Effect of Spinal Microinjections of an Antagonist to Substance P or Somatostatin on the Exercise Pressor Reflex

L. Britt Wilson, P. Tim Wall, Kanji Matsukawa, and Jere H. Mitchell

The purpose of this study was to determine the heart rate and arterial blood pressure changes to isometric skeletal muscle contraction and muscle stretch before and after microinjecting an antagonist to substance P (SP) or somatostatin (SOM) into the L-7 dorsal horn region of the spinal cord of anesthetized cats. Anesthesia was induced by administering an anesthetic gas mixture and was subsequently maintained with α-chloralose. Triceps surae contraction was induced by electrically stimulating the L-7 ventral root. Three muscle manipulations (all 1 minute in duration) were performed: 1) continuous tetanic contraction, 2) intermittent tetanic contractions (1 second of contraction, 1 second of relaxation), and 3) passive muscle stretch. Saline microinjections had no effect on the cardiovascular responses to these muscle manipulations. However, both peptide antagonists blunted the pressor response to a continuous tetanic contraction as mean arterial pressure increased 47±4 and 44±4 mm Hg before and 28±3 and 28±4 mm Hg after microinjecting the SP or SOM antagonist, respectively. In contrast, neither antagonist influenced the increase in mean arterial pressure produced by passive stretch; values were 43±6 versus 41±6 mm Hg (SP antagonist) and 39±7 versus 42±7 mm Hg (SOM antagonist) before and after injections, respectively. Microinjecting the SOM antagonist attenuated the pressor response to intermittent tetanic contractions (44±4 mm Hg before SOM antagonist versus 26±4 mm Hg after SOM antagonist), whereas the SP antagonist had no effect (35±3 mm Hg before SP antagonist versus 32±4 mm Hg after SP antagonist). These data suggest that the spinal release of SP and SOM plays a role in eliciting the cardiovascular responses to isometric muscle contraction. Furthermore, the release of these peptides appears to be the result of activation of contraction, not mechanically sensitive muscle afferents. (Circulation Research 1992;70:213–222)

Isometric skeletal muscle contraction, in anesthetized animals, increases arterial blood pressure, heart rate (HR), cardiac output, and myocardial contractility.1–7 This response is abolished by transecting the appropriate spinal roots, indicating that it is a reflex arising from the contracting muscles.1–4 These cardiovascular changes evoked by isometric skeletal muscle contraction are commonly referred to as the “exercise pressor reflex.”6,7

The exercise pressor reflex is mediated by contraction-induced activation of group III and group IV muscle afferents.1,2 However, the discharge pattern of these afferents in response to a variety of muscle manipulations is different. Group III afferents appear to be primarily sensitive to the mechanical events that occur during muscular contraction, because they discharge at the onset of the contraction and the activity is related to the developed tension.5,8–11 Also, these mechanosensitive afferents are activated by passive muscle stretch.5,10 In contrast, the majority of group IV afferents are activated by nonmechanical muscular events, because increased neural discharge occurs later and is enhanced by ischemic muscle contraction.5,8–11 Changes in muscle metabolism and/or temperature may be the stimulus for activating these afferents.5,8–10 Thus, different types of muscle manipulations may preferentially stimulate the mechanically or metabolically sensitive muscle afferents. For instance, passive muscle stretch or brief, intermittent tetanic (IT) contractions may primarily stimulate mechanically sensitive afferents, whereas a continuous tetanic (CT) contraction activates both afferent populations.
Although the afferents that mediate the exercise pressor reflex are known, very little knowledge exists about the central neural mechanisms involved. The majority of thinly myelinated (group III) and unmyelinated (group IV) muscle afferents synapse in the dorsal horn of the spinal cord.\textsuperscript{12} Immunocytochemical studies have shown that this region of the spinal cord contains a variety of neurochemically active substances, including the peptides substance P (SP) and somatostatin (SOM).\textsuperscript{13–16} Both of these peptides are found in the nerve terminals of small-diameter afferents, but they arise from separate populations.\textsuperscript{17–19} Although recent evidence suggests this may be somewhat controversial.\textsuperscript{20} Intrathecal SP administration increases arterial blood pressure and HR.\textsuperscript{21,22} Sciatic nerve stimulation increases the spinal release of SP, but only at intensities that activate groups III and IV afferents.\textsuperscript{23,24} SOM also appears to be released by activation of group IV afferents.\textsuperscript{19} Furthermore, recent studies have shown that intrathecal administration of polyclonal antibodies to SP or an antagonist to SP or SOM blunts the exercise pressor reflex.\textsuperscript{25–27} Thus, the cardiovascular responses to static skeletal muscle contraction can be mediated, at least in part, by the spinal release of SP and SOM from the spinal afferents.

