Effects of Clonidine on the Reflex Cardiovascular Responses and Release of Substance P During Muscle Contraction

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Abstract The effects of microdialyzing clonidine into the L-7 dorsal horn on the cardiovascular responses, renal sympathetic nerve activity (RSNA), and release of substance P (SP) evoked by static contraction of the triceps surae muscle were studied using anesthetized cats. A microdialysis probe was inserted into the spinal cord ipsilateral to the muscle being contracted or stretched. Contraction, evoked by stimulation of the distal ends of the cut L-7 and S-1 ventral roots for 1 minute, increased mean arterial pressure (MAP), heart rate (HR), and RSNA by 48±6 mm Hg, 18±2 beats per minute, and 66±5%, respectively. Passive stretch of the same muscle for 1 minute also increased MAP, HR, and RSNA by 51±6 mm Hg, 17±2 beats per minute, and 50±3%, respectively. Microdialysis of clonidine (380 μmol/L) blunted the contraction-evoked responses: MAP, HR, and RSNA increased by 19±4 mm Hg, 7±1 beats per minute, and 24±5%, respectively. The increases elicited by passive stretch were also attenuated (MAP, 22±4 mm Hg; HR, 6±1 beats per minute; and RSNA, 15±4%). This attenuation by clonidine was dose dependent (3.8 μmol/L, 38 μmol/L, 380 μmol/L, and 3.8 mmol/L). Preadministration of the α2-adrenergic antagonist yohimbine (3 mmol/L) blocked the effect of clonidine (380 μmol/L) on the cardiovascular and RSNA responses to muscle contraction. Clonidine (380 μmol/L) did not alter the release of SP in the dorsal horn during contraction (before clonidine, 0.380±0.018 fmol/100 μL; after clonidine, 0.356±0.012 fmol/100 μL). These results demonstrate that stimulation of α2-adrenergic receptors in the L-7 dorsal horn attenuates the cardiovascular responses and RSNA changes to static contraction and passive stretch. This attenuation by clonidine appears not to be mediated through presynaptic inhibition of SP release. (Circ Res. 1994;75:567-575.)

Key Words • exercise pressor reflex • passive stretch • sympathetic nerve activity • α2-adrenergic receptor • yohimbine

Static muscle contraction evokes increases in arterial pressure (AP), heart rate (HR), cardiac contractility, and renal sympathetic nerve activity (RSNA) in anesthetized cats.1-6 These changes are a reflex arising from the contracting muscle, which is commonly known as the “exercise pressor reflex.”7 The reflex cardiovascular and sympathetic nerve activity responses are initiated by a contraction-induced activation of thinly myelinated (group III) and unmyelinated (group IV) muscle afferents.3 The majority of the group III afferents display mechanosensitivity; i.e., their endings respond to mechanical stimuli, whereas the majority of the group IV afferents respond to metabolic changes.7-8

The dorsal horn of the spinal cord is the site of the first synapse of the majority of group III and group IV muscle afferents.9,10 Thus, this region may play an important role in the reflex cardiovascular changes observed during muscle contraction. A variety of neurotransmitters and/or neuromodulators of group III and group IV afferents have been identified in the dorsal horn, substance P (SP) being an example.11-14 In addition, it has been recently reported that static muscle contraction causes the release of SP in the dorsal horn region of the spinal cord and that this release is related to the developed tension.15,16

Autoradiographic studies have identified significant levels of α2-adrenergic receptors in the dorsal horn.17,18 Stimulation of α2-adrenergic receptors in the dorsal horn of the cat spinal cord modulates sensory transmission.19-22 It has been recently reported that stimulation of α2-adrenergic receptors in the spinal cord by the intrathecal administration of clonidine attenuates the reflex cardiovascular and ventilatory responses to static muscle contraction.23 In addition, it has been shown that α2-adrenergic receptor agonists inhibit the spinal release of SP evoked by electrical stimulation of the sciatic nerve at intensities that activate group III and group IV afferents.12 Since SP appears to play a role in the reflex cardiovascular changes evoked by muscle contraction,15,16,24,25 it is possible that clonidine might attenuate the responses through a reduction in the SP release. Therefore, the purpose of the present study was to determine whether microdialysis of clonidine into the L-7 dorsal horn region of the spinal cord attenuates the cardiovascular responses and RSNA to static muscle contraction and passive stretch. Stretching of the muscle served to primarily activate the mechanosensitive afferents. In addition, the present study investigated whether microdialysis of clonidine attenuates the cardiovascular and RSNA responses during static contraction through a reduction in the release of SP in the dorsal horn of the spinal cord.
Materials and Methods

