Characterization of Biphasic Modulation of Spinal Nociceptive Transmission by Neurotensin in the Rat Rostral Ventromedial Medulla

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Urban, M. O. and G. F. Gebhart. Characterization of biphasic modulation of spinal nociceptive transmission by neurotensin in the rat rostral ventromedial medulla. J. Neurophysiol. 78: 1550–1562, 1997. Modulation of spinal nociceptive transmission by neurotensin microinjected in the rostral ventromedial medulla (RVM) was examined in anesthetized, paralyzed rats. Forty-three spinal dorsal horn neurons in the L3–L5 spinal segments responding to mechanical and noxious thermal stimulation (50°C) of the plantar surface of the ipsilateral hind foot were studied. Spinal units were classified as either wide dynamic range or nociceptive specific and were located in spinal laminae I–V. Microinjection of neurotensin (0.03 pmol/0.2 μl) into the RVM produced a significant facilitation (135% of control) of spinal unit responses to noxious thermal stimulation (50°C) that lasted ~12 min. In contrast, injection of greater doses of neurotensin (300 or 3,000 pmol) produced an inhibition of spinal unit responses to noxious heat (51.7 and 10.6% of control, respectively) that had a longer duration (60–120 min). The effects of neurotensin on wide-dynamic-range and nociceptive-specific neuron responses to noxious heat were qualitatively and quantitatively similar. Spinal unit responses to graded heating of the skin (42–50°C) were completely inhibited after microinjection of 3,000 pmol of neurotensin into the RVM. Injection of a lesser dose of neurotensin (300 pmol), however, resulted in a partial inhibition of spinal unit responses and significantly reduced the slope of the stimulus-response function to graded heating of the skin. Transection of either the ipsilateral or contralateral dorsolateral funiculus (DLF) significantly reduced the inhibition of spinal nociceptive transmission produced by neurotensin (3,000 pmol) in the RVM, whereas bilateral transection of the DLFs completely blocked the effect. In contrast, bilateral transection of the DLFs had no effect on facilitation of spinal nociception by neurotensin (0.03 pmol) in the RVM. The inhibition of spinal nociceptive transmission by neurotensin (3,000 pmol) in the RVM was completely blocked by injection of the nonpeptide neurotensin receptor antagonist SR48692 (30 fmol) into the RVM 10 min before neurotensin. To confirm a specific block of neurotensin-receptor-mediated effects by the antagonist, a subsequent injection of L-glutamate into the RVM was performed. L-Glutamate (100 nmol) was found to inhibit the nociceptive responses of those spinal units whose responses were no longer inhibited by neurotensin. In contrast, injection of SR48692 (30 fmol) into the RVM failed to block the facilitation of spinal unit responses to noxious heat produced by a subsequent injection of neurotensin (0.03 pmol) into the same site. The present series of experiments demonstrate a specific role for neurotensin in the RVM in the modulation of spinal nociceptive transmission, because the peptide was found to both facilitate and inhibit spinal neuron responses to noxious thermal stimulation. Additionally, the facilitatory and inhibitory effects of neurotensin appear to occur via interaction with multiple neurotensin receptors in the RVM that activate independent systems that descend in the ventrolateral funiculi and DLFs, respectively. The results from these experiments are consistent with prior studies demonstrating that the RVM both facilitates and inhibits spinal nociceptive transmission, and they complement previous work showing that neurotensin in the RVM modulates spinal nociceptive behavioral responses.

INTRODUCTION

It is well established that spinal nociceptive input is modulated by systems that descend from supraspinal sites in the CNS (for reviews see Basbaum and Fields 1984; Gebhart and Randich 1990; Hammond 1986). The rostral ventromedial medulla (RVM) has been identified as an important supraspinal site that modulates spinal nociceptive transmission and includes the nucleus raphe magnus, nucleus reticularis gigantocellularis, and nucleus reticularis paragigantocellularis lateralis (Fields et al. 1991). Initial investigations of the role of the RVM in nociceptive processing focused on inhibition of spinal nociception, because this area was implicated as an important relay in an endogenous descending pain inhibitory system originating from the midbrain periaqueductal gray (Basbaum and Fields 1984).

Recent studies, however, have demonstrated that the RVM both inhibits and facilitates spinal nociceptive transmission. Zhuo and Gebhart (1990a, 1992) demonstrated that low-intensity electrical stimulation or low-dose glutamate injection into the RVM facilitates spinal behavioral and dorsal horn neuron responses to noxious thermal stimulation. In contrast, greater-intensity electrical stimulation or greater doses of glutamate at the same sites in the RVM inhibit these responses. Descending facilitatory and inhibitory systems from the RVM have been found to be anatomically independent; facilitation of spinal nociception involves descending axons in the ventrolateral funiculi (VLF), whereas inhibition is mediated via axons in the dorsolateral funiculi (DLF) (Zhuo and Gebhart 1991a, 1992). These anatomically independent descending facilitatory and inhibitory systems appear to involve different spinal mediators (Ren et al. 1991; Zhuo and Gebhart 1990b, 1991b) and may also be related to physiologically distinct classes of neurons within the RVM (Fields et al. 1991). Thus facilitation and inhibition of spinal nociceptive transmission from the RVM involves distinct, independent systems that may be anatomically, pharmacologically, and physiologically differentiated.