The purpose of this study was to determine the cardiovascular responses to three different muscle manipulations before and after microinjection of an antagonist to SP\textsuperscript{28,29} or SOM\textsuperscript{30} into the L-7 dorsal horn region of the spinal cord. The three interventions, involving the triceps surae muscle of the cat, were 1) a CT contraction, 2) IT contractions, and 3) passive muscle stretch. This study should provide insight into the potential role of SP and SOM in mediating or modulating the cardiovascular responses to muscle afferent activation, as well as the population of afferents that may release these peptides.

Materials and Methods

Surgical Preparation

Twenty-two mongrel cats (mean weight, 3.5 kg) were anesthetized by inhalation of a halothane–nitrous oxide–oxygen mixture. An endotracheal tube was inserted into the airway via a tracheotomy, and catheters were put into a jugular vein and a carotid artery. After catheterization, the anesthetic gas mixture was removed, and anesthesia was maintained with α-chloralose (60 mg/kg i.v.). Anesthesia was supplemented with α-chloralose (5–10 mg/kg i.v.) or sodium pentobarbital (1–2 mg/kg) as necessary. The lungs were ventilated with room air using a respirator (model 661, Harvard Apparatus, South Natick, Mass.). Arterial blood gases and pH were periodically checked and maintained within normal limits by adjusting the ventilator or injecting sodium bicarbonate intravenously. Body temperature was continuously monitored with a rectal probe and was maintained between 37°C and 38.5°C by a water-perfused heating pad and an external heat lamp.

A laminectomy was performed, exposing the lower lumbar and upper sacral portions of the spinal cord. The dura was opened, and the spinal roots were visually identified. The L-7 dorsal and ventral roots were carefully separated, and the ventral root was cut close to its entrance into the spinal cord. The S-1 and L-6 spinal (dorsal and ventral) roots were sectioned. Previous work has shown that this does not affect the exercise pressor reflex.\textsuperscript{31} The calcaneal bone was cut, allowing the Achilles tendon to be connected to a force transducer (model F10, Grass Instrument Co., Quincy, Mass.). The pelvis was stabilized in a spinal unit, and the knee joint was secured by attaching the patellar tendon to a steel post. The peripheral end of the cut L-7 ventral root was placed on a platinum bipolar stimulating electrode, and the exposed spinal cord region was covered with warm mineral oil.

Protocol

After surgery, the cats were allowed to stabilize for at least 30 minutes. After this period, HR and arterial blood pressure responses to three different muscle manipulations were measured. All three muscle interventions involved the triceps surae muscle of the hind limb and lasted for 1 minute. The three muscle manipulations were 1) CT contraction induced by electrically stimulating (three times the motor threshold, 40 Hz, and 0.1 msec) the L-7 ventral root, 2) IT contractions (1 second of contraction, 1 second of relaxation) using the electrical parameters described above, and 3) passively stretching the muscle to a length that matched the peak tension produced by the CT contraction. The order of these interventions was randomized.

