Effects of Graded Muscle Contractions on Spinal Cord Substance P Release, Arterial Blood Pressure, and Heart Rate

L. Britt Wilson, Ingbert E. Fuchs, Jere H. Mitchell

The release of substance P (SP)–like immunoreactivity (SP-LI) in the dorsal horn of the spinal cord and the cardiovascular changes to both high-tension (HT) and low-tension (LT) contractions were determined using α-chloralose–anesthetized cats. Over a 10-minute period, seven contractions (HT or LT) were induced. Each contraction was 20 seconds in duration and was followed by an 80-second quiescent period. The tension-time index (TTI) for the HT contractions was 2751±348 kg·s (mean±SD), which was greater than the TTI of 813±167 kg·s for the LT contractions. The HT contractions caused a greater release of SP-LI than the LT contractions: SP-LI increased from 0.18±0.02 to 0.32±0.03 fmol/100 μL and from 0.18±0.02 to 0.25±0.04 fmol/100 μL for the two types of contractions, respectively. Concomitant with this greater SP-LI release, HT contractions caused larger increases in mean arterial pressure (34±16 versus 11±4 mm Hg) and heart rate (18±7 versus 8±4 beats per minute) than did the LT contractions. These changes in SP-LI, mean arterial pressure, and heart rate were virtually abolished when the contractions were repeated after sectioning the L-5–S-2 dorsal and ventral roots or when the electrical stimulation of the ventral roots was repeated after muscle paralysis with gallamine triethiodide. These results demonstrate that contraction-evoked SP-LI release in the dorsal horn is related to the developed tension. Furthermore, these data provide additional support for the hypothesis that the release of SP from the central terminations of muscle afferents plays a role in mediating the cardiovascular responses to static contraction of skeletal muscle. (Circ Res. 1993;73:1024-1031.)

KEY WORDS • substance P • exercise pressor reflex • cats • dorsal horn • microdialysis

It has been demonstrated using anesthetized cats that static contraction of the triceps surae muscle increases arterial blood pressure, heart rate, myocardial contractility, and renal sympathetic nerve activity.1-6 These changes are a reflex arising from the contracting muscle, because they are abolished by cutting the appropriate spinal roots.2,3,5,6 The reflex cardiovascular and sympathetic responses are mediated by a contraction-induced activation of group III (thick myelinated) and group IV (unmyelinated) muscle afferents.2 These afferents appear to respond to the mechanical and metabolic alterations that occur during the contraction.7-9 Furthermore, the magnitude of afferent activation and the reflex autonomic responses are related to the developed tension.1,5,8,10,11

The majority of group III and IV fibers synapse in the dorsal horn of the spinal cord.12-14 Thus, this region may be the site of the first synapse for the reflex cardiovascular changes seen during muscle contraction. The dorsal horn of the spinal cord contains numerous compounds that may be the neurotransmitters/neuromodulators of primary afferents.15-17 Substance P (SP) is one such compound.18-20

Several studies have investigated the possibility that the release of SP in the dorsal horn plays a role in mediating the reflex changes elicited by static contraction.21-25 Administration of an antagonist to SP, either intrathecally or directly into the dorsal horn, blunts the cardiovascular changes elicited by static contraction.21,24 Hill et al25 showed that the intrathecal administration of a neurokinin-1 (NK-1; this receptor preferentially binds SP) antagonist attenuates the pressor response elicited by muscle contraction. Furthermore, we have recently demonstrated that static muscle contraction increases the release of SP in the dorsal horn.25 Thus, the release of SP in the spinal cord as a result of muscle afferent activation appears to play a role in mediating the reflex cardiovascular and renal sympathetic increases caused by static contraction. Because the reflex autonomic changes are related to the developed tension,1,5,10,11 we hypothesized that the release of SP in the dorsal horn is also influenced by the degree of force production.

