Segmental effect of spinal NK-1 receptor blockade on the pressor reflex

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Wilson, L. Britt, and Gregory A. Hand. Segmental effect of spinal NK-1 receptor blockade on the pressor reflex. Am. J. Physiol. 275 (Heart Circ. Physiol. 44): H789–H796, 1998.—The physiological effects of substance P (SP) are mediated via activation of neurokinin-1 (NK-1) receptors. The purpose of this study was to test the hypothesis that blockade of NK-1 receptors in the dorsal horn, both at the site of entry for the primary afferent neurons and adjacent spinal segments, attenuates the pressor reflex evoked by static contraction and stretch of skeletal muscle. Cats were anesthetized with α-chloralose and urethane, and a laminectomy was performed. With the exception of the L7 dorsal root, the dorsal and ventral roots from L5 to S2 were sectioned on one side of the spinal cord. Thus the primary afferent fibers mediating the pressor reflex enter the spinal cord via the L7 dorsal root in these experiments. Based on dose-response data, dialysis of the NK-1 receptor antagonist CP-96,345 (5 mM for 2 h) into the L7 dorsal horn ipsilateral to the contracting muscle attenuated the pressor response to static contraction (75 ± 15 vs. 46 ± 7 mmHg; n = 5 cats) but not muscle stretch (60 ± 12 vs. 50 ± 8 mmHg). Administration of the inactive enantiomer of CP-96,345, CP-96,344 (5 mM for 2 h), into the L7 dorsal horn failed to alter the cardiovascular changes elicited by contraction (45 ± 7 vs. 43 ± 6 mmHg) and stretch (31 ± 8 vs. 32 ± 11). Dialysis of 5 mM CP-96,345 into the dorsal horn at the L6 and S1 segments for 2 h decreased the peak pressor response to static contraction (58 ± 9 vs. 31 ± 6 mmHg; n = 7) and muscle stretch (61 ± 6 vs. 44 ± 8 mmHg). These data suggest that the activation of NK-1 receptors, both at the site of entry and in regions outside of the entry site for afferent neurons, is involved in the spinal processing that produces the pressor reflex evoked by static contraction of skeletal muscle.

cats; exercise pressor reflex; substance P; dorsal horn; blood pressure

Previous studies have shown that static contraction of skeletal muscle can reflexly increase heart rate and arterial blood pressure, and this is commonly referred to as “the exercise pressor reflex” or simply “the pressor reflex” (3, 18, 22, 34). The increases in cardiovascular function associated with this reflex are mediated by a contraction-induced activation of group III and IV muscle afferent fibers (18). These afferent fibers respond to the mechanical and metabolic changes that occur within the contracting muscle (13, 15, 22). Most, if not all, of these muscle afferent neurons synapse in the dorsal horn of the spinal cord (11, 21). Thus the dorsal horn of the spinal cord serves as the site of the first synapse for the pressor reflex evoked by static contraction of skeletal muscle (34).

Afferent fibers primarily enter the spinal cord via dorsal roots. Upon entering the spinal cord, the primary afferent neuron typically bifurcates into an ascending and a descending branch. These branches often traverse the entire rostrocaudal extent of a given spinal segment by coursing through the spinal cord in the marginal zone of the dorsal horn, Lissauer’s tract, the midline of the spinal cord dorsal to the central canal, and the dorsal columns (2, 26, 31). Numerous collaterals arise from these branches, ultimately making synaptic contacts with cells of the dorsal horn. Thus a single primary afferent neuron makes multiple synaptic connections within the dorsal horn of the segment in which it enters. In addition, the ascending and descending branches of the primary afferent fiber often travel beyond the segment of entry sending collaterals that terminate in the dorsal horn (2, 26, 31). For example, an afferent fiber that enters the spinal cord in the L7 dorsal root makes synaptic contacts not only in the L7 dorsal horn but also in the dorsal horn of L8 and S1 as well. Thus stimulation of a primary afferent neuron can activate cells in the dorsal horn of several spinal segments (31).