Surgical Preparation

Experiments were performed on 27 spontaneously breathing anesthetized cats of either sex, weighing between 1.8 and 3.6 kg. Anesthesia was induced by inhalation of a 2.5% to 5.0% halothane–nitrous oxide–oxygen mixture. An endotracheal tube was inserted into the trachea, and a jugular vein and carotid artery were catheterized. Gaseous anesthesia was then replaced by administration of α-chloralose (60 mg/kg IV). Throughout the experiment, supplemental doses of α-chloralose (5 to 10 mg/kg IV) were given if the cat’s pupils became dilated and/or a corneal reflex was evident and/or if withdrawal of the limb occurred in response to noxious pinch of the paw. Arterial blood gases and pH were periodically checked (model ABL-3, Radiometer) and maintained within normal limits (pH 7.30 to 7.40; PaCO2, 32 to 36 mm Hg; PaO2, >80 mm Hg) by injecting sodium bicarbonate intravenously, by brief hyperventilation using a respirator (model 661, Harvard Apparatus), or by administering oxygen. 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.

For the studies in experiment 1 (see below), the left kidney was exposed retroperitoneally, and a branch of the renal nerve was isolated from the renal plexus and surrounding connective tissue with the aid of a dissecting microscope (Zeiss). A pair of silver-wire electrodes was carefully wound around the nerve and then covered with silicone gel. The electrodes were connected to a high-impedance probe (model HIP511, Grass Instrument Co), and the raw renal nerve activity was amplified by a differential preamplifier (Grass model P511K) with the low- and high-band-pass filters set at 30 and 3000 Hz, respectively. The amplified output was rectified by a full-wave rectifier circuit and then integrated with a time constant of 1 second. The integrated nerve signal was used for the analysis of RSNA. In each experiment, the background noise level was checked after the animal was euthanized with an overdose of sodium pentobarbital (120 mg/kg IV). The noise level was subtracted from the integrated output signal.

The calcaneal bone was cut, allowing the Achilles tendon to be connected to a force transducer (Grass model FT10). The pelvis was stabilized in a spinal unit (Kopf Instruments), and the knee joint was secured by attaching the patellar tendon to a steel post.

A laminectomy was performed, exposing the lower lumbar and upper sacral portions of the spinal cord, and the right L-5–S-2 spinal roots were isolated. The L-7 and S-1 dorsal and ventral roots were separated, and the ventral roots were severed close to the spinal cord. The S-1 dorsal root and the L-5, L-6, and S-2 dorsal and ventral roots were cut. Only the L-7 dorsal root was left intact. The peripheral ends of the transected L-7 and S-1 ventral roots were placed on bipolar platinum stimulating electrodes. The exposed spinal cord was kept moist with physiological saline.

Microdialysis

A microdialysis probe (model CMA-10, Bioanalytical Systems Inc) with a 3-mm membrane tip was inserted into the right L-7 dorsal horn region with a Kopf carrier (Kopf Instruments) so that the entire membrane was submerged in the cord tissue. The probe was then continuously perfused at a rate of 5 μL/min with artificial extracellular fluid (ECF) 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, HCO₃⁻ 13, and Mg²⁺ 1.3. The pH was adjusted to 7.4. This solution was made fresh for each experiment. The drugs used in the present study, clonidine hydrochloride and yohimbine hydrochloride (Sigma Chemical Co), were dissolved in this ECF solution. To stabilize the probe, a mound of agar was formed surrounding the probe, the height of which was at the same level as the muscles of the back. Then, a piece of parafilm was placed around the shaft of the probe on top of the agar to form a bridge, which was fixed to the shaft of the probe and adjoining muscles of the back by applying a rapid-setting silicone elastomer (A6653, Smith and Nephew, Rolyan Co). The Kopf carrier was removed from the shaft of the probe once the elastomer had set. A new probe was used for each animal experiment.

Radioimmunoassay for SP-like Immunoreactivity

The radioimmunoassay (RIA) procedure used has been previously described in detail. Briefly, removable microplate wells (Removawell Immuno 4, Dynatech) were coated with 1 μg purified protein A (purified recombinant protein A, binding 1.3 mg rabbit IgG per milligram, Pierce) 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 antiserum, diluted in RIA buffer, was added. The wells were then 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. Again, after washing three times with the RIA buffer, standards in triplicate and samples (100 μL) were added to the wells and incubated for 24 hours at 4°C. [125I]-Labeled [125I]SP-(1-11) (110 μL) was added to the standards and the samples. These were incubated for 24 hours at 4°C. Wells were then washed three times with RIA buffer, separated, and counted for 10 minutes in a 20-well gamma counter; a cubic spline algorithm was used for data processing.