In previous studies, we focused on the role of neurotensin in the RVM in the modulation of spinal nociceptive behavioral responses. Neurotensin is a tridecapeptide that is distributed throughout the CNS, including the RVM, and has been implicated in pain modulation (Behbehani 1992; Beitz
Following intracisternal administration or microinjection into various brain loci, neurotensin was found to produce antinociception in models of both cutaneous and visceral nociception (Clesmeschmidt and McGuffin 1977; Kalivas et al. 1982). Additionally, in a preliminary study, injection of neurotensin into the RVM was found to dose-dependently inhibit the spinal nociceptive tail-flick reflex in anesthetized rats (Fang et al. 1987). Recent studies investigating effects of neurotensin in the RVM on spinal nociceptive transmission demonstrated neurotensin to have a biphasic effect on the spinal tail-flick reflex, because injection of lesser doses facilitates the response whereas greater doses inhibit it (Urban and Smith 1993). An interaction of neurotensin with independent descending facilitatory and inhibitory systems is supported by the observation that these dual effects are anatomically differentiated within the RVM (Urban and Smith 1994). Additionally, descending facilitation and inhibition of spinal nociceptive transmission by neurotensin in the RVM involves different spinal mediators (Urban et al. 1996).

The present series of experiments were designed to expand on the behavioral data by investigating the effects of neurotensin in the RVM on spinal dorsal horn neuron responses to noxious thermal stimulation. A limitation of behavioral nociceptive tests is that the response involves both a sensory and motor component. Therefore, to preclude any effect of neurotensin on motor function, these experiments directly focus on effects of neurotensin in the RVM on spinal nociceptive sensory input.

METHODS

Animal preparation

Adult male Sprague-Dawley rats (400–450 g; Harlan, Indianapolis, IN) were used in all experiments. Animals were anesthetized with an intraperitoneal injection of pentobarbital sodium (45–50 mg/kg) and catheters were inserted into the trachea for mechanical ventilation, into the femoral vein to administer drugs, and into the femoral artery for measurement of blood pressure and heart rate (HR). Animals were paralyzed with pancuronium bromide (0.2 mg iv) and mechanically ventilated for the remainder of the experiment with room air. Anesthesia was maintained with a continuous intravenous infusion of pentobarbital sodium (3–10 mg·kg⁻¹·h⁻¹) and supplemental doses of pancuronium bromide (0.2 mg iv) were administered to maintain paralysis. Arterial blood pressure and HR were continuously monitored, and the animals were kept at physiological temperatures with a hot-water heating pad and overhead lamps. The lumbar and upper sacral spinal cord was exposed by laminectomy between vertebrae T₁₃ and L₄, and the animal was suspended in vertebral clamps with the head fixed in a stereotaxic apparatus. The dura mater was cut, and skin flaps were tied to the stereotaxic frame to form a pool in which warm mineral oil (37°C) was placed to prevent dehydration. A guide cannula through which drugs could be delivered (26-gauge needle shaft) was stereotaxically implanted and maintained 3 mm above the medial RVM (nucleus raphe magnus). The final coordinates for the RVM relative to the interaural line were −2.0 mm rostral-caudal, 0 mm medial-lateral, and −9.5 mm dorsal-ventral (Paxinos and Watson 1986). Tungsten microelectrodes (Micro Probe, Clarksburg, MD, 0.5–1.2 MΩ) were used for extracellular single-unit recording of neurons in the L₁–L₅ spinal segments (0–2.0 mm lateral, 0–1.1 mm from the cord dorsum). Single spinal units were isolated with the use of a window discriminator, amplified through a low-noise amplifier, and displayed on an oscilloscope, and responses were quantified on-line with the use of a computer (CED; Spike2 Analysis software).

Noxious stimulation

Mechanical stimulation (touch, brush, or pinch) of the plantar surface of the ipsilateral hind foot was used as a search stimulus for neurons in the L₁–L₅ spinal segments. Neurons were classified as wide dynamic range on the basis of their ability to respond to noxious pinch with the use of forceps and noxious heat (50°C) as well as nonnoxious light touch (light pressure and brush). Nociceptive-specific neurons were identified on the basis of their ability to respond to noxious pinch and heat, but not nonnoxious light touch. Isolated units were subsequently tested for responsiveness to noxious heat (50°C) in the center of the receptive field. Radiant heat was supplied from a projector lamp containing a copper-constantan thermocouple to allow feedback control for selection of specific temperatures. Heat stimuli were applied for a 10-s duration at 3-min intervals to minimize tissue damage and allow stable unit responses throughout the experiment.