After measuring these initial cardiovascular responses, the injector needles were inserted 1.5–2.0 mm into the L-7 dorsal horn region, ipsilateral to the triceps surae muscle that was contracted or stretched. Figure 1 is a schematic diagram illustrating the injector needles used in this study. Briefly, three 30-gauge needles were bound together, −1 mm apart. In turn, these needles were attached to stainless-steel (27-gauge) tubes that were connected to 1-ml syringes (Hamilton Co., Reno, Nev.) by polyethylene (PE-10) tubing. The syringes were filled with saline and placed in an infusion pump (model CMA-10, Bioanalytical Systems, Inc., West Lafayette, Ind.). A removable connection between the syringes and the injector needles allowed the needles to be flushed and refilled with any desired solution. Because the syringes and the injector needles were connected with PE-10 tubing, the solution in the injector needles was expelled when saline was pushed into the PE-10 tubing by the pump.

After the injector needles were inserted into the saline or a solution containing 100–500 ng of the SP antagonist d-Pro\textsuperscript{2–5}·Phe\textsuperscript{2–5}·Trp\textsuperscript{5–7}·substance P\textsuperscript{28,29} (Peninsula Laboratories, Inc., Belmont, Calif.) or 100–500 ng of the SOM antagonist cyclo[7-aminoheptanoyl-phenylalanyl-d-tryptophyl-lysyl-threonyl-

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FIGURE 1. Schematic diagram illustrating the injector needles and exposed portion of the spinal cord (dura has been opened). The injectors were 30-gauge needles and were inserted into the L-7 region of the spinal cord. Note that the L-6 and S-1 spinal roots, as well as the L-7 ventral root, have been cut.

[benzyl]\textsuperscript{30} (Sigma Chemical Co., St. Louis, Mo.) was microinjected into the spinal cord. Larger doses did not produce a greater effect. Both antagonists were dissolved in saline. Microinjections of SP (0.1–100 \( \mu \text{g} \)) or SOM (0.1–100 \( \mu \text{g} \)) failed to elicit changes in HR or arterial blood pressure. The injector needles were removed from the spinal cord, and HR and blood pressure responses to the three muscle manipulations were again determined. Saline microinjection always preceded the peptide antagonist injection. When it became apparent that saline had no effect on the cardiovascular responses to the muscle interventions (see “Results”), this injection was eliminated. In four cats, both antagonists were tested, but not on the same side of the spinal cord. After euthanasia, the spinal cord was removed, and the tissue was fixed by placing it in a 10\% formalin solution. In eight cats, 1 \( \mu \text{l} \) Evans blue dye was microinjected (1 \( \mu \text{l}/\text{min} \)) into the dorsal horn region before euthanasia. Transverse frozen sections of the spinal cord (50 \( \mu\text{m} \)) were cut to determine the location of the injector tracts and the dye distribution.

In summary, the cardiovascular responses evoked by two different types of isometric contractions and passive stretch of the triceps surae muscle were determined before and after microinjecting 1 \( \mu \text{l} \) saline, an SP antagonist, or an SOM antagonist into the L-7 dorsal horn region of the spinal cord. Thus, the potential role of the spinal release of these peptides in modulating or mediating this reflex was tested. A CT contraction activates both mechanically sensitive (primarily group III) and nonmechanically sensitive (primarily group IV) muscle afferents, whereas passive muscle stretch should only stimulate mechanically sensitive fibers. IT contractions should primarily stimulate mechanically sensitive afferents, but this is a contraction-induced (not passive) activation. A \( >20 \text{ mm Hg} \) increase in arterial blood pressure in response to a muscle manipulation, before antagonist injection, was a criterion used in this study. It should be noted that not all the interventions elicited this magnitude of a pressor response in a given cat. Thus, it was not possible to obtain data for all three muscle manipulations in every cat, although one or two of the interventions may have been analyzed. A portion of these cats were included in a separate study.\textsuperscript{31}