The cross-reactivity at IC₅₀ was 100% for SP-(1-11) and SP-(2-11); 98% for [125I]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); and <0.01% for SP-(7-11), SP-(8-11), SP-(9-11), neurokinin A, neurokinin B, somatostatin, neuropeptide K, kassinin, edenisin, and physalaemin. The detection limit of the assay was 0.07 fmol/100 μL, and the coefficients of variation for 0.1 fmol/100 μL and 0.5 fmol/100 μL were 6.3% and 2.1%, respectively.

Histology

At the end of all the experiments, the probe was perfused with a solution containing ferric chloride for 40 minutes, followed by another 10-minute dialysis of distilled water to minimize accumulation of ferric chloride at the site of the probe insertion. The animal was euthanized with an intravenous injection of sodium pentobarbital (120 mg/kg). The L-5–S-2 region of the spinal cord was removed and placed in a solution of 10% phosphate-buffered formalin with 1% potassium ferrocyanide and potassium ferricyanide to elicit a Prussian blue reaction. On a separate day, 50-μm frozen transverse sections of the spinal cord were cut using a cryostat (2800 Frigocut E, Reichert-Yeung, Cambridge Instruments) to determine the location of the tract and the distribution of the Prussian blue reaction.

Experiment 1 (Protocol)

Eighteen cats were used in this protocol. The preparation was allowed to stabilize for 2 hours, during which the ECF was continuously dialyzed. Then, a static contraction of the right triceps surae muscle was performed, and AP, mean arterial pressure (MAP), HR, RSNA, and muscle tension were recorded. The contraction was induced by simultaneous electrical stimulation (three times motor threshold, 40 Hz, 0.1 millisecond) of the peripheral cut ends of the L-7 and S-1 ventral roots for 1 minute. After measuring these control cardiovascular responses, the dose-response effect of microdialyzing clonidine was determined. This was done by perfusing clonidine at doses of 3.8 μmol/L, 38 μmol/L, 380 μmol/L, and
3.8 mmol/L sequentially for \( \approx 40 \) minutes. After each dose, the muscle contraction was repeated as described previously. The dose of 380 \( \mu \)mol/L clonidine was chosen for subsequent experiments (see "Results").

For the experiments in which 380 \( \mu \)mol/L clonidine was tested \((n=8)\), \( \approx 15 \) minutes after the control contraction of the triceps surae, the same muscle was passively stretched for 1 minute to a tension equal to that elicited by the contraction. Then, clonidine was microdialyzed for \( \approx 40 \) minutes, and the muscle contraction was repeated. After \( \approx 15 \) minutes, the passive stretch was repeated as described previously. In six of these eight cats, the ECF solution was microdialyzed for 2 hours after discontinuing the perfusion of clonidine, and both muscle contraction and passive stretch were performed to determine if the cardiovascular and RSNA responses returned to their control levels (recovery). A separate set of cats \((n=5)\) was used to determine the effects of the \( \alpha_2 \)-adrenergic receptor antagonist yohimbine on the cardiovascular and RSNA responses to muscle contraction and passive stretch. After determining the control responses to contraction and stretch, yohimbine \((3 \ \text{mmol/L})\) was dialyzed for \( \approx 1 \) hour. Next, the muscle contraction and passive stretch were repeated. Then, clonidine \((380 \ \text{mmol/L})\) was dialyzed into the spinal cord for another 40 minutes, the muscle manipulations were repeated, and the responses were recorded. Clonidine microdialysis was performed for an additional 40 minutes (total, 80 minutes), and the contraction and stretch were repeated. If the effects of clonidine were still blunted by yohimbine after an 80-minute dialysis period, this would further support the specificity of \( \alpha_2 \)-adrenergic receptors.