Microinjection procedure

Microinjection of drugs into the RVM was performed by lowering a 33-gauge needle through the guide cannula and delivering 0.2 μl of drug during 30 s. A Hamilton 10-μl syringe was connected to the injection needle with polyethylene tubing (PE 10) containing an air bubble to monitor flow of the drug solution.

Spinal DLF transection

The cervical spinal cord was exposed, and a small amount of Gelfoam, soaked in dilute lidocaine, was applied. The ipsilateral and/or contralateral DLF was then pinched with the use of a pair of fine forceps (Janss and Gebhart 1988).

Histology

At the conclusion of each experiment, rats were killed with an intravenous overdose of pentobarbital sodium, and anodal electrolytic lesions were made in the spinal cord to mark the site of spinal recording. All neurons that were studied were located in dorsal horn laminae I–V, 0–1,100 μm from the dorsum of the spinal cord. Methylene blue was injected into the site of drug administration in the RVM. The brain and spinal cord were removed, stored in 10% Formalin, and frozen, and coronal sections were cut for histological verification of injection, transection, and recording sites.

Data and statistics

Spontaneous unit activity was counted for the initial 10-s period of analysis followed by total number of unit responses to noxious heat during a 15-s period beginning at the onset of stimulation (peristimulus time histogram, 1-s binwidth). These recordings were made for three consecutive trials, with an intertrial interval of 3 min. The total number of counts during the period of stimulation (10 s), and 5 s thereafter, were averaged for the three trials (counts/15 s). The response was considered stable if the intratrial variation was <10%. Stimulus-response functions were generated for various intensities of heat stimulation (42–50°C) with the data represented as counts per 15 s or normalized to a percentage of the response at the greatest stimulation intensity (50°C). After neurotensin injection, time-response functions were generated, with the response (counts/15 s) represented as a percentage of
TABLE 1. Summary of spinal unit sample

<table>
<thead>
<tr>
<th></th>
<th>Wide Dynamic Range</th>
<th>Nociceptive Specific</th>
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<tbody>
<tr>
<td>$n$</td>
<td>35</td>
<td>8</td>
</tr>
<tr>
<td>Spontaneous activity, impulses per 10 s</td>
<td>$20 \pm 7.4$</td>
<td>$10.7 \pm 5.6$</td>
</tr>
<tr>
<td>Response to 50°C, impulses per 15 s</td>
<td>$294 \pm 28.0$</td>
<td>$330 \pm 68.7$</td>
</tr>
<tr>
<td>Percent facilitation, 0.03 pmol NT</td>
<td>$33 \pm 8.2$</td>
<td>$44 \pm 10.0$</td>
</tr>
<tr>
<td>Percent inhibition, 300 pmol NT</td>
<td>$50 \pm 6$</td>
<td>$45 \pm 12$</td>
</tr>
<tr>
<td>Percent inhibition, 3000 pmol NT</td>
<td>$87 \pm 10$</td>
<td>$94 \pm 15$</td>
</tr>
</tbody>
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Values, except $n$ values, are means ± SE. NT, neurotensin.

the predrug (control) response. Dose-response relationships were generated by recording responses following neurotensin injection at the time of maximal effect (represented as % of the control response). Effects of neurotensin on stimulus-response functions were determined by generating a stimulus-response function during the time period in which neurotensin’s effects were stable and maximal. Comparisons of effects of a neurotensin receptor antagonist or spinal DLF transection on neurotensin responses were made in separate experiments because repeated administration of neurotensin into the RVM resulted in a tachyphylaxis (see RESULTS).

The data are represented as means ± SE of measurement. Comparisons between two groups were performed with the use of a $t$-test, whereas statistical analysis for multiple comparisons was carried out with the use of analysis of variance with Fisher’s test for post hoc comparisons. The slopes of stimulus-response functions were determined by linear regression with the use of a curve-fitting computer program (GraphPad Inplot). A $P$ value <0.05 was considered statistically significant in all tests.

Drugs

Drugs used in the present series of experiments were neurotensin, L-glutamate, and sodium nitroprusside (Sigma Chemical, St. Louis, MO) and SR48692 (provided as a gift from SanoBet Recherche, Toulouse, France). All drugs were dissolved in saline, with the exception of SR48692, which was dissolved in 20% dimethyl sulfoxide. The lack of effect of vehicle injection into the RVM was previously determined.