We have recently investigated the possibility that activation of dorsal horn cells in several spinal segments is involved in producing the pressor reflex (6, 7). Blocking the N-methyl-D-aspartic acid (NMDA) subtype of excitatory amino acid receptors in dorsal horn regions rostral and caudal to the entry site of the afferent fibers attenuates the pressor response to static contraction and muscle stretch (6). Likewise, administration of an antagonist to the non-NMDA subtype of excitatory amino acids receptors into the same regions attenuates the pressor reflex (7). These results suggest that neurochemical events in the dorsal horn outside of the entry site for the afferent neurons play a role in producing the pressor reflex elicited by static contraction and stretch of skeletal muscle.

Substance P (SP) is a putative neurotransmitter/neuromodulator found within afferent fibers synapsing in the dorsal horn of the spinal cord (10, 19). The postsynaptic actions of SP are mediated via binding to neurokinin-1 (NK-1) receptors, and these receptors are located within the dorsal horn of the spinal cord (8, 16). SP is located in group IV afferent fibers, one of the classes of neurons that mediates the pressor reflex (19), and previous work has suggested that the release of this neuropeptide into the dorsal horn plays a role in producing the pressor reflex to static contraction (9, 12,
14, 32, 33, 36). For example, our previous work demonstrated that static contraction of skeletal muscle increases SP release in the L7 dorsal horn (20, 23, 25, 33). Hill et al. (9) showed that the intrathecal administration of the selective NK-1 receptor antagonist CP-96,345 attenuates the pressor reflex. In addition, Wilson et al. (36) showed that microinjecting a nonspecific antagonist to SP receptors into the L7 dorsal horn attenuates the pressor response evoked by static muscle contraction. However, these studies did not examine the possibility that SP exerts its effect over several spinal levels when the afferent input is limited to a single spinal segment.

The purpose of the current study was to examine the possibility that SP release in the dorsal horn partially mediates the pressor reflex, and that this neuropeptide exerts its effect at multiple spinal segments. Specifically, we tested the hypothesis that the pressor reflex to static contraction is attenuated by administering an antagonist to NK-1 receptors into the dorsal horn in segments that are rostral and caudal to the site of entry for the primary afferent neurons. For these studies, the selective NK-1 antagonist CP-96,345 was locally administered into the dorsal horn region using microdialysis (28). Because the antagonist used in our previous work was not specific for NK-1 receptors (36), we also tested the hypothesis that local administration of CP-96,345 into the dorsal horn at the segment of entry attenuates the pressor reflex evoked by static contraction. Furthermore, we examined whether or not NK-1 receptor blockade in the dorsal horn alters the pressor response to muscle stretch.

METHODS

Surgical preparation. Twenty-one adult cats of either sex were used for this study. Cats were anesthetized by inhaling a halothane (5%)-nitrous oxide (1–3 l/min)-oxygen (1–3 l/min) mixture. Polyethylene catheters were inserted into an external jugular vein and a common carotid artery. The inhalation anesthetic was removed, and anesthesia was maintained by an intravenous injection of α-chloralose (80 mg/kg) and urethane (200 mg/kg). The trachea was exposed and an endotracheal tube inserted into the airway. If a corneal reflex abcesses.

Animals were mechanically ventilated (model 661, Harvard Apparatus). Arterial blood gases were periodically measured and maintained within normal limits (pH 7.35–7.40; Pco2, 35–40 Torr; Po2 > 80 Torr) by adjusting the ventilator, administering intravenous sodium bicarbonate, and/or providing supplemental oxygen. Body temperature was continuously monitored using a rectal probe (Yellow Spring Instruments series 400) and maintained between 36.0 and 38.0°C with a heating pad and lamp. A laminectomy was performed exposing the spinal cord, and the animal was placed in a spinal unit (Kopf) to stabilize the vertebral column. The dura of the spinal cord was opened, allowing visual identification of the L5–S2 spinal roots. With the exception of the L7 dorsal root, the L5–S2 spinal (dorsal and ventral) roots were unilaterally severed. A cavern was formed around the exposed neural and muscular tissue by suturing skin flaps to brass bars and was filled with warm (37°C) mineral oil. The hindlimb containing the triceps surae muscle, ipsilateral to the cut spinal roots, was fixed in one position by clamping the foot, and the knee joint was secured by placing a string around the patellar tendon and tying it to a post. Muscle tension was determined by cutting the calcaneal bone and attaching the Achilles tendon to a force transducer (Grass FT10). Because the positions of the force transducer and the knee were fixed, all muscle contractions were isometric.