**Experiment 2 (Protocol)**

Nine cats were used to determine the effect of microdialyzing clonidine \((380 \ \text{mmol/L})\) on the release of SP in the dorsal horn of the spinal cord in response to static contraction. After inserting the microdialysis probe, six 20-minute collection periods were performed (Table 1). These control collections were performed so that basal SP release was attained before muscle contraction. Once the control collections were completed, a 5- to 8-minute static contraction was evoked by alternately stimulating the L-7 and S-1 ventral roots (three times motor threshold, 30 Hz, 0.1 millisecond duration) during a 10-minute collection period (Table 1). The alternate stimulation-evoked contraction was performed so that the muscle did not fatigue rapidly. Next, the muscle was allowed to recover during a 40-minute (two times for 20 minutes each) collection period. The muscle contraction was then repeated during another 10-minute collection. After this, another 40-minute (two times for 20 minutes each) collection period was performed. The dialysates from the two collections were then combined so that the final sample volume was 100 \( \mu \)L. Then, clonidine \((380 \ \mu \text{L})\) was microdialyzed for 40 minutes (two collection periods for 20 minutes each), and the contraction protocol was repeated as described above. The MAP and HR responses to the muscle contractions before and after clonidine were also recorded during this protocol. The samples were stored in a freezer \((-80^\circ \text{C})\), and on a separate day, the SP-like immunoreactivity (SP-LI) of the perfusate was measured by RIA (see above). The tubes containing the dialysis samples were coded, and the person who performed the assays was unaware of the code or the protocol.

**Data Analysis**

Data were continuously recorded on an eight-channel chart recorder (Gould model 2800S). The carotid artery catheter was connected to a pressure transducer (Statham model P231D) for measuring AP. MAP was obtained by integrating the AP signal with a time constant of 4 seconds. HR was derived from the AP pulse (Biotach, Gould). Muscle tension was measured by the force transducer.

A 60-second period of RSNA \((cRSNA, \text{in microvolts})\) was measured before each muscle manipulation. Since the absolute voltage (in microvolts) of RSNA varied between animals, the precontraction and prestretch control values were defined as 100%. Percent change in RSNA \((\text{RSNA}\%)\) from control values during each muscle manipulation were calculated by using the following formula:

\[
\text{RSNA}\% = \frac{[c\text{RSNA} - \Delta c\text{RSNA}] \times c\text{RSNA}}{100}
\]

Data are expressed as mean\(\pm\)SEM. A one-way ANOVA with repeated measures \((\text{RM-ANOVA})\) was used to compare the dose-response effect of clonidine. Peak changes in MAP, HR, and RSNA elicited by muscle contraction before and 40 minutes after clonidine administration were compared by paired Student’s \(t\) test. The same comparisons were performed for the responses evoked by passive stretch. The hemodynamic, RSNA, and tension data were analyzed by one-way RM-ANOVA. Comparisons of changes in cardiovascular variables and RSNA measured before, 40 minutes after clonidine, and 2 hours after discontinuation of clonidine (recovery) were performed by one-way RM-ANOVA. Also, the one-way RM-ANOVA was used for comparison of changes in MAP, HR, and RSNA before yohimbine, after yohimbine, and after clonidine. Basal and peak changes in SP-LI elicited by muscle contraction before and after clonidine were compared by one-way RM-ANOVA. The cardiovascular variables measured in experiment 2 were also analyzed by paired Student’s \(t\) test. Post hoc analyses for the ANOVAs were performed by Tukey’s \(t\) test.\(^{26}\) For all analyses, \(P<.05\) was considered to be statistically significant.

**Results**

**Experiment 1**

**Dose-Response Effect of Clonidine**

The muscle contraction elicited an increase in MAP, which was attenuated by microdialysis of clonidine in a dose-dependent manner (Fig 1). However, the lowest dose \((3.8 \ \mu\text{mol/L})\) did not blunt the contraction-induced increase in MAP. The increases in HR and RSNA evoked by muscle contraction were also attenuated dose-dependently by clonidine. The developed muscle tensions across all the doses were similar. Microdialysis of clonidine between each dose did not alter the baseline cardiovascular variables or RSNA. However, the highest dose \((3.8 \ \text{mmol/L})\) tended to decrease the baseline MAP in one of the three cats. Because of this possible peripheral effect, the dose of 380 \( \mu \)mol/L clonidine was used in subsequent experiments.
Contraction

Before and After Clonidine (380 μmol/L)

The typical tracings of changes in the AP, MAP, HR, RSNA, and tension evoked by passive stretch of the triceps surae muscle before (control) and 40 minutes after clonidine (380 μmol/L) in the same cat are illustrated in Fig 4. Passive stretch produced an increase in MAP, HR, and RSNA, whereas after microdialysis of clonidine, these responses were blunted. The peak tensions before and after clonidine were the same. The peak increases in MAP and RSNA in response to stretch under control conditions and after 40 minutes of microdialyzing clonidine are shown in Fig 3B. The baseline and peak cardiovascular variables, RSNA values, and tension data in response to passive stretch before and after microdialyzing clonidine are shown in Table 2.