RESULTS

Spinal unit sample

Forty-three spinal dorsal horn neurons in the L₁–L₅ spinal segments (laminae I–V) responding to mechanical (touch, brush, or pinch) and noxious thermal (50°C) stimulation of the plantar surface of the ipsilateral hind foot are identified (Table 1). The majority of spinal units was classified as wide dynamic range ($n = 35$) and the remainder was classified as nociceptive specific ($n = 8$). The spontaneous activity and responses to noxious heat were quantitatively similar for both wide-dynamic-range and nociceptive-specific spinal units. In addition, facilitation and inhibition of responses to noxious heat by neurotensin in the RVM were both qualitatively and quantitatively similar for wide-dynamic-range and nociceptive-specific spinal units in laminae I–V (Table 1). Accordingly, data from wide-dynamic-range and nociceptive-specific neurons recorded in laminae I–V are reported below as a single group.

Effects of neurotensin in the RVM

DOSE AND TIME DEPENDENCE. After characterization of spinal unit responses to noxious heat, neurotensin (0.2 μl) was microinjected into the medial RVM (nucleus raphe magnus) (Fig. 1). Injection of 0.03 pmol neurotensin into the RVM produced a facilitation of spinal unit responses ($n = 6$) to noxious heat; an example is illustrated in Fig. 2. The facilitatory effect had a relatively short duration of 15 min and was maximal ($135.0 \pm 8\%$ of control, mean ± SE) 3 min after injection (data summarized in Fig. 5). Microinjection of a greater dose of neurotensin (3,000 pmol) into the RVM significantly inhibited spinal unit responses ($n = 6$) to noxious heat; an example is illustrated in Fig. 3. Inhibition of spinal nociception was maximal 9 min after injection ($10.6 \pm 5\%$ of control) and had a duration of action of 60–120 min (data summarized in Fig. 5). In 50% of the spinal units studied (3 of 6), 3,000 pmol of neurotensin completely inhibited responses to noxious heat. Spinal unit responses
to noxious heat were significantly attenuated, but not inhibited after injection of 300 pmol neurotensin \((n = 6)\) into the RVM \((51.7 \pm 6\% \text{ of control})\); an example is illustrated in Fig. 4 (data are summarized in Fig. 5).

**Intensity Coding.** The effects of neurotensin injection into the RVM on the stimulus-response function to graded heating \((42–50^\circ \text{C})\) of the ipsilateral hind foot were determined in 12 experiments during the time that the response to neurotensin was near maximal and relatively stable (between 6 and 24 min). Injection of neurotensin \((3,000 \text{ pmol}; \ n = 6)\) into the RVM significantly reduced the slope of the stimulus-response function to graded heating \((\text{from } 12.0 \pm 0.7 \text{ to } 1.2 \pm 0.1\% \text{ /deg C}; \text{ Fig. 6})\) and completely inhibited responses to all intensities of thermal stimulation in four of six spinal units. Injection of a lesser dose of neurotensin \((300 \text{ pmol}; \ n = 6)\) partially inhibited responses at all stimulation intensities, resulting in a significantly reduced slope for the stimulus-response function to graded heat \((\text{from } 12.0 \pm 0.7 \text{ to } 7.4 \pm 0.9\% \text{ /deg C}; \text{ Fig. 6})\). The effects of a facilitatory dose of neurotensin on the stimulus-response function to graded heat could not be determined because of the short duration of this effect.

**Tachyphylaxis.** Because we planned to study the effects of neurotensin before and after transection of the DLF, the effects of multiple injections of neurotensin in the RVM on spinal unit responses to noxious heat were determined in a separate series of experiments. Following injection of a facilitatory dose of neurotensin \((0.03 \text{ pmol})\), a subsequent injection of neurotensin \((0.03 \text{ pmol})\) was made 15 min later, after the response to heat had returned to the basal level. In all cases, this subsequent neurotensin injection was found to have no effect (Fig. 7). To preclude the possibility of nonselective damage to the injection area, a third injection of an inhibitory dose of neurotensin \((300 \text{ pmol})\) was made into the RVM. In all cases \((n = 3)\), the inhibitory effect of neurotensin was apparent and did not differ in magnitude from the effect produced by 300 pmol neurotensin given as a first injection (Fig. 7). Similarly, in one experiment following injection of an inhibitory dose of neurotensin \((300 \text{ pmol})\) into the RVM, a subsequent injection of the same
FIG. 3. A: PSTHs (1-s binwidth) and corresponding oscillographic records illustrating an example of a control response to 50°C heating of plantar surface of ipsilateral hind foot and complete inhibition of response 15 min post NT injection (3,000 pmol) into RVM. B: stimulus-response function for unit illustrated in A before (control) and 15 min after NT injection. Data are expressed as response to heat (impulses/15 s) 15 min following injection of NT into RVM. C: receptive field (shaded area) of spinal unit in A. D: illustration of spinal recording site for unit in A.