For these experiments, microdialysis was used to deliver drugs to the dorsal horn region. The microdialysis probe (BAS, CMA-10) has a diameter of 500 µM at the membrane, which is 3 mm in length. For the first three protocols indicated below, following the surgical procedure, a single microdialysis probe was inserted into the L7 dorsal horn region using a Kopf carrier. For the last protocol, a microdialysis probe was inserted into the dorsal horn at L6, a minimum of 4 mm from the most rostral aspect of L7. Likewise, a second probe was inserted into the dorsal horn at S1, a minimum of 4 mm from the most caudal aspect of L7. This placement of the probes ensures against spillover into the L7 dorsal horn when dialyzing into L6 and S1 (20, 23). The probes were inserted so that the entire membrane was submerged in spinal cord fluid. For all experiments, the probes were continuously perfused (5 µl/min) with an artificial extracellular fluid containing 0.2% bovine serum albumin, 0.1% bacitracin, and the following ions (in mM): 6.2 K+, 134 Cl−, 2.4 Ca2+, 150 Na+, 1.3 P−, 13 HCO3−, and 1.3 Mg2+. This solution was made fresh for each experiment. After the probe(s) was inserted, the cat was allowed to stabilize for at least 2 h.

For all of the following protocols, contraction of the triceps surae muscle was induced by electrically stimulating the peripheral ends of the cut L7 and S1 ventral roots at three times the motor threshold, 40 Hz, and 0.1-ms duration for 1 min. Before each contraction, the resting tension on the muscle was set at 1 kg, which represents L7 (optimal length) in this preparation (6, 7, 20, 23, 33). For protocols II-IV, the triceps surae muscle was stretched by manually displacing the force transducer until the tension approached that produced by the contraction. Because the L5–S2 spinal (dorsal and ventral) roots were unilaterally severed, with the exception of the L7 dorsal root, all of the afferent information mediating the pressor reflex from the triceps surae muscle entered the spinal cord via the L7 dorsal root (3, 18, 35). At least 15 min were allotted between successive muscle perturbations.

Protocol I: Dose response. After a minimum of two contractions of the triceps surae muscle in which the pressor reflex was reproducible, doses of 0.01, 0.1, 1.0, 5.0, and 10 mM of the NK-1 antagonist CP-96,345 were microdialyzed sequentially into the L7 dorsal horn of four cats. Each dose was dialyzed for ~1 h, at which time the muscle was contracted and we recorded the cardiovascular changes.

Protocol II: Single dose of CP-96,345 in L7. Because the aforementioned dose-response protocol is rather long, our a priori design is to test a single dose based on the dose-response curve, using a separate group of cats, thereby minimizing any possible confounding effect of time. Thus protocol II was designed to determine the effect of dialyzing 5 mM CP-96,345 into the L7 dorsal horn on the reflex cardiovascular responses to static contraction and stretch of the triceps surae muscle (n = 5 cats). When at least two reproducible responses to contraction and stretch were obtained, the dialysis of CP-96,345 was initiated. After ~45 min of this dialysis, the reflex responses to a contraction or a stretch were determined. Fifteen minutes later, either a stretch or a contraction followed, respectively. The contraction and stretch were performed in a counterbalance fashion. The contraction and stretch were repeated, again separated by 15 min, after...
CP-96,345 had been dialyzed for about 120 min. Thus the reflex cardiovascular responses to contraction and stretch were tested after ~1 and ~2 h of CP-96,345 dialysis into the L7 dorsal horn. Next, the contraction and stretch were repeated after a 20% lidocaine solution had been dialyzed for ~1 h. This was done as a functional test for placement of the probe. The data from a given animal were excluded if the pressor response to static contraction was not reduced by at least 70% relative to control.