Materials and Methods

Experimental Preparation

Mongrel male cats (mean weight, 3.9 kg; n=8) were anesthetized by breathing a gaseous mixture containing halothane (2% to 5%), nitrous oxide (1 to 3 L/min), and oxygen (1 to 3 L/min). An endotracheal tube was inserted into the airway via a tracheotomy, and cathe-
ters were placed 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 IV). If the animal’s pupils became dilated and/or they exhibited a corneal reflex or withdrawal of the limb in response to noxious pinch of the paw, additional α-chloralose (5 to 10 mg/kg IV) or sodium pentobarbital (1 to 2 mg/kg) was administered to maintain an adequate depth of anesthesia. In those animals that were paralyzed (see below), additional anesthesia was provided if arterial blood pressure and/or heart rate increased after inducing the paralysis. The lungs were ventilated with room air using a respirator (model 552, Harvard Instruments, South Natick, Mass). Arterial blood gases and pH were periodically checked and maintained within normal limits (pH, 7.30 to 7.40; Pco2, 32 to 36 mm Hg; Po2, >80 mm Hg) by adjusting the ventilator, providing supplemental oxygen, or injecting sodium bicarbonate intravenously. Rectal temperature was continuously monitored and maintained between 37° 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 and S-1 dorsal and ventral roots were carefully separated, and the ventral roots were cut close to the spinal cord. The L-5, L-6, and S-2 dorsal and ventral roots were sectioned. Cutting these roots should have no effect on the reflex cardiovascular responses induced by static contraction.26 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 (David Kopf Instruments, Tujunga, Calif), and the knee joint was secured by attaching the patellar tendon to a steel post. The exposed spinal cord region was kept moist with saline.

Arterial blood pressure was measured by connecting the carotid artery catheter to a pressure transducer (model P23ID, Statham, Oxnard, Calif). Mean arterial pressure was obtained by integrating the arterial signal with a time constant of 4 seconds. Heart rate was derived from the arterial pressure pulse by a biotachometer (Gould Instruments, Cleveland, Ohio), and muscle tension was measured using the force transducer. The tension-time index (TTI) for each contraction was determined by measuring the area under the tension trace. The TTI for the high-tension (HT) or low-tension (LT) contractions represents the sum of the TTI values for the 14 contractions (see below). All measured variables were continuously recorded on an eight-channel chart recorder (Gould, 2800S). Control values were determined by analyzing at least 30 seconds of the data immediately before muscle contraction.

**Microdialysis**

After isolating the dorsal and ventral roots, a microdialysis probe (model CMA-5410, 3 mm membrane, Bioanalytical Systems Inc, West Lafayette, Ind) was lowered into the center of the rostral-caudal extent of the L-7 dorsal horn region using a stereotaxic carrier (David Kopf Instruments). The probe was inserted so that the entire membrane was submerged in the spinal cord tissue. The probe was continuously perfused at a rate of 5 μL/min with a physiological salt solution containing 0.2% bovine serum albumin, 0.1% bacitracin, and the following ions (mmol/L: K+, 6.2; Cl−, 134; Ca2+, 2.4; Na+, 150; P−, 1.3; HCO3−, 13; and Mg2+, 1.3). This solution was made fresh for each experiment (in vivo and in vitro, see below). After probe insertion, timed collections (20 minutes) were begun. During the six collection periods, the probe was surrounded by agar so that the height of the agar mound was even with the muscles of the back. Next, a piece of parafilm was placed around the shaft of the probe, on top of the agar.

A quick-setting silicone elastomer (A643-K, Smith and Nephew Rolyan Co, Menomonee Falls, Wis) was applied to the top of the parafilm so that the parafilm was fixed to the shaft of the probe and the muscles of the back. This procedure allowed the carrier to be removed from the shaft of the probe; thus, the probe remained in the same relative position despite any movement of the back. On a separate day, the concentration of SP-like immunoreactivity (SP-LI) of the perfusates was measured using a radioimmunoassay (RIA; see below). The RIA was performed in a blinded fashion.