Dose 60 min later was found to be ineffective, whereas a third injection of a facilitatory dose (0.03 pmol) of neurotensin was still effective (data not shown).

**Descending pathways mediating neurotensin effects from the RVM**

The effects of transection of the dorsolateral funicular tracts (DLF) on descending modulation of spinal nociception by neurotensin in the RVM were determined. In three experiments, the effects of ipsilateral and subsequent bilateral DLF transection on neurotensin-induced inhibition of spinal nociceptive transmission were determined. Following characterization of spinal unit responses to noxious heat (50°C), transection of the DLF ipsilateral to the spinal recording site had no effect on either spontaneous activity (from 10.3 ± 8 to 11 ± 8.3 impulses/10 s) or heat-evoked responses (from 169 ± 51 to 157 ± 47 impulses/15 s) of spinal units (n = 3). Inhibition of spinal unit responses to noxious heat following injection of neurotensin (3,000 pmol) into the RVM was apparent, but significantly attenuated after transection of the ipsilateral DLF (Fig. 8A). A subsequent transection of the contralateral DLF (resulting in a final bilateral transection) during the period of action of neurotensin completely reversed the remaining inhibition of spinal nociceptive transmission by neurotensin in the RVM (Fig. 8A). Similarly, initial transection of the DLF contralateral to the spinal recording site did not affect spontaneous activity (from 38 ± 24 to 15 ± 12 impulses/10 s) or heat-evoked responses (from 366 ± 122 to 336 ± 130 impulses/15 s) of spinal units (n = 3), but significantly attenuated the inhibition of spinal unit responses to noxious heat by neurotensin in the RVM (Fig. 8A). A subsequent transection of the ipsilateral DLF (resulting in a bilateral transection) during the period of action of neurotensin completely blocked neurotensin-induced inhibition of spinal nociceptive transmission from the RVM (Fig. 8B).

Because the facilitatory effect of neurotensin on spinal nociceptive transmission has a relatively short duration, the
effects of immediate bilateral transection of the DLF on neurotensin-induced facilitation were determined \((n = 3)\). After characterization of spinal unit responses to noxious heat, the DLF were transected bilaterally. In contrast to neurotensin-induced inhibition, bilateral DLF transection had no effect on facilitation of spinal unit responses to noxious heat by injection of neurotensin \((0.03 \text{ pmol})\) into the RVM (Fig. 8C).

**Effects of a neurotensin receptor antagonist on neurotensin responses**

To determine whether the effects of neurotensin on spinal nociceptive transmission from the RVM are neurotensin receptor mediated, the effects of the nonpeptide neurotensin receptor antagonist SR48692 injected into the RVM before neurotensin were determined. Injection of SR48692 \((30 \text{ fmol})\) into the RVM did not affect spontaneous activity \((65 \pm 25 \text{ and } 61 \pm 20 \text{ impulses/10 s, pre- and postinjection, respectively})\) or heat-evoked responses of spinal units \((440 \pm 123 \text{ and } 456 \pm 146 \text{ impulses/15 s, pre- and postinjection, respectively, } n = 6)\). SR48692 \((30 \text{ fmol})\) injected into the RVM 10 min before neurotensin \((3,000 \text{ pmol})\) however, completely blocked the inhibition of spinal unit responses to noxious heat by neurotensin \((n = 3; \text{Fig. } 9A)\). To confirm a selective block of neurotensin-receptor-mediated effects by SR48692, L-glutamate \((100 \text{ nmol})\) was subsequently injected into the RVM. L-Glutamate injection into the RVM produced a rapid, short-lived inhibition of spinal unit responses to noxious heat following SR48692 and neurotensin injections (Fig. 9A).

In contrast to the effect of SR48692 on neurotensin-induced inhibition, injection of SR48692 \((30 \text{ fmol})\) into the RVM 10 min before the facilitatory dose of neurotensin \((0.03 \text{ pmol})\) had no effect on neurotensin-induced facilitation of spinal nociception \((n = 3; \text{Fig. } 9B)\). A subsequent injection of L-glutamate into the RVM was found to produce a short-lived inhibition of spinal unit responses.
Effects of neurotensin on cardiovascular responses

Neurotensin microinjection into the RVM in doses that inhibited spinal nociceptive transmission (300 or 3,000 pmol) produced a dose-dependent decrease in mean arterial blood pressure (MAP) and HR (Table 2). Injection of a dose of neurotensin into the RVM that facilitated spinal nociceptive transmission (0.03 pmol), however, had no effect on either MAP or HR. To preclude the possibility that neurotensin-produced inhibition of spinal nociceptive transmission is due to changes in blood pressure and not to a direct effect on spinal sensory processing, the effect of the direct-acting vasodilator sodium nitroprusside on spinal unit responses to noxious heat was determined. Sodium nitroprusside was administered in a dose (8 μg/kg iv) that produced a decrease in MAP similar to that observed after injection of 3,000 pmol neurotensin into the RVM (ΔMAP = −31.3 ± 7.0 and −28.6 ± 3.2 mmHg for neurotensin and sodium nitroprusside, respectively, n = 3). In contrast to neurotensin, however, sodium nitroprusside had no effect on spinal unit responses to noxious thermal stimulation (n = 3; Fig. 10).