Muscle Contraction and Passive Stretch During Recovery

For the six cats in which recovery was tested, clonidine blunted the MAP and RSNA responses to muscle contraction; however, after 2 hours of microdialyzing ECF, the responses recovered (Fig 5A). This indicates that the clonidine-induced attenuation of the pressor and RSNA responses were due to the effects of the drug. The increases in MAP and RSNA in response to passive stretch also recovered (Fig 5B). Likewise, the peak changes in HR to both contraction and stretch recovered. For contraction, the HR changes in the control condition, during clonidine administration, and in recovery were 19±2, 7±2, and 20±3 beats per minute, respectively; for stretch, the changes were 18±2, 7±2, and 19±2 beats per minute, respectively. Peak tensions were the same throughout the protocol.

Effects of Yohimbine on Muscle Contraction and Passive Stretch

Yohimbine had no effect on baseline MAP, HR, and RSNA (n=5). Moreover, yohimbine had no influence

FIG 1. Bar graph showing the effects of microdialysis of clonidine (solid bar) on the changes in mean arterial pressure (MAP) in response to static contraction of the triceps surae muscle for 1 minute in anesthetized cats. Open bar represents control response before clonidine (n=5). Clonidine, in doses of 3.8 μmol/L (n=3), 38 μmol/L (n=4), 380 μmol/L (n=5), and 3.8 mmol/L (n=3) produced a dose-dependent attenuation of the pressor response. Values are mean±SEM. *P<.05 vs value before clonidine.

Fig 2. Tracings showing changes in arterial pressure (AP), mean arterial pressure (MAP), heart rate (HR), renal sympathetic nerve activity (RSNA), and tension during a 1-minute static contraction of the triceps surae muscle before (control) and after microdialysis of clonidine (380 μmol/L) for 40 minutes in the same anesthetized cat.
on the responses evoked by muscle contraction and passive stretch (Fig 6). Subsequent microdialysis of clonidine (380 μmol/L) for 40 minutes did not produce any attenuation of the pressor or RSNA responses to the muscle contraction and passive stretch (Fig 6). The dialysis of clonidine for another 40 minutes (total, 80 minutes) also had no effect on the responses to either muscle contraction or passive stretch (Fig 6). Changes in the HR response to contraction during the control condition, after yohimbine, and after 40 and 80 minutes of clonidine were 17±2, 17±3, 18±3, and 15±2 beats per minute, respectively. For passive stretch, the HR changes were 16±1, 15±3, 15±1, and 13±2 beats per minute, respectively.

Experiment 2

Effects of Clonidine on SP-LI During Muscle Contraction

The basal release of SP-LI for this preparation (Fig 7) is similar to that shown in a previous study. During the control condition, muscle contraction evoked an increase in the concentration of SP-LI. The basal release of SP was increased during the dialysis of clonidine. Subsequent muscle contraction also elicited an increase in SP-LI, which was not different from that before clonidine (Fig 7). The tensions developed by the contracting muscle before and after clonidine were the same. The peak changes in MAP and HR in response to muscle contraction before microdialysis of clonidine were 32±3 mm Hg and 14±1 beats per minute, respectively, whereas after clonidine, the MAP and HR responses were 14±2 mm Hg and 9±1 beats per minute, respectively (P<.05 versus values before clonidine).

Histology

Sections of the spinal cord, viewed with a microscope, revealed the Prussian blue reaction in the dorsal horn (Fig 8). The tract made by the microdialysis probe was seen entering through the L-7 dorsal horn to a depth of ≈3 mm. The tip of the probe was at the level of the lamina VII. The rostral-caudal spread of the blue reaction was ≈4 mm. It was not possible to obtain histological data for the spinal cords of five cats because of technical difficulties. However, the cardiovascular and RSNA responses to muscle contraction in these experiments were similar to those in the others. Thus, these data were not excluded.