A new probe was used for each experimental animal. The percent recovery of SP-LI for five of the eight probes was tested in vitro on a separate day. The probe was perfused at the same rate as used for the animal experiment (5 μL/min). The probe was inserted into vials containing varying concentrations of SP. A minimum of three timed collections (20 minutes) was taken from each vial, and the percent recovery from the first collection was omitted to ensure that stabilization of the concentration gradient occurred. The average percent recovery from the remaining two collections was calculated to determine the percent recovery for each probe. For three of the probes, this in vitro test was performed before use in the animal. The remaining two probes were tested after the animal experiment. The recovery for the three probes that were tested before the experiment was 12.3 ± 1.9% (mean ± SD), which was similar to the other two probes (14.4 ± 1.7%). The average recovery of SP-LI for the five probes was 13.1 ± 2.0%.

**RIA for SP-LI**

This procedure has been previously described in detail.25 Briefly, removable microplate wells (Removewell Immulon 4, Dynatech, Rochester, NY) were coated with 1 μg purified protein A (Purified Recomb Protein A, binding 1.3 mg rabbit IgG per mg, Pierce Chemical Co, Rockford, Ill) in 100 μL of 0.2 mol/L sodium borate (pH 9.0). After a 24-hour incubation period at 4°C, wells were washed three times with an RIA buffer (0.05 mol/L sodium phosphate buffer, pH 7.4). Fifty microliters of the purified SP antiserner, diluted in RIA buffer, was added. The wells were again washed three times with the RIA buffer after 8 hours of incubation at 4°C. Remaining protein binding sites on the polystyrene-like wells were saturated by incubating with 3% bovine serum albumin in phosphate-buffered saline overnight at 4°C. After washing three times with the RIA buffer, standards in triplicate and samples (90 μL) were added to the wells and incubated for 24 hours at 4°C. 125I-labeled [Tyr5]SP-(1-11) (110 μL) was added to standards and samples and incubated for 24 hours at 4°C. Wells were washed three times with RIA buffer, separated, and counted for 10 minutes in a 20-well
TABLE 1. Microdialysis Protocol

<table>
<thead>
<tr>
<th>Collection Time, min</th>
<th>Collection Period</th>
</tr>
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<tbody>
<tr>
<td>6×20</td>
<td>After insertion of probe</td>
</tr>
<tr>
<td>10</td>
<td>Contraction periods (HT or LT, 7 total)</td>
</tr>
<tr>
<td>2×20</td>
<td>After contraction</td>
</tr>
<tr>
<td>10</td>
<td>Repeat contractions</td>
</tr>
<tr>
<td>2×20</td>
<td>After contraction</td>
</tr>
<tr>
<td>10</td>
<td>Contraction periods (HT or LT, 7 total)</td>
</tr>
<tr>
<td>2×20</td>
<td>After contraction</td>
</tr>
<tr>
<td>10</td>
<td>Repeat contractions</td>
</tr>
<tr>
<td>2×20</td>
<td>After contraction</td>
</tr>
</tbody>
</table>

HT indicates high tension; LT, low tension.

gamma counter, using a cubic spline algorithm for data processing.

The cross-reactivity at IC_{50} was 100% for SP-(1-11) and SP-(2-11); 98% for ^{125}I[Tyr^6]SP; 95% for SP-(1-9); 85% for SP-(1-7); 75% for SP-(3-11); 60% for SP-(1-6); 45% for SP-(1-4); 12% for SP-(4-11) and SP-(5-11); 5% for SP-(6-11); <0.01% for SP-(7-11), SP-(8-11), SP-(9-11), neurokinin A (NKA), neurokinin B (NKB), somatostatin, neuropeptide K, kassinin, edoleisin, and physalaemin. The detection limit of the assay was 0.07 fmol/100 μL, and the mean±SD for the IC_{20}, IC_{50}, and IC_{90} was 0.32±0.08, 0.98±0.12, and 3.80±1.3 fmol/100 μL, respectively.