Measured and Calculated Variables

Arterial blood pressure was measured by connecting the carotid artery catheter to a pressure transducer (Statham P23ID, Gould, Cleveland, Ohio). Mean arterial pressure (MAP) was obtained by integrating the arterial signal with a time constant of 4 seconds or was calculated from the pulsatile arterial blood pressure tracing. HR was derived from the arterial pressure pulse by a biotachometer (Gould), and muscle tension was measured using the force transducer. Muscle tension is reported as the difference between peak tension and resting tension. Pulsatile arterial pressure, MAP, HR, and tension were simultaneously recorded on an eight-channel chart recorder (model 2800S, Gould). Time course data were measured in 10-second intervals from the onset to the end of the contraction or stretch, and 30- and 60-second time points after manipulation were also determined. In some experiments, the data were analyzed on line with a minicomputer (DEC PDP 11/23).

Data Analysis

Data are expressed as mean±SEM. Time course data were analyzed using a two-way analysis of variance. Tukey’s test was performed when a significant \( F \) value was found. A paired Student’s \( t \) test, using the Bonferroni modification, was performed to compare resting values, before and after microinjection, to the peak changes produced by a given muscle manipulation.\textsuperscript{32} For both analyses, \( p<0.05 \) was used as the level of statistical significance.

Results

Saline Microinjections

A CT contraction, IT contractions, and passive muscle stretch were performed before and after microinjecting 1 \( \mu \text{l} \) saline into the L-7 dorsal horn region of the spinal cord. All three muscle perturbations increased MAP and HR, and these changes were not diminished by microinjecting saline into the spinal cord (Table 1). Also, saline microinjections had no effect on the time course of the responses to the three muscle manipulations. In experiments performed on five separate cats, cardiovascular responses to a CT contraction were not altered even after five microinjections of saline. This suggests that the injectant volume did not elicit extensive spinal damage resulting in a nonspecific reduction in the pressor and HR responses to isometric muscle contraction or passive muscle stretch. Resting MAP was
lower after the saline injection for the CT contraction, and there was also a tendency for it to be less for the other two manipulations. This is probably the result of time, since the presaline manipulations were performed before insertion of the injector needles into the spinal cord (see "Materials and Methods").

**SP Antagonist Microinjections**

Figures 2–4 are original tracings from one cat and illustrate the cardiovascular responses to the three muscle manipulations before and after microinjecting the SP antagonist into the L-7 dorsal horn region of the spinal cord. Figure 2 shows that the SP antagonist blunted the cardiovascular responses to a CT contraction even though tension was the same before and after microinjection. The SP antagonist failed to attenuate the pressor and HR responses to muscle stretch (Figure 3) or IT contractions (Figure 4) in the same cat.

The time course of the changes in MAP in response to a CT contraction before and after SP antagonist microinjection is illustrated in Figure 5A. MAP increased within 10 seconds after the onset of muscle contraction and peaked ~10–20 seconds later. This increase was maintained throughout the remainder of the contraction. MAP subsequently decreased after the contraction, but it remained above the precontraction level for the 1-minute post-contraction time period. Microinjecting the SP antagonist did not influence the time course of the pressor response to the CT contraction, but it did attenuate the magnitude (Figure 5A and Table 2). The SP antagonist also blunted the HR response to a CT contraction, and there was also a tendency for it to be less for the other two manipulations. This is probably the result of time, since the presaline manipulations were performed before insertion of the injector needles into the spinal cord (see "Materials and Methods").

**TABLE 1. Hemodynamic and Tension Data Before and After Saline Injection**

<table>
<thead>
<tr>
<th></th>
<th>Tension before saline injection</th>
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<td></td>
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<td>MAP (mm Hg)</td>
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<td>HR (bpm)</td>
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<td>HR (bpm)</td>
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<tr>
<td>Tension (kg)</td>
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<td>11.5±0.7*</td>
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<tr>
<td>IT contractions (n=7)</td>
<td></td>
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</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>116±10.9</td>
<td>156±10.5*</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>189±4.7</td>
<td>212±6.6*</td>
</tr>
<tr>
<td>Tension (kg)</td>
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<td>10.1±1.1*</td>
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</table>

Values are mean±SEM. CT, continuous tetanic; MAP, mean arterial pressure; HR, heart rate; bpm, beats per minute; IT, intermittent tetanic. Peak tension is the difference between the absolute maximum and resting tension. *Significant increase above control (p<0.05). †Significant difference before vs. after saline injection (p<0.05).
contraction, but tension development was not different (Table 2).