Table 2. Hemodynamic, Renal Sympathetic Nerve Activity, and Tension Data Before and 40 Minutes After Microdialysis of Clonidine (380 μmol/L)

<table>
<thead>
<tr>
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<th>Before Clonidine</th>
<th>After Clonidine</th>
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<td></td>
<td>n</td>
<td>Baseline Peak</td>
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<tr>
<td><strong>Contraction</strong></td>
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<tr>
<td>MAP, mm Hg</td>
<td>8</td>
<td>109±5 156±5*</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>8</td>
<td>187±8 205±7*</td>
</tr>
<tr>
<td>RSNA, %</td>
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<td>100 166±5*</td>
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<tr>
<td>Tension, kg</td>
<td>8</td>
<td>1.0 10.3±0.8</td>
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<td><strong>Passive stretch</strong></td>
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<tr>
<td>MAP, mm Hg</td>
<td>8</td>
<td>104±6 155±65*</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>8</td>
<td>184±7 200±6*</td>
</tr>
<tr>
<td>RSNA, %</td>
<td>6</td>
<td>100 150±3*</td>
</tr>
<tr>
<td>Tension, kg</td>
<td>8</td>
<td>1.0 10.4±0.5</td>
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n indicates number of animals; MAP, mean arterial pressure; HR, heart rate; and RSNA, renal sympathetic nerve activity. Values are mean±SEM.

*P<.05 vs corresponding control value; †P<.05 vs preclonidine peak value.
Discussion

The present study determined the effects of clonidine, microdialyzed into the L-7 dorsal horn, on the reflex increases in MAP, HR, and RSNA in response to static contraction and passive stretch of the triceps surae muscle. In the present study, clonidine attenuated the reflex increases in MAP, HR, and RSNA to muscular contraction and passive stretch. The attenuations by clonidine were blocked by prior microdialysis of yohimbine, the α2-adrenergic receptor antagonist. These results demonstrate that stimulation of α2-adrenergic receptors in the L-7 dorsal horn of the spinal cord attenuates the cardiovascular and RSNA changes to static muscle contraction and passive stretch. In addition, clonidine did not change the release of SP in the dorsal horn in response to muscle contraction. Therefore, these data do not support the hypothesis that clonidine attenuates the cardiovascular responses to muscle contraction through an inhibition of the release of SP in the dorsal horn of the spinal cord.

 Autoradiographic visualization of the rat and cat spinal cord sections has demonstrated a dense distribution of α2-adrenergic receptor binding sites in the dorsal horn. Microinjection of norepinephrine into the dorsal horn of the cat spinal cord produces an inhibition of C-fiber (ie, group IV afferent) reflexes evoked by either electrical stimulation of a cutaneous nerve or noxious radiant heat applied to the foot pad. Furthermore, iontophoretically applied norepinephrine suppresses dorsal horn neuronal activity evoked by noxious stimuli. It has also been reported that administration of norepinephrine into the dorsal horn neurons

**Fig 4.** Tracings showing changes in arterial pressure (AP), mean arterial pressure (MAP), heart rate (HR), tension, and renal sympathetic nerve activity (RSNA) during a 1-minute passive stretch of the triceps surae muscle before and after microdialysis of clonidine (380 μmol/L) for 40 minutes in the same anesthetized cat.

**Fig 5.** Bar graphs showing peak changes in mean arterial pressure (MAP) and renal sympathetic nerve activity (RSNA) in response to static muscle contraction (A) and passive stretch (B) before clonidine (open bar), after 40 minutes of clonidine (380 μmol/L) microdialysis (solid bar), and 2 hours after discontinuing clonidine (recovery, crosshatched bar). Values are mean±SEM. *P<.05 vs before clonidine (for MAP studies, n=6; for RSNA studies, n=5).
causes primary afferent depolarization. Thus, activation of α2-adrenergic receptors in the dorsal horn of the spinal cord appears to inhibit spinal nociceptive and sensory transmission.

The activation of group III and group IV muscle afferents arising from the contracting muscle has been shown to involve the afferent arm of the reflex cardiovascular changes to static contraction. The dorsal horn is the site of the first synapse of the majority of the group III and group IV muscle fibers; after reaching the first synapse, impulses evoked by activation of these afferents are transmitted through the second-order neurons in the dorsal horn to higher brain centers. A previous report has shown that the intrathecal administration of clonidine into the lumbosacral spinal cord attenuates the cardiovascular responses to muscle contraction through stimulation of α2-adrenergic receptors in the spinal cord. However, a more localized site of action of clonidine in the spinal cord has not been investigated. In the present study, all the dorsal roots of the spinal cord from L-5 to S-2, excluding L-7, were severed. Thus, the contraction- or stretch-induced activation of the group III and group IV muscle afferents was transmitted only through the L-7 dorsal root. The distribution of the Prussian blue reaction in the L-7 dorsal horn region, extending ≈4 mm rostrocaudally, suggests the probable diffusion of clonidine in the dorsal horn. Thus, it may be presumed that clonidine exerted its effect on the L-