**DISCUSSION**

The results from the present series of experiments complement previous behavioral data demonstrating biphasic modula-
FIG. 7. Effects of NT in RVM on spinal unit responses to noxious heat (50°C) following sequential injection of a facilitatory dose (0.03 pmol) followed by an inhibitory dose (300 pmol). Although a subsequent injection of 0.03 pmol was ineffective, 300 pmol significantly inhibited spinal unit responses to noxious heat (ANOVA, $P < 0.05, n = 3$). Data expressed as % of control (% of response to 50°C before NT) over time.

Neurotensin Modulation of Spinal Nociception

FIG. 7. Effects of NT in RVM on spinal unit responses to noxious heat (50°C) following sequential injection of a facilitatory dose (0.03 pmol) followed by an inhibitory dose (300 pmol). Although a subsequent injection of 0.03 pmol was ineffective, 300 pmol significantly inhibited spinal unit responses to noxious heat (ANOVA, $P < 0.05, n = 3$). Data expressed as % of control (% of response to 50°C before NT) over time.

The area of the RVM has been identified as an important supraspinal site that can both facilitate and inhibit spinal nociception, presumably via distinct, independent descending systems. Zhuo and Gebhart (1990a, 1992) reported that low-intensity electrical stimulation or low doses of glutamate injected into the RVM facilitate spinal behavioral and dorsal horn neuron responses to noxious stimulation, whereas greater-intensity electrical stimulation or greater doses of glutamate inhibit these responses. Additionally, physiologically distinct classes of neurons have been characterized in the RVM on the basis of their activity during various conditions of nociceptive responsiveness (Fields et al. 1991). “OFF Cells” have been identified as exhibiting increased activity during inhibition of nociception, and are thus believed to mediate descending inhibition of nociception from the RVM. In contrast, “ON cells” show increased activity during periods of enhanced nociceptive responsiveness and are believed to mediate descending facilitation of nociception. That two opposing effects on spinal nociceptive transmission may be produced from the RVM, presumably mediated by distinct classes of neurons, suggests that facilitation and inhibition of spinal nociceptive transmission from the RVM involves distinct, independent descending systems. This notion is further supported by the observation that descending facilitation and inhibition involve different spinal mediators; facilitation involves spinal serotonin$_1$, kappa opioid, and cholecystokinin$_B$ receptors, whereas inhibition involves descending cholinergic and noradrenergic systems (Ren et al. 1991; Urban et al. 1996; Zhuo and Gebhart 1990b, 1991b). Additionally, descending facilitation/inhibition of spinal nociceptive transmission by neurotensin appears to be anatomically differentiated within the RVM, because descending inhibition is localized within the medial RVM (nucleus raphe magnus) whereas descending facilitation involves a larger area of the RVM, including the nucleus raphe magnus, nucleus reticularis gigantocellularis, and nucleus reticularis paragigantocellularis lateralis (Urban and Smith 1994). In the current study, all microinjections were confined to the medial RVM (nucleus raphe magnus), and, consistent with the behavioral studies, both facilitatory and
inhibitory effects on spinal nociceptive transmission could be elicited following neurotensin microinjection into this site. It is unlikely that the dual effects of neurotensin on spinal nociceptive transmission are the result of diffusion to other sites. In previous studies in which dyes and radiolabeled substances were used, 0.5-μl intracerebral microinjections were found to diffuse through a volume of tissue having a radius of 0.5 mm from the injection site (Myers 1966; Myers and Hoch 1978). The localization of neurotensin effects within the RVM is further supported in a study that found that microinjection of 0.5 μl of neurotensin into the nucleus raphe magnus produced antinociception, whereas microinjection of 1 mm bilaterally (nuclei reticularis paragigantocellularis lateralis) was without effect (Urban and Smith 1994). Given the smaller microinjection volume used in the current study (0.2 μl), the effects of neurotensin were likely occurring through an action in the medial RVM (nucleus raphe magnus). Additionally, it was found that following injection of either an inhibitory or facilitatory dose of neurotensin into the RVM, a subsequent injection of the identical dose was without effect, whereas injection of a dose producing the opposite response was still effective. These results suggest the occurrence of an acute desensitization or tachyphylaxis to neurotensin effects within the RVM. That repeated stimulation of the same biological system may produce a tachyphylaxis is well documented (see O’Brien 1996 for review). Therefore the apparent lack of cross-tolerance between lesser and greater doses of neurotensin within the RVM further supports an interaction with distinct, independent descending facilitatory and inhibitory systems.
NEUROTENSIN MODULATION OF SPINAL NOCICEPTION