Protocol III: Single dose of CP-96,345 in L7. It has been reported that CP-96,345 can block Ca2+ channels (27). Thus we determined whether dialysis of 5 mM CP-96,344, the inactive enantiomer of CP-96,345, influenced the pressor responses evoked by static contraction and muscle stretch (28). CP-96,344 exhibits the same Ca2+-blocking properties as CP-96,345 but fails to block NK-1 receptors. For this study, the protocol described in the preceding section was repeated on seven cats, except 5 mM CP-96,344 was dialyzed into the dorsal horn at L7. The exclusionary criteria for lidocaine were maintained for this study.

Protocol IV: Single dose of CP-96,345 in L6 and S1. This protocol was designed to test the hypothesis that blockade of NK-1 receptors in regions of the dorsal horn outside of the entry segments for the afferent neurons attenuates the reflex cardiovascular responses evoked by static contraction and stretch of the triceps surae muscle. As described above, microdialysis probes were placed in the dorsal horn at L6 and S1, a minimum of 4 mm rostral and caudal to L7, respectively. Thereafter, the protocol described above (protocol II) was repeated on seven cats, except for the exclusionary criteria for lidocaine. In this study, the data were omitted if lidocaine failed to reduce the pressor response to contraction by at least 50%. This change was made because in our previous work we found that lidocaine was less effective in reducing the pressor reflex when dialyzed into L6 and S1 than when administered into L7 (6, 7). This is expected because the L7 dialysis of lidocaine blocks not only synaptic connections in the dorsal horn but also the conduction from the primary afferents as they enter the dorsal horn, including the afferent branches.

Measured and calculated variables. Arterial blood pressure was measured by connecting the carotid artery catheter to a pressure transducer (Statham P23 ID). Mean arterial blood pressure (MAP) was obtained by integrating this signal with a time constant of 4 s. Heart rate (HR) was derived from the arterial pressure pulse by a biotachometer (Gould), and muscle tension was measured using the force transducer. The cardiovascular and tension variables were continuously monitored on an eight-channel chart recorder (Gould). Baseline values were determined by averaging at least 30 s of data immediately before a given muscle manipulation (passive stretch or contraction). Peak values represent the peak level immediately before a given muscle manipulation (passive stretch or contraction). Peak values represent the change in MAP at 10-s time points compared with baseline during the 1-min contraction and was done for control (predrug), 1 h, and 2 h of drug dialysis. The time-course data were analyzed using a three-way analysis of variance (ANOVA), with the three factors being contraction time (0–60 s at 10-s intervals), duration of dialysis (control, 1 h, and 2 h), and animal [considered a random effect variable; (37)]. The three-way ANOVA was also used to compare baseline and peak hemodynamic and tension data. The changes in MAP, HR, and tension produced by contraction and stretch were analyzed using a two-way ANOVA, with the factors being the change in the variable and animal. Tukey’s test was performed when a significant F value was found. For all analyses, P < 0.05 was used as the level of statistical significance.

RESULTS

Dose response. The effects of microdialyzing increasing doses of CP-96,345 into the L7 dorsal horn for ~1 h on the pressor response to static contraction are shown in Fig. 1. The two lowest doses (0.01 and 0.1 mM) had no effect on the pressor response evoked by static contraction. On the other hand, the next three doses showed a progressive reduction in magnitude of the pressor response, and it reached statistical significance at the 10 mM dose. However, the pH of the 10 mM solution of CP-96,345 did not remain at 7.4 throughout the dialysis period because it decreased several minutes after the solution was prepared. Thus, we could not rule out the possibility that changes in pH contributed to the reduction in the magnitude of the pressor reflex. For the subsequent single dose studies, we used the 5.0 mM solution of CP-96,345, because this dose elicited a similar level of attenuation and the pH of the solution remained at 7.4. The peak tensions developed at each dose were not statistically different (in kg: control, 9.0 ± 0.9; 0.01 mM, 8.4 ± 1.2; 0.1 mM, 8.4 ± 1.1; 1.0 mM, 8.0 ± 1.2; 5.0 mM, 8.0 ± 1.2; 10 mM, 7.1 ± 0.9).

