at this site. There are also some reports implicating cholinergic activity in the NAS in the processing of aversion. More specifically, it has been reported that extracellular levels of acetylcholine in the NAS are increased in animals experiencing either aversive somatic withdrawal symptoms (Rada et al., 1991, 1996), or conditioned taste aversion (Mark et al., 1995), and in animals exposed to aversive handling stimulation (Boix et al., 1994;

Pfister et al., 1994). Finally, there is also evidence implicating cholinergic activity in reward. For instance, it has been reported that administration of the cholinergic antagonist scopolamine enhances rewarding hypothalamic brain self-stimulation (Stephens & Herberg, 1979) and amphetamine-induced conditioned place preference (Lynch, 1991). All of these findings, together with those described previously suggesting that that the inhibition of tonic pain is due to the alleviation of aversive negative affect, and that reward and analgesia appear to be mediated by a common neural substrate, suggest that decreased cholinergic activity in the NAS might be involved in mediating the analgesic effects induced by tachykinin agonists and psychostimulants. Future studies should be conducted to evaluate the effect of manipulations of the cholinergic system, especially in the NAS, on responses to tonic pain.

Alternatively, it is possible that intra-VTA tachykinin agonists, intra-VTA morphine, and intra-NAS amphetamine induce analgesia in the formalin test by decreasing the activity of serotonergic systems. As mentioned previously, manipulations that deplete serotonin potentiate the analgesic effects of morphine in the formalin test (Abbott & Melzack, 1982; Abbott et al., 1982; Abbott & Young, 1988; Dennis & Melzack, 1979), whereas those that enhance serotonin attenuate morphine analgesia in the test (Abbott & Young, 1988; Dennis & Melzack, 1979). Likewise, there is evidence from the clinical literature that decreased serotonergic function potentiates morphine analgesia (Franklin et al., 1990). In addition, there is evidence that manipulations of serotonergic activity modulate the rewarding effects of psychostimulant drugs. For instance, it has been reported that manipulations that decrease serotonin enhance amphetamine self-administration (Lyness et al., 1980; Lyness & Moore, 1983), whereas those that increase serotonin attenuate this effect (Lyness, 1983) as well as attenuate operant responding to a conditioned reward (Fletcher, 1995). Thus, the findings that decreased serotonergic function potentiates

both the analgesic and rewarding effects of psychostimulant drugs, and that analgesia in the formalin test and reward appear to be associated behavioural phenomena suggest that intra-VTA tachykinin agonists, intra-VTA morphine, and intra-NAS amphetamine may induce analgesia in the formalin test by depressing serotonergic function. To the extent that decreased serotonergic activity might be involved in the analgesic effects induced by these drugs, however, this neurochemical mechanism is likely to take place at a site other than the NAS because there is evidence that intra-VTA DiMe-C7 does not affect extracellular levels in the NAS of the serotonin metabolite, 5-hydroxyindoleactic acid, in animals experiencing formalin pain (Altier, 1993).

It is also possible that the analgesia observed in the present studies was due to the actions of tachykinin agonists, morphine, and amphetamine on noradrenergic systems. As mentioned previously, it has been shown that administration of the alpha adrenergic agonist clonidine induces analgesia and potentiates morphine analgesia in the formalin test (Dennis & Melzack, 1980; Tchakarov et al., 1985). The evidence that amphetamine, which causes the synaptic release and re-uptake blockade of noradrenaline, in addition to DA, administered either systemically or in the NAS induces analgesia in the formalin test lends support to this idea.

Finally, in the case of tachykinin agonists and morphine acting in the VTA, it is possible that, while midbrain DA neurons are critical in mediating the analgesic effects induced by these compounds, those projecting to forebrain sites other than the NAS are important. For instance, it is possible that midbrain DA neurons projecting to the caudate are involved in morphine (Clarke & Franklin, 1994) and SP-induced analgesia in the formalin test. Another possibility is that the DA neurons projecting from the VTA to the medial thalamus, including the paraventricular and mediodorsal nuclei, mediate the analgesic effects of intra-VTA SP and morphine. The evidence that pain-sensitive

neurons are present in these regions (Casey and Jones, 1978; Giesler et al. 1979; Craig and Burton, 1981; Kevetter and Willis, 1982; Albe-Fessard et al. 1985) supports this possibility. Finally, it is also possible that the direct descending projections from the VTA to the periaqueductal gray are involved in mediating the analgesic effects of SP and morphine. Support for this idea comes from the findings that morphine infusions or electrical stimulation into the periaqueductal gray induce analgesia in the formalin test (Manning et al., 1994; Vaccarino & Chorney, 1994).