Stretching the triceps surae muscle increased MAP in a manner similar to the CT contraction, but arterial blood pressure appeared to decrease more rapidly after the stretch (Figure 5B). Microinjections of the SP antagonist into the L-7 dorsal horn region did not affect the time course or the peak MAP and HR changes elicited by muscle stretch (Figure 5B and Table 2). Peak tension before and after the microinjection was the same (Table 2).

Figure 5C illustrates the time course for the changes in MAP in response to IT contractions before and after the SP antagonist injection. MAP increased within 20 seconds after the onset and peaked 30–40 seconds from the start of the contractions. MAP remained elevated after the cessation of the IT contractions. Microinjecting the SP antagonist had no effect on the time course or the peak changes in MAP and HR (Figure 5C and Table 2). Peak muscle tension was the same before and after injection (Table 2).

**SOM Antagonist Microinjections**

Similar to the SP antagonist, microinjecting the SOM antagonist attenuated the pressor response to the CT contraction but did not alter the time course (Figure 6A and Table 3). Peak tension was the same before and after SOM antagonist injection, and there was a tendency for the HR change to be blunted (Table 2). Original tracings demonstrating the cardiovascular changes produced by a CT contraction before and after SOM antagonist injection were similar to those seen in Figure 2. Thus, both the SOM antagonist and the SP antagonist blunted the pressor reflex evoked by a CT contraction.

The cardiovascular responses to muscle stretch were unaffected by microinjecting the SOM antagonist into the L-7 dorsal horn region of the spinal cord.
There was no difference in the peak changes in MAP, HR, and tension before and after SOM antagonist injection or in the time course of these responses (Figure 6B and Table 3). Original tracings demonstrating the cardiovascular changes produced by passive muscle stretch before and after SOM antagonist injection were similar to those seen in Figure 3. Thus, neither the SOM antagonist nor the SP antagonist blunted the cardiovascular responses elicited by passive muscle stretch.

In contrast to the SP antagonist, the SOM antagonist attenuated the pressor response to IT contractile (Figure 6C). Only the magnitude of the response was reduced, because the time course was the same (Figure 6C and Table 3). Also, there was a tendency for the HR response to be blunted, but it did not reach statistical significance (Table 3). Peak tension was slightly reduced after microinjecting the SOM antagonist. It is unlikely that this reduction in tension is the cause of the attenuated cardiovascular responses to IT contractions after injection of the SOM antagonist. First of all, this is a small reduction in peak tension (~1 kg). Furthermore, in five of the seven cats, the pressor and HR responses to IT contractions returned to or were near the same level as that evoked before the SOM antagonist injection after a period of time (3–7 hours), despite a further reduction in peak tension.

In two of the cats in which the SOM antagonist was microinjected, the cardiovascular responses to passive stretch were determined before and after microinjecting 2% lidocaine into the L-7 dorsal horn region of the spinal cord. Each muscle manipulation began at time 0 and was 1 minute in duration. Data are mean±SEM. *Significant increase above control (p<0.05). †Significant difference before vs. after antagonist injection (p<0.05).

![Figure 5](image_url)

**Figure 5.** Time course data for the change in mean arterial pressure (MAP) in response to a continuous tetanic (CT) contraction (panel A, n=11), passive stretch (panel B, n=7), and intermittent tetanic (IT) contractions (panel C, n=7) of the triceps surae muscle before (open diamonds) and after (closed diamonds) microinjecting an antagonist to substance P into the L-7 dorsal horn region of the spinal cord. Each muscle manipulation began at time 0 and was 1 minute in duration. Data are mean±SEM. *Significant increase above control (p<0.05). †Significant difference before vs. after antagonist injection (p<0.05).