Protocol II: Single dose of CP-96,345 in L7. From the aforementioned dose response data, we tested the effect of microdialyzing 5 mM CP-96,345 into the L7 dorsal horn on peak pressor response to static contraction and stretch of the triceps surae muscle. Table 1 denotes the baseline, peak, and change in MAP, HR, and tension before and during the dialysis of CP-96,345. Before the administration of CP-96,345, static contraction increased MAP 75 ± 15 mmHg and HR 31 ± 6 beats/min (Table 1). These contraction-induced changes in MAP and HR were significantly
Table 1. Hemodynamic and tension data in response to static contraction and muscle stretch before and during 5 mM CP-96,345 dialysis in L7 dorsal horn

<table>
<thead>
<tr>
<th>Contraction (n = 5)</th>
<th>Control</th>
<th>1 h</th>
<th>2 h</th>
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<tr>
<td>MAP, mmHg</td>
<td>Baseline</td>
<td>Peak</td>
<td>Change</td>
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<tr>
<td>HR, beats/min</td>
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<tr>
<td>Tension, kg</td>
<td>1.0</td>
<td>9.0</td>
<td>8.0</td>
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<tr>
<td>Stretch (n = 5)</td>
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<tr>
<td>MAP, mmHg</td>
<td>108 ± 6</td>
<td>168 ± 16</td>
<td>60 ± 12</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>163 ± 7</td>
<td>184 ± 10</td>
<td>21 ± 4</td>
</tr>
<tr>
<td>Tension, kg</td>
<td>1.0</td>
<td>8.4 ± 0.3</td>
<td>7.4 ± 0.3</td>
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Values are means ± SE; n, no. of cats. MAP, mean arterial pressure; HR, heart rate. *P < 0.05 compared with corresponding control value; †P < 0.05 baseline vs. peak; ‡P < 0.05 compared with control.

Reduced to 46 ± 7 mmHg and 23 ± 4 beats/min, respectively, after 2 h of CP-96,345 dialysis but not after 1 h of dialysis (56 ± 10 mmHg and 24 ± 3 beats/min, respectively).

The time course of the pressor response evoked by static contraction before and during the dialysis of CP-96,345 into the L7 dorsal horn is depicted in Fig. 2A. Statistical analysis showed a significant main effect for time (0–60 s: P = 0.0001), indicating that MAP increased during the 1-min static contraction. Also, there was a significant main effect for duration of drug dialysis (control, 1 h, and 2 h: P = 0.0134) and a significant interaction (P = 0.0201) between time and duration. Thus CP-96,345 caused a downward shift in the time course of the pressor response to static contraction. On the other hand, CP-96,345 did not cause a statistically significant downward shift in the time course of the HR responses.

Similar to static contraction, stretch of the triceps surae muscle increased MAP 60 ± 12 mmHg and HR 21 ± 4 beats/min (Table 1). However, blockade of NK-1 receptors in the L7 dorsal horn failed to reduce the stretch-evoked changes in MAP and HR after 1 h (52 ± 11 mmHg and 21 ± 4 beats/min, respectively). However, CP-96,345 caused a downward shift in the time course of the stretch-induced pressor reflex (Fig. 2B). There was a significant main effect for time (P = 0.0001) and drug duration (P = 0.0490). In addition, the interaction between time and drug duration was significant (P = 0.0320). The time course was reduced after 2 h only, and post hoc analysis showed a difference between control and 2 h at the 20-, 30-, and 40-s time points. Thus blocking NK-1 receptors in the dorsal horn reduced the rate at which MAP changed during muscle stretch but not the maximum change.