1559
tion of the VLF, however, was found to be ineffective in attenuating these responses (Jones and Gebhart 1987). In contrast, facilitation of spinal nociceptive transmission by RVM or vagal afferent stimulation appears to be attenuated by VLF inactivation (Ren et al. 1989; Zhuo and Gebhart 1991a).

Although it is generally accepted that descending inhibition and facilitation are mediated via spinal DLF and VLF pathways, respectively, exceptions to this notion have been reported. Jones and Gebhart (1987) found descending inhibition from the locus coeruleus to involve the VLF and not the DLF. Furthermore, long-train DLF stimulation has been found to facilitate dorsal horn neuron responses (McMahon and Wall 1988), and the DLF have been implicated in descending facilitation in a model of illness-induced hyperalgesia (Watkins et al. 1994). In the present study, both ipsilateral and contralateral transection of the DLF was found to attenuate the inhibition of spinal nociceptive transmission produced by neurotensin (3,000 pmol) in the RVM. Bilateral transection of the DLF completely blocked neurotensin-induced inhibition of nociception. These results are consistent with those obtained in previous studies and suggest that neurotensin-induced inhibition of spinal nociceptive transmission is mediated bilaterally through the DLF. In contrast, bilateral transection of the DLF was ineffective in attenuating neurotensin-induced facilitation of nociception, suggesting that this effect is not mediated through the DLF and is likely occurring via descending tracts in the VLF. In the current study, DLF lesion alone had no effect on spinal neuron responses to noxious heat, which is consistent with results reported in other studies (Jones and Gebhart 1987; Zhuo and Gebhart 1992). There appears to be conflicting data regarding this subject, however, because certain other studies demonstrated that DLF lesion enhanced spinal nociceptive neuron responses and thereby revealed a tonic descending inhibitory influence (Pubols et al. 1991; Sandkuhler et al. 1987). Although it is unclear why contradictory results have been reported concerning this manipulation, the lack of effect of DLF lesions in the current study precludes the possibility that effects on basal neuron responses account for the block of neurotensin-induced inhibition of spinal nociceptive transmission. These results support the existence of distinct, independent descending systems through which neurotensin is interacting in the RVM to differentially modulate spinal nociceptive transmission.

**TABLE 2. Summary of cardiovascular effects of neurotensin in the RVM**

<table>
<thead>
<tr>
<th>Neurotensin Dose, pmol</th>
<th>ΔMAP mmHg</th>
<th>ΔHR bpm</th>
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<tbody>
<tr>
<td>0.03</td>
<td>1.1 ± 2.3</td>
<td>−2.5 ± 0.5</td>
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<tr>
<td></td>
<td>(−0.8 ± 1.8%)</td>
<td>(−0.9 ± 0.2%)</td>
</tr>
<tr>
<td>300</td>
<td>−17.3 ± 4.0</td>
<td>−72.5 ± 2.5</td>
</tr>
<tr>
<td></td>
<td>(−13.8 ± 3.2%)</td>
<td>(−25.0 ± 0.9%)</td>
</tr>
<tr>
<td>3,000</td>
<td>−31.3 ± 7.0</td>
<td>−73.6 ± 8.6</td>
</tr>
<tr>
<td></td>
<td>(−25.0 ± 5.6%)</td>
<td>(−25.4 ± 3.0%)</td>
</tr>
</tbody>
</table>

Values for change in mean arterial pressure (ΔMAP) and change in heart rate (ΔHR) are means ± SE. RVM, rostral ventrolateral medulla.

**FIG. 9.** Significant block of NT-induced inhibition (A), but not facilitation (B), of spinal unit responses to noxious heat by 30 fmol SR48692 injected into RVM 10 min before NT (○: NT, n = 3; ●: SR48692 + NT, n = 3; ANOVA, P < 0.05). A subsequent injection of L-glutamate (100 nmol) produced a significant inhibition of response (ANOVA, P < 0.05). Data expressed as % of control (% of response 50°C before NT) over time.