Experimental Protocol

After inserting the microdialysis probe, six 20-minute collection periods (flow rate, 5 μL/min) were performed (Table 1). These control collections were performed so that basal SP-LI release was attained before any muscle manipulation. After a sample was collected, it was stored in a freezer (−80°C) until the assay was performed. The probe was continuously perfused throughout the experiment.

Once the control collections were completed, a series of HT or LT contractions was evoked during a 10-minute collection period (Table 1). A total of seven contractions were evoked for each of the 10-minute collection periods. The duration of each contraction was 20 seconds, and each was separated by an 80-second quiescent period. The HT contractions were elicited by simultaneously stimulating the peripheral ends of the cut L-7 and S-1 ventral roots (3× motor threshold, 40 Hz, 0.1 millisecond) with a resting tension of 1.0 kg set on the muscle. LT contractions were evoked by stimulating only the L-7 ventral root (1.5 to 2.0× motor threshold, 40 Hz, 0.1 millisecond), and resting tension was set at 200 g. Before the electrical stimulation, the ventral roots were placed on the electrodes, and the saline covering the spinal cord was removed. Thus, only air surrounded the ventral roots while they were stimulated. The contractions began approximately 20 seconds after the onset of the 10-minute collection period. Next, the muscle was allowed to recover during two 20-minute collection periods. This pattern was repeated until four sets of contractions were performed. Thus, a total of 28 contractions were performed (14 HT and 14 LT contractions). The dialysates from the HT contractions were then combined so that the sample volumes remained equal. Likewise, the dialysates from the LT contractions were combined. Whether the HT or LT contractions were performed first was randomized. However, the contractions always occurred consecutively; ie, if the HT contractions were performed first, then the next series of contractions was HT. This protocol was performed on all the cats.

After the aforementioned procedure had been accomplished, one of two different protocols was performed on a given cat. The L-7 and S-1 dorsal roots were cut on some of the cats (n=5), and then the protocol described in Table 1 was repeated. This was done to test if the neuropeptide release was the result of activation of muscle afferents. For three of the animals, the L-7 and S-1 ventral roots were stimulated while the cat was paralyzed (3 mg/kg gallamine triethiodide) to determine if changes in peptide release were the result of muscle contraction and not current spread associated with the electrical stimulus. The electrical parameters for this test were 5× motor threshold, 50 Hz, and 0.1 millisecond, which were greater than those used for the HT contractions (see above). The animals were euthanized (120 mg/kg sodium pentobarbital IV), and the L-6–S-1 region of the spinal cord was removed and placed in 10% formalin for tissue fixation. Once the tissue was adequately fixed, 50-μm frozen (transverse) sections of the L-7 region of the spinal cord were cut using a cryostat (2800 Fricogut E, Reichert-Young, Cambridge Instruments, Buffalo, NY) to determine the location of the tract caused by the microdialysis probe.

Statistical Analysis

All data are expressed as mean±SD. SP-LI values during the six precontraction collections were analyzed using a one-way ANOVA. The ANOVA was also used to compare the absolute concentration of the precontraction and HT and LT contraction SP-LI values (Fig 2). Tukey's test was performed when a significant F value was found. For the SP-LI, hemodynamic, and tension data, a paired Student's t test was performed to compare the changes elicited by the HT versus LT contractions. For all analyses, P<.05 was used as the level of statistical significance.

Results

The concentration of SP-LI for the six precontraction collections is illustrated in Fig 1. Initially, SP-LI was relatively high but decreased thereafter. The SP-LI values during the last two collections were essentially identical, suggesting that this was the basal release for this preparation. The last bar represents the precontraction SP-LI concentration for the HT or LT contractions that were subsequently performed. Both HT and LT contractions increased SP-LI release in the dorsal horn of the spinal cord (Fig 2). However, the HT contractions evoked a larger increase in SP-LI compared with the LT contractions.