Protocol III: Single dose of CP-96,344 in L7. The baseline, peak, and changes in the cardiovascular and tension data produced by static contraction and muscle stretch were unaltered by dialysis of CP-96,344 into the L7 dorsal horn (Table 2). In addition, CP-96,344 failed to alter the time courses of the pressor response for contraction and stretch (Fig. 3). For static contraction (Fig. 3), there was a significant main effect for time (P = 0.0001) but not for drug duration (P = 0.8124) or the interaction of the two (P = 0.9670). Likewise, there was a significant effect for time (P = 0.0103), but not drug duration (P = 0.9150) or interaction (P = 0.2452) for the time course of the pressor response to muscle stretch.

Protocol IV: Single dose of CP-96,345 in L6 and S1. To determine whether blockade of NK-1 receptors in re-
regions of the dorsal horn outside the entry site for the afferent neurons attenuates the pressor reflex, we dialyzed CP-96,345 into the dorsal horn at L6 and S1. The probes were placed in such a manner as to ensure against spillover into the L7 dorsal horn (see METHODS). The changes in MAP produced by static contraction were significantly reduced from 58 ± 9 to 39 ± 10 and 31 ± 6 mmHg after 1 and 2 h of CP-96,345 dialysis, respectively (Table 3). The stretch-evoked change in MAP was significantly reduced from 61 ± 6 to 44 ± 8 mmHg after 2 h of CP-96,345 dialysis but not after 1 h (51 ± 7 mmHg; Table 3). On the other hand, the reflex change in HR produced by these muscle perturbations was unaltered (Table 3). Peak muscle tensions were similar during drug dialysis (Table 3).

Dialysis of CP-96,345 into L6 and S1 suppressed the time course of the pressor response to static contraction at both 1 and 2 h (Fig. 4A). The statistical analysis showed significant main effects for time (P = 0.0001), drug duration (P = 0.0007), and interaction (P = 0.0001). Similar to contraction, the time course of the pressor response to stretch was attenuated by CP-96,345 dialysis into L6 and S1 (Fig. 4B). However, this suppression of the time course occurred only after 2 h of CP-96,345 dialysis. Again, statistical analysis showed significance for time (P = 0.0001), drug duration (P = 0.0057), and interaction (P = 0.0001). Thus blockade of NK-1 receptors in the dorsal horn of L6 and S1 diminished the pressor response to both static contraction and muscle stretch.

**DISCUSSION**

For this study, we investigated the influence that SP may exert in the dorsal horn of several spinal segments on the cardiovascular changes evoked by static muscle contraction and muscle stretch, while limiting the afferent input to a single segment (L7). The actions of SP were blocked using the selective antagonist for NK-1 receptors CP-96,345 (28). In high concentrations, CP-96,345 can block calcium channels (27). CP-96,344, an enantiomer of CP-96,345, fails to block NK-1 receptors but retains the calcium channel-blocking properties. A 2-h dialysis of CP-96,344 into the L7 dorsal horn failed to alter the pressor reflex to static contraction or muscle stretch, indicating that the potential calcium-blocking properties were not a complicating factor in this study. The results of this study provide several new findings. First, local administration of a selective NK-1 blocker to the L7 dorsal horn, the segment of afferent fiber entry, attenuated the pressor reflex produced by static contraction. This administration of CP-96,345 into L7 failed to alter the change in MAP and HR evoked by muscle stretch. On the other hand, the NK-1 antagonist slightly reduced the time course of the MAP changes elicited by muscle stretch. Second, blockade of

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**Table 2. Hemodynamic and tension data in response to static contraction and muscle stretch before and during 5 mM CP-96,344 dialysis in L7 dorsal horn**