Analgesia and Locomotor Activity

In addition to inducing analgesia, it was observed that intra-VTA tachykinin agonists, intra-VTA morphine, and intra-NAS amphetamine stimulated locomotor activity, as others have reported (e.g., Elliott et al., 1991; Joyce et al., 1981; Kalivas et al., 1985, 1986; Kelly & Iversen, 1976; Latimer et al., 1987; Stinus et al., 1978). It could therefore be argued that increased locomotor activity might have interfered with the animals' ability to display pain responses, thereby accounting for the analgesic effects reported here. There are several lines of evidence, however, indicating that locomotor activity levels and analgesia in the formalin test are dissociable. First, there is a discrepancy in the time-course between the locomotor stimulant and analgesic effects induced by tachykinin agonists and psychostimulants. More specifically, the locomotor-activating effects of intra-VTA infusions of either SP and DiMe-C7 (e.g., Elliott et al., 1986) or mu receptor agonists (e.g., Joyce et al., 1981; Kalivas & Richardson-Carlson, 1986) and intra-NAS infusions of amphetamine (e.g., Boss et al., 1988) greatly outlast the analgesic effects induced by similar manipulations. For instance, in a previous study, it was found that intra-VTA infusions of DiMe-C7 in animals experiencing formalin-induced pain cause increases in locomotor activity that last at least 110 minutes (Altier, 1993). Pain responses, however, are gradually reinstated approximately 30

minutes following identical manipulations (Experiments 3, 6, and 7). Second, the issue has been examined directly by Abbott (1981), who showed that levels of locomotion and analgesia in the formalin test are uncorrelated. In the same vein, it was observed in Experiment 2 that animals infused with the lowest dose of the NK-1 agonist, GR-73632 (i.e., 0.005 nmol/side), into the NAS were hyperactive and yet displayed pain scores no different from animals infused with saline at this site. Finally, it was observed in the present studies, as was in other studies (Clarke & Franklin, 1992; Manning et al., 1994; Morgan & Franklin, 1991) that animals can locomote (e.g., on three paws) while engaging in pain behaviours. In fact, it was found in Experiment 1 that hyperactive animals infused with analgesic-inducing doses of GR-73632 (i.e., 0.05 and 0.5 nmol/side) into the VTA still spent between 220 and 300 seconds licking or chewing their injured paw, and between 613 and 840 seconds raising their injured paw over a time period of 1800 seconds (see Figure 2).

Probably the strongest evidence that high levels of locomotor activity are not responsible for analgesia in the formalin test comes from the fact that footshock stress-induced analgesia is not accompanied by increased locomotor activity and, just as is the case for systemic or intra-VTA morphine-induced analgesia, is prevented by intra-VTA infusions of either an opioid receptor antagonist (Altier & Stewart, 1996; Morgan, 1990) or Neuropeptide FF (Altier & Stewart, 1997). Other lines of evidence that secondary motor effects do not interfere with an animal's ability to display pain behaviour comes from studies showing that animals that are either cataleptic (Matthies & Franklin, 1992) or hypoactive due to DA receptor antagonist pretreatment (Morgan & Franklin, 1991) display high pain scores in the formalin test. Thus, based on all these findings, it is unlikely that the effects on tonic pain reported in the present experiments are due to secondary motor deficits.

Concluding Remarks

In conclusion, several findings revealed throughout this thesis suggest that part of a pain-suppression system in the central nervous system that serves to inhibit tonic pain depends upon the activation of midbrain ascending DA neurons projecting to the NAS, and that their activation by endogeneous SP released in the VTA is part of a mechanism underlying stress and/or pain-induced inhibition of tonic pain. This idea may provide a resolution to the paradox that exposure to both appetitive (e.g., drug-related cues, palatable food, sexually receptive mate) and aversive (e.g., stress, pain) stimuli activate the same midbrain DA ascending neurons. It may be that these stimuli, although opposing, activate the same neural systems because their effects on behaviour are the same. Indeed, exposure to either type of stimulus motivates organisms, through the activation of the same neural substrate, to engage in behaviours that have a survival value. More specifically, engaging in consummatory behaviours (e.g., feeding, copulation) following exposure to an appetitive stimulus and experiencing relief from persistent pain under aversive stressful conditions are adaptive because they promote survival of the individual and species. Thus, it would appear that midbrain ascending DA neurons are, along with other brain structures, essential to survival.

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