<p>| Table 2. Hemodynamic and Tension Data Before and After Injection of Substance P Antagonist |
|--------------------------------------------------|--|--------|--|--------|--|--------|--|--------|
| Tension before SP antagonist injection | Tension after SP antagonist injection |</p>
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<th>Change</th>
<th>Control</th>
<th>Peak</th>
<th>Change</th>
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<td>8.9±0.8*</td>
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<tr>
<td>MAP (mm Hg)</td>
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<td>Tension (kg)</td>
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<td><strong>IT contractions</strong> (n=7)</td>
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<td>MAP (mm Hg)</td>
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<td>HR (bpm)</td>
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<td>1.0</td>
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</table>

Values are mean±SEM. SP, substance P; CT, continuous tetanic; MAP, mean arterial pressure; HR, heart rate; bpm, beats per minute; IT, intermittent tetanic. Peak tension is the difference between the absolute maximum and resting tension.

*Significant increase above control (p<0.05).
†Significant difference before vs. after SP antagonist injection (p<0.05).
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Histology

In the experiments in which 1 µl Evans blue dye was microinjected into the L-7 dorsal horn region of the spinal cord, the injector needles were inserted into the same tracks and to the same depth as the previous injections for that individual cat. Figure 7 represents the relative dye distribution for these cats. The dye covered the entire dorsal horn region and extended ventrally to at least the level of the central canal. The rostral-caudal spread was ~7 mm. The injection tracts from the remaining spinal cords (n = 10, no dye injection) were similar to those seen in the dye-injected cords. Thus, the dye distribution illustrated in Figure 7 probably reflects the spread of the microinjection of saline and the peptide antagonists performed in these studies. However, it should be noted that the microinjections spread to the ventral horn in at least three cats. It was not possible to obtain histological data from the spinal cords of four cats because of technical difficulties.

Discussion

The purpose of this study was to determine if microinjecting an antagonist to SP or SOM into the L-7 dorsal horn region of the spinal cord blunts the cardiovascular responses elicited by two different patterns of isometric skeletal muscle contractions or passive muscle stretch. Both antagonists attenuated the pressor response to the CT contraction, yet neither antagonist affected the MAP and HR increases produced by stretching the triceps surae muscle. Peak muscle tension for the two conditions

FIGURE 6. Time course data for the change in mean arterial pressure (MAP) in response to a continuous tetanic (CT) contraction (panel A, n = 7), passive stretch (panel B, n = 6), and intermittent tetanic (IT) contractions (panel C, n = 7) of the triceps surae muscle before (open diamonds) and after (closed diamonds) microinjecting an antagonist to somatostatin into the L-7 dorsal horn region of the spinal cord. Each muscle manipulation began at time 0 and was 1 minute in duration. Data are mean ± SEM. *Significant increase above control (p < 0.05). †Significant difference before vs. after antagonist injection (p < 0.05).

TABLE 3. Hemodynamic and Tension Data Before and After Somatostatin Antagonist Injection

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<tr>
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<tr>
<td>MAP (mm Hg)</td>
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<td>Tension (kg)</td>
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<td>HR (bpm)</td>
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<tr>
<td>Tension (kg)</td>
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<tr>
<td>IT contractions (n=6)</td>
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<tr>
<td>MAP (mm Hg)</td>
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<tr>
<td>Tension (kg)</td>
<td>1.0</td>
<td>8.5±1.0*</td>
</tr>
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</table>

Values are mean ± SEM. SOM, somatostatin; CT, continuous tetanic; MAP, mean arterial pressure; HR, heart rate; bpm, beats per minute; IT, intermittent tetanic. Peak tension is the difference between the absolute maximum and resting tension.