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<th>Control (n = 5)</th>
<th>1 h</th>
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<tr>
<td></td>
<td>Baseline</td>
<td>Peak</td>
<td>Change</td>
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<tr>
<td><strong>Contraction</strong></td>
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<tr>
<td>MAP, mmHg</td>
<td>101 ± 10</td>
<td>147 ± 14†</td>
<td>45 ± 7</td>
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<tr>
<td>HR, beats/min</td>
<td>163 ± 8</td>
<td>178 ± 10†</td>
<td>15 ± 4</td>
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<tr>
<td>Tension, kg</td>
<td>1.0</td>
<td>8.1 ± 1.5†</td>
<td>7.1 ± 1.5</td>
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Values are means ± SE; n, no. of cats. †P < 0.05 baseline vs. peak.
Table 3. Hemodynamic and tension data in response to static contraction and muscle stretch before and during 5 mM CP-96,345 dialysis in L6 and S1 regions of dorsal horn

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<th>Control</th>
<th>1 h</th>
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<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Peak</td>
<td>Change</td>
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<tr>
<td>MAP, mmHg</td>
<td>100 ± 6</td>
<td>159 ± 12†</td>
<td>58 ± 9</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>187 ± 11</td>
<td>207 ± 11†</td>
<td>20 ± 5</td>
</tr>
<tr>
<td>Tension, kg</td>
<td>1.0</td>
<td>9.1 ± 0.8†</td>
<td>8.1 ± 0.6</td>
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Values are means ± SE; n, no. of cats. *P < 0.05 compared with corresponding control value; †P < 0.05 baseline vs. peak.

dorsal horn NK-1 receptors in segments that are rostral (L6) and caudal (S1) to the segment of entry for the afferent neurons attenuated the pressor response but not the HR response to muscle contraction. Third, dialysis of CP-96,345 into the dorsal horn L6 and S1 reduced the MAP changes elicited by muscle stretch. Thus the results of this study support the hypothesis that SP release in the dorsal horn plays a role in mediating the pressor responses elicited by static contraction and stretch of skeletal muscle. Furthermore, this study provides evidence for a multisegmental influence of this neuropeptide.

SP is an undecapeptide found in the spinal terminations of afferent neurons (10, 25, 30). Several lines of evidence indicate that this peptide is found in group IV (unmyelinated) afferent fibers, one of the afferent types mediating the pressor reflex. Electron microscopic analysis showed SP in unmyelinated neurons (19). Local application of capsaicin to a peripheral nerve, which causes destruction of group IV fibers, markedly reduces the SP content in the dorsal horn (19). Furthermore, the release of SP in the spinal cord in response to electrical stimulation of the afferent neurons only occurs at intensities that activate group III and IV fibers (1, 5). Thus SP exists in the same class of afferent neurons that elicit the pressor reflex.

SP is a member of a family of structurally similar peptides called tachykinins (8, 16). The other mammalian tachykinins include neurokinin A (NKA) and neurokinin B (NKB). Previous work has shown that, similar to SP, NKA is present in afferent neurons (24). Also, Duggan et al. (4) showed that static contraction increases NKA release in the dorsal horn of the spinal cord. Although structurally similar, NKA exerts its actions by binding to NK-2 receptors, whereas SP binds NK-1 receptors (8, 16). The antagonist used in the current study CP-96,345 is selective for the NK-1 receptor (28). Furthermore, a previous study showed that blockade of spinal NK-2 receptors failed to alter the pressor reflex (9). Thus a functional role for the contraction-evoked release of NKA, as it applies to the pressor reflex, is still unknown.

We have previously demonstrated with the use of microinjections that blocking SP receptors in the L7 dorsal horn attenuates the pressor reflex evoked by static contraction (36). However, the antagonist used in that study was not selective for NK-1 receptors, the receptor subtype that SP preferentially binds. Thus the use of a selective NK-1 antagonist in the current study extends our previous work. In addition, our current study supports previous work showing that the intrathecal administration of CP-96,345 attenuates the reflex cardiovascular changes seen during static contraction (9). Further evidence supporting a role for SP in...
producing the pressor reflex is that static contraction increases the release of this neuropeptide in the L7 dorsal horn (20, 23, 32). Also, SP release in the L7 dorsal horn, as well as the cardiovascular changes, are influenced by the magnitude of the developed tension (33). Considered together, these studies provide strong evidence supporting the hypothesis that SP release in the L7 dorsal horn partially mediates the pressor reflex evoked by static contraction of skeletal muscle.