An original recording from one cat illustrating the cardiovascular responses to the HT and LT contractions is shown in Fig 3. During a 10-minute collection period, a series of seven HT contractions was evoked (upper panel of Fig 3). During a subsequent 10-minute collection period, a series of seven LT contractions was elicited (lower panel of Fig 3). This recording demon-
strates that the HT contractions caused larger increases in mean arterial pressure and heart rate compared with the LT contractions. The average data for TTI, change in SP-LI, and hemodynamic responses to the HT and LT contractions are illustrated in Fig 4. As designed, the HT contractions produced a higher TTI compared with the LT contractions (Fig 4A). A greater SP-LI release occurred in response to this larger TTI (Fig 4B). Likewise, there were greater increases in mean arterial pressure (Fig 4C) and heart rate (Fig 4D) in response to the HT contractions. Thus, the release of SP and the cardiovascular responses to static contraction are related to the developed tension. The precontraction hemodynamic variables were not different (Table 2).

The relation between TTI, change in SP-LI, and change in mean arterial pressure is depicted in Fig 5. The upper and middle graphs show that the change in SP-LI and the change in mean arterial pressure are related to the TTI. A relation exists between the change in SP-LI and the change in mean arterial pressure, but it appears to be weaker than the previous relations, as indicated by the overlap of the deviation bars (Fig 5, lower graph).

The precontraction and contraction SP-LI values before and after cutting the L-7 and S-1 dorsal roots for the five cats in which this procedure was performed are depicted in the upper panel of Fig 6. Before sectioning the dorsal roots, both HT and LT contractions increased SP-LI release. However, these increases were virtually abolished after cutting the L-7 and S-1 dorsal roots. Thus, the changes in SP-LI release in the dorsal horn in response to static contraction are the result of muscle afferent activation. The lower panel of Fig 6 illustrates that the contraction-induced release of SP-LI is abolished if the contraction is prevented by muscle paralysis. This indicates that the changes in SP-LI release demonstrated in this study are not the result of direct activation of muscle afferents by the electrical stimulus.

The position of the probe was determined histologically. The results verified that the probe was in the dorsal horn. The central canal was the approximate lateral level of the tip of the membrane, ie, in the ventral portion of lamina VII or the most dorsal aspect of lamina VIII. This is similar to the location found in our previous study and suggests that the extracellular space of the entire dorsal horn was probably sampled.

Discussion

The purpose of this study was to determine if the contraction-induced increases in the release of SP in the dorsal horn, as well as the cardiovascular changes, are related to the developed tension. The developed tension was much higher for the HT compared with the LT contractions. The reflex cardiovascular changes were related to force production, a finding that has been shown previously.1,5,10,11 An additional observation was that HT contractions elicited greater increases in the release of SP in the dorsal horn of the spinal cord compared with the LT contractions. Thus, the release of SP in the dorsal horn from muscle afferents is related to the developed tension.

Using microdialysis, Brodin et al10 demonstrated that electrical stimulation of the sciatic nerve increases SP release in the dorsal horn of anesthetized cats. However, this increase only occurred if the intensity of the stimulus was such that it activated group III and IV afferents. A similar finding was reported by Go and Yaksh,12 who used an in vitro spinal cord preparation. Thus, SP may play a role in sensory transmission at the level of the spinal cord. Along these lines, several studies have provided evidence that the spinal release of SP plays a role in mediating the reflex cardiovascular responses to muscle contraction.21-25 The present study provides additional support that the release of SP from muscle afferents is involved in the reflex autonomic changes produced by static contraction.