*Significant increase above control (p < 0.05).
†Significant difference before vs. after SOM antagonist injection (p < 0.05).
was the same. Microinjecting the SOM antagonist attenuated the pressor response produced by IT contractions, but the SP antagonist had no effect. These results suggest that the spinal release of SP and SOM plays a role in mediating the exercise pressor reflex.

Immunocytochemical studies have demonstrated that afferent fibers synapsing within the dorsal horn of the spinal cord contain SP and SOM, although the population of nerve fibers containing these peptides is different.13–17 Administering capsaicin, a compound that causes the degeneration of group IV (unmyelinated) primary afferents, markedly depletes the spinal content of SP and SOM.33,34 Also, electron-microscopic analysis of dorsal roots indicates that these peptides are found exclusively in group IV fibers.35 Furthermore, the spinal release of SP can be evoked by electrically stimulating primary afferent nerve fibers, but only at intensities that activate group III and group IV afferents.23,24 Thus, anatomic and physiological studies suggest that SP and SOM may be spinal neurotransmitters or neuromodulators released in response to activating group IV afferents.36,37

Although both peptides have been associated with group IV afferents, the modality of the sensory stimulus that elicits their spinal release may be different. Previous studies have shown that noxious mechanical stimuli cause the spinal release of SP, while SOM-containing fibers are activated by thermal stimuli.18,19,38 Innocuous stimuli did not alter the release of these peptides. These studies suggested that SP-containing primary afferents are polymodal nociceptors, whereas SOM may be released by thermosensitive fibers.18,19,38 However, skin fibers were the afferents tested in the aforementioned experiments. It is unclear if modality-specific release of these peptides exists for muscle afferents.

In this study, microinjecting an antagonist to SP or SOM into the L-7 dorsal horn region of the spinal cord blunted the exercise pressor reflex. This supports previous work suggesting that the spinal release of these peptides plays a role in mediating the exercise pressor reflex.25–27 However, this study differs from the previous investigations25,27 because the antagonists were microinjected directly into the L-7 dorsal horn region as opposed to intrathecal administration. This microinjection provides a more localized application of the peptide antagonist (Figure 7). Nevertheless, whether the spinal release of these peptides is the result of activation of mechanosensitive or metabosensitive (or both) afferents is unknown. Thus, additional muscle manipulations were performed in an attempt to answer this question.

In contrast to the CT contraction, neither antagonist blunted the increase in MAP or HR elicited by passive muscle stretch. This manipulation should have only activated mechanosensitive afferents, the majority of which appear to be group III fibers.8,10 Stebbins et al.30 demonstrated that passive stretch of the triceps surae muscle of the cat increases arterial blood pressure and HR without altering the chemical composition of the venous blood draining the muscle. Thus, it is unlikely that metabolically sensitive afferents are activated by this maneuver. The fact that microinjecting the antagonists into the spinal cord failed to alter the cardiovascular changes evoked by muscle stretch suggests that the MAP and HR responses to activation of mechanosensitive group III afferents is not mediated by the spinal release of SP and SOM. On the other hand, the population of mechanosensitive afferents stimulated by the CT contraction may be different from the ones activated by muscle stretch. Thus, the mechanosensitive afferents activated by a CT contraction may release SP and SOM into the dorsal horn, but these same fibers may not be stimulated by muscle stretch. It is unknown which, if either, explanation is correct.

IT contractions primarily activate the mechanically sensitive group III afferents.8,10 However, this is a contraction-induced stimulation; thus, metabolic changes will occur. Microinjecting the SOM antagonist attenuated the cardiovascular changes to IT contractions, whereas the SP antagonist had no effect. It is possible that contraction-induced increases in muscle temperature may have activated group IV afferents containing SOM. This is supported by other studies that have suggested that skin thermosensitive afferents (group IV) contain SOM.18,19,38 On the other hand, the group III afferents activated by IT contractions may release SOM. This seems unlikely, because microinjecting the SOM antagonist had no effect on the cardiovascular responses to passive muscle stretch. Passive muscle stretch should have primarily activated group III afferents.8,10 Thus, the spinal release of SOM is probably not the result of group III afferent activation, but this possibility cannot be eliminated.