**Descending spinal pathways**

Neuronal projections from the RVM descend through the spinal funiculi and terminate on spinal dorsal horn neurons in laminae I–III and V–VII (Basbaum and Fields 1979; Holstege and Kuypers 1982). It has been generally found that inhibition of spinal nociceptive transmission from supraspinal sites occurs via bilateral descending projections in the DLF, whereas facilitatory influences involve the VLF. Inhibition, but not facilitation, of spinal nociceptive transmission by stimulation in the RVM or by stimulation of vagal afferents is attenuated by unilateral transection and abolished by bilateral transection of the DLF (Fields et al. 1977; McCreery et al. 1979; Ren et al. 1989; Sandkuhler et al. 1987; Zhuo and Gebhart 1992). Additionally, direct stimulation of the DLF has been shown to inhibit spinal nociceptive neurons (McMahon and Wall 1988). Inacti-
Neurotensin receptors in the RVM

The nonpeptide neurotensin receptor antagonist SR48692 (Gully et al. 1993) was used in the current study to confirm that neurotensin effects in the RVM on spinal nociceptive transmission were neurotensin receptor mediated. SR48692 has been previously used as a tool to discriminate potential neurotensin receptor subtypes. Dubuc et al. (1994) found SR48692 to inhibit neurotensin-induced hypomotility, but not hypothermia or analgesia in the mouse and rat. In a separate study, SR48692 was shown to inhibit neurotensin-induced hypomotility, but not stimulation of dopamine release in the nucleus accumbens (Steinberg et al. 1994). Additionally, SR48692 was found to be ineffective in inhibiting neurotensin-induced excitation of substantia nigra neurons in vitro (Pinnock and Woodruff 1994). This selective inhibition of neurotensin effects by SR48692 suggests the existence of potential neurotensin receptor subtypes within the CNS. In the current study, injection of SR48692 into the RVM had no effect on either spontaneous unit activity or heat-evoked activity of spinal neurons, suggesting that neurotensin in the RVM does not have a tonic action. However, SR48692 injection into the RVM blocked neurotensin-induced inhibition, but not facilitation of spinal nociceptive transmission. To preclude the possibility that the lack of effect of neurotensin was the result of nonselective inactivation of the RVM by SR48692, L-glutamate was subsequently injected at a dose previously reported to cause a short-lived inhibition of spinal nociceptive transmission (Zhuo and Gebhart 1990a, 1992). Because L-glutamate was found to be effective in inhibiting spinal nociceptive transmission following SR48692 + neurotensin injection, the prevention of neurotensin-induced inhibition by SR48692 was likely due to a selective block of the neurotensin receptor. The selective block of neurotensin-induced inhibition, but not facilitation, suggests a possible interaction with multiple neurotensin receptor subtypes. This notion is supported by the large difference in doses required for the production of these effects. These results are consistent with data demonstrating that SR48692 blocks neurotensin-induced inhibition but not facilitation of the tail-flick reflex following sequential RVM injection in awake rats (Smith et al. 1996). The results from that study support the existence of multiple neurotensin receptors within the RVM that can be discriminated by SR48692. Interestingly, the dose range found to selectively block neurotensin-induced inhibition of the tail-flick reflex (0.03–30 fmol) is consistent with the dose of SR48692 found to selectively block inhibition of spinal nociceptive transmission by neurotensin in the current study (30 fmol).

Cardiovascular effects

In the current study, injection of neurotensin into the RVM in doses that facilitated and inhibited spinal nociceptive
NEUROTENSIN MODULATION OF SPINAL NOCICEPTION

transmission differentially affected resting MAP and HR. The RVM has been implicated in modulating cardiovascular function because this area receives afferent input from the nucleus tractus solitarius and sends descending projections to the thoracic and lumbar intermedullary cell columns (Basbaum et al. 1978; Beitz 1982b; Holstege and Kuypers 1982; see Foreman and Blair 1988 for review). Electrical stimulation in the RVM at intensities that attenuate spinal nociceptive transmission has been shown to produce depressor effects, whereas RVM stimulation at facilitatory intensities is without effect on either MAP or HR (Zhuo and Gebhart 1990a, b, 1992). The effects of neurotensin on MAP and HR observed in the current study are consistent with these results and demonstrate that neurotensin injection in the RVM produces depressor responses at nociceptive inhibitory doses while having no effect on cardiovascular function at facilitatory doses. The inhibition of spinal nociceptive transmission following neurotensin injection into the RVM cannot be attributed to changes in arterial pressure, because intravenous injection of the direct-acting vasodilator sodium nitroprusside produced a depressor response similar in magnitude to that produced by neurotensin while causing no inhibition of spinal neuron responses to noxious stimulation.

In summary, these results complement previous behavioral data and suggest that neurotensin in the RVM can both facilitate and inhibit spinal dorsal horn neuron responses to noxious, cutaneous thermal stimulation at lesser and greater doses, respectively. The facilitatory and inhibitory actions of neurotensin in the RVM on spinal nociceptive transmission are possibly due to an interaction with multiple neurotensin receptors that activate distinct, independent systems that descend through the VLF and DLF, respectively.

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