SP is a member of a class of neuropeptides collectively referred to as tachykinins.32,33 The other members of this class found in mammals are NKA and NKB. Three different receptors mediate the actions of these tachykinins: NK-1, NK-2, and NK-3.33 The actions of SP are thought to be mediated by the NK-1 receptor,
Fig 3. Original tracings from one cat demonstrating the tension and cardiovascular responses to a series of high-tension contractions (top) and low-tension contractions (bottom). HR indicates heart rate; AP, arterial pressure.

whereas NKA preferentially binds to NK-2. SP and NKA are found in the afferents synapsing in the dorsal horn, and in some of these fibers, the neuropeptides may be colocalized. Because of this, it may be speculated that NKA is involved in the reflex responses to muscle contraction. Duggan et al. demonstrated that muscle contraction increases NKA release in the dorsal horn of the spinal cord. However, Hill et al. showed that injecting an NK-2 antagonist into the intrathecal space of the lumbar spinal cord fails to alter the reflex pressor response to muscle contraction. Injection of an NK-1 antagonist blunted these reflex changes. Thus, the possible role of NKA in the reflex responses to muscle contraction is unclear. Any confounding influence of NKA release in this study is unlikely, because the antibody used in the RIA showed virtually no cross-reactivity to NKA.

There appeared to be a relation between TTI and the change in mean arterial pressure (Fig 5). Previous studies have shown that the reflex cardiovascular responses to static muscle contraction are related to the developed tension. There is also a tendency for the reflex changes in renal sympathetic nerve activity evoked by static contraction to be related to the developed tension. Using anesthetized cats, Perez-Gonzalez examined the relation of the arterial blood pressure changes to three different indexes of tension: (1) peak tension, (2) mean tension, and (3) TTI. The

![Graph](http://circres.ahajournals.org/)

TABLE 2. Precontraction Hemodynamic Data

<table>
<thead>
<tr>
<th></th>
<th>HT Contractions</th>
<th>LT Contractions</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mm Hg</td>
<td>137±10</td>
<td>143±14</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>209±26</td>
<td>204±36</td>
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HT indicates high tension; LT, low tension; MAP, mean arterial pressure; HR, heart rate; and bpm, beats per minute. Values are mean±SD (n=8).

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Fig 4. Bar graphs depicting the tension-time index (TTI, A), change in substance P-like immunoreactivity (SP-LI, B), change in mean arterial pressure (MAP, C), and the change in heart rate (HR, D) in response to high-tension (HT) contractions (open bars) and low-tension (LT) contractions (filled bars). Data are mean±SD (n=8). *Significant difference compared with HT contractions.
On the other hand, because of the rather large deviation bars, the relation between the change in SP-LI and the change in mean arterial pressure appears weak (Fig 5, lower graph). This is not surprising, since the release of SP from muscle afferents represents the first synapse in the reflex arc. Because an intact medulla is required for full expression of the cardiovascular responses to muscle contraction, a minimum of two more synapses in the central nervous system must be involved in this reflex. Thus, multiple sites exist for integration and/or modulation of the signal arising from the second-order neuron. Further, the exact relation between neurally released SP and the discharge of dorsal horn cells is unknown. This is also complicated by the fact that SP does not evoke fast excitatory postsynaptic potentials when applied to dorsal horn cells. Thus, it may be quite difficult to demonstrate a strong relation between spinal cord SP release and the end organ response (change in mean arterial pressure). However, this should not distract from the relation between SP release and the change in mean arterial pressure for the two levels of tension that is illustrated in Fig 5. In our previous study, cutting the L-6-S-1 dorsal and ventral roots marked attenuated, but did not abolish, contraction-induced SP release. In the

strongest relation that existed (r=0.87) was for TTI. Further, this correlation between the pressor responses and TTI only existed for tetanic, not rhythmic, contractions. In the present study, only tetanic contractions were evoked. Thus, the relation between the change in mean arterial pressure and TTI found in this study is in agreement with Perez-Gonzalez.

A new finding from the present study is that the release of SP in the dorsal horn of the spinal cord is also related to the TTI (Fig 5). The discharge of mechanically sensitive muscle afferents is related to developed tension. Although not tested, it is quite plausible that the discharge of metabolically sensitive afferents is related to TTI, since muscle oxygen consumption is predominantly influenced by this parameter. Further, it appears that activation of metabolically sensitive muscle afferents is necessary to evoke SP release in this model, since microinjection of an antagonist to this neuropeptide has no effect on the reflex responses elicited by muscle stretch. Thus, the relation between SP release and TTI provides further support for the hypothesis that the release of this neuropeptide into the dorsal horn plays a role in mediating the reflex cardiovascular responses to static muscle contraction.