The results from this study also suggest that activation of NK-1 receptors in segments of the spinal cord rostral and caudal to where the afferent fibers enter partially mediates the pressor reflex. Afferent fibers primarily enter the spinal cord via the dorsal root and collaterals from these neurons synapse in the dorsal horn at the spinal segment in which they enter. However, branches of the afferent neurons course in a rostrocaudal direction in the spinal cord synapsing in the dorsal horn of segments that are removed from the segment in which they entered. The presence of these branches has been demonstrated by anatomical and neurophysiological studies (2, 26, 31). We have previously demonstrated that activation of excitatory amino acid receptors in spinal segments that are adjacent to the segment in which the afferent neurons enter the spinal cord partially mediates the pressor reflex (6, 7). However, whether or not NK-1 receptor activation in these sites is involved in mediating the pressor reflex has not been tested to date. In the model used in the current study, all of the afferent neurons mediating the pressor reflex enter the spinal cord via the L7 dorsal root (see methods). The results from the current study demonstrate that blockade of NK-1 receptors in the dorsal horn at L5 and S1 reduced the increase in MAP produced by static contraction. This suggests that activation of NK-1 receptors in spinal segments adjacent to the entry site is involved in evoking the reflex increases in arterial blood pressure that occur during static contraction of skeletal muscle.

Stretch of the triceps surae muscle increased HR and MAP, as has been demonstrated in previous studies (17, 29, 36). Presumably, muscle stretch activates mechanically sensitive afferent neurons (13, 15). Similar to our previous work (36), blockade of SP receptors in the L7 dorsal horn failed to attenuate the increase in MAP evoked by muscle stretch. However, unlike the previous study, dialysis of CP-96,345 into L6 and S1 provide additional support for this concept. After 2 h of CP-96,345 dialysis into L6 and S1, the time course and the change in MAP elicited by muscle stretch were attenuated. Thus the results of the current study suggest that the reflex arterial blood pressure changes elicited by muscle mechanoreceptor activation involve multisegmental activation of dorsal horn cells and that SP is one mediator involved in this reflex.

The results from this study, as well as our previous work (6, 7), indicate that synaptic transmission in several spinal segments is responsible for evoking the pressor reflex, even though the afferent input is limited to one segment. Thus during muscular activity in an intact animal, when the afferent input is not limited to one segment, dorsal horn cells within a given spinal segment can be activated from afferent fibers arising from numerous spinal levels. This multisegmental activation may represent a type of intraspinal redundancy for dorsal horn cells associated with primary afferent neurons. On the other hand, the neurons that activated cells within the dorsal horn of L6 and S1 may arise from the L7 dorsal horn. In other words, the primary afferent fibers activated by static contraction may have stimulated cells in the dorsal horn at the site of entry, i.e., L7. In turn, these L7 neurons could project to and stimulate the cells in L6 and S1. However, this seems unlikely with respect to SP, since this neuropeptide appears to be very scarce in intraspinal neurons (10). Nevertheless, this multisegmental activation of dorsal horn cells, whether from primary afferent fibers or intraspinal neurons, suggests that the spinal cord serves as an important integrative site for the coupling of skeletal muscle afferent neurons and cardiovascular function.

To summarize, static contraction and muscle stretch of the triceps surae muscle reflexly increased MAP and HR in anesthetized cats. Blockade of NK-1 receptors in the dorsal horn attenuated these pressor responses. This occurred both at the segment where the afferent fibers mediating the reflex enter the spinal cord, as well as in segments where collaterals from rostral and caudal branches of afferent fibers synapse. Thus these data support the hypothesis that the pressor reflex is partially mediated by activation of NK-1 receptors in the dorsal horn of several spinal cord segments.

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