Microinjecting the SP antagonist attenuated the cardiovascular responses to the CT contraction but had no effect on the changes elicited by IT contractions. This discrepancy may be the result of differences in the blood flow perfusing the contracting regions of the spinal cord.
muscles. A sustained isometric contraction of the magnitude produced in this study may decrease the blood flow through the muscle, thereby allowing some accumulation of metabolic byproducts. These metabolic products probably stimulate group IV muscle afferents that are sensitive to these chemical changes. On the other hand, blood flow is not impeded for at least the 1-second time interval between each of the IT muscle contractions, thus preventing the accumulation of metabolic byproducts in the muscle. This type of contraction has been shown to preferentially activate group III afferents. Also, microinjecting the SP antagonist did not affect the cardiovascular responses to group III activation evoked by passively stretching the triceps surae muscle. Thus, the results of this study support the contention that the spinal release of SP is the result of group IV muscle afferent activation.

The effect of the antagonists on the cardiovascular changes produced by the isometric contractions was probably not the result of some nonspecific action of the compounds or extensive spinal trauma elicited by the injection itself. Microinjecting saline into the L-7 dorsal horn failed to alter the increases in MAP and HR produced by the three muscle manipulations, even after five injections. In some cats, the magnitude of the cardiovascular responses to the CT and/or IT muscle contractions returned to the preinjection level after a period of time (3–7 hours). It is unclear why this occurred in only a portion of the cats, but the preparation may have deteriorated because of the length of time. More convincing evidence that the peptide antagonists did not exert a nonspecific reduction in spinal neuronal excitability is the fact that both antagonists attenuated the cardiovascular responses to only one (or two) but not all three muscle manipulations. If the SP or SOM antagonist had exerted a neurotoxic effect, then the increases in MAP and HR produced by all three muscle manipulations would have been reduced.

Although the peptide antagonist did not exhibit a neurotoxic effect, this does not provide the specificity of these compounds. It must be kept in mind that SP is a member of a class of closely related neuropeptides collectively referred to as tachykinins. Furthermore, at least three different receptors seem to be involved in the physiological actions of the mammalian neurokinins. The antagonist used in this study may have nonselectively blocked all three receptor subtypes, thereby not only blocking the actions of SP but the other neurokinins as well. Thus, the possibility exists that neurokinins other than SP, either alone or in combination, may play a role in mediating the exercise pressor reflex. A similar situation regarding SOM may exist; recent evidence suggests that there are receptor subtypes mediating the actions of this neuropeptide.

One limitation of this study is the inability to test the efficacy of the antagonists. As stated in “Materials and Methods,” microinjecting the peptide agonist (SP or SOM) had no effect on MAP or HR. Thus, it was not possible to accurately test the antagonists’ efficacy in blocking the actions of SP and SOM. However, this limitation should not detract from the results and interpretations of this study, because it is unlikely that the peptide antagonists exerted a nonspecific effect on the spinal neurons (see preceding paragraph).

In conclusion, the increases in MAP and HR produced by a CT contraction were attenuated by microinjecting an antagonist to SP or SOM into the L-7 dorsal horn region of the spinal cord. This suggests that the spinal release of these peptides plays a role in mediating the exercise pressor reflex. Furthermore, the SOM antagonist blunted the cardiovascular responses to IT contractions, but the SP antagonist had no effect. Neither antagonist altered the cardiovascular changes evoked by muscle stretch. These results indicate that the spinal release of SP and SOM is the result of activation of nonmechanically sensitive muscle afferents, although the stimulus that activates the afferents containing these peptides may be different.

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