**Fig 5.** Top and middle, Graphs illustrate the relation of tension-time index (TTI) and the change in substance P–like immunoreactivity (SP-LI) and TTI and the change in mean arterial pressure (MAP) for low-tension (LT) contractions (Δ) and high-tension (HT) contractions (▲). Bottom, Graph depicts the relation of the change in SP-LI and the change in MAP. Symbols are the same as for the upper two graphs. Data are mean±SD (n=8).

**Fig 6.** Top, Bar graph shows the substance P–like immunoreactivity (SP-LI) values before (open bars) and during high-tension (HT) contractions (filled bar) and low-tension (LT) contractions (hatched bar) before and after cutting the L-7 and S-1 dorsal root (DR) for the five cats in which this was performed. Bottom, The first four bars of the bar graph illustrate the concentration of SPLI before (open bars) and during HT contractions (filled bar) and LT contractions (hatched bar) before muscle paralysis for the three cats in which this was performed. The second two bars represent the SPLI values before (open bar) and during (cross-hatched bar) electrical stimulation of the L-7 and S-1 ventral roots (VR). Data are mean±SD.
The present study, cutting the L-5–S-2 dorsal and ventral roots virtually abolished the increases in SP. This suggests that activation of fibers entering the spinal cord via the L-5 and S-2 spinal roots elicits small increases in SP release in the L-7 dorsal horn. Further, these results suggest that movement of the lower back during a muscle contraction is not responsible for the evoked increases in SP release. In addition, the changes in SP release are not the result of electrical activation of the afferents, because changes in peptide release did not occur when the contractions were prevented.

In the present study, the HT contractions were evoked by stimulating the L-7 and S-1 ventral roots, whereas only the L-7 was stimulated to elicit the LT contractions. This was done because it is our experience that the peak tension evoked by stimulating both roots is more sustained than if the L-7 root is stimulated alone. Because the design of the study was to compare HT and LT contractions and because multiple contractions were required, we wanted to ensure that the HT contractions were reproducible and that the peak tension was sustained as long as possible. However, this raises the possibility that the greater release of SP during the HT contractions was the result of this neuropeptide being released at different spinal levels. Since both ventral roots innervate the triceps surae muscle, the afferents activated by a contraction elicited by L-7 and S-1 stimulation should be the same as the ones activated by L-7 stimulation alone (if equal tensions are produced). Thus, it is very unlikely that the rostral-caudal extent of SP release is different. Further, the probe was placed in the center of the rostral-caudal extent of the L-7 dorsal horn. Unfortunately, we do not know the rostral-caudal extent from which the microdialysis probe samples. The ability to determine this is a difficult problem, because numerous factors (diffusion coefficients, rate of removal from the extracellular space, size of the membrane, flow rate, etc) influence the size of the sample area. Bungay et al have suggested that the extent of delivery of solutes to the extracellular space may be as great as 3 mm (depending on the aforementioned variables). The L-7 region of the cat extends 7 to 11 mm, depending on the size of the cat. Further, it should be kept in mind that the L-5, L-6, and S-2 dorsal and ventral roots were sectioned before inserting the microdialysis probe. Also, the release of contraction-induced SP was abolished, as were the cardiovascular changes, when the contractions were repeated after cutting the L-7 and S-1 dorsal roots. Thus, it is very unlikely that the SP measured in this study was from an area outside the L-7 region.

In summary, HT contractions elicited greater increases in the release of SP in the L-7 dorsal horn region of the spinal cord compared with LT contractions. This indicates that the release of SP is related to the developed tension. Concomitant with the increased SP release, HT contractions evoked larger cardiovascular changes. Thus, the results of the present study suggest that the release of SP in the spinal cord plays a role in mediating the cardiovascular responses produced by static contraction of skeletal muscle.

Acknowledgments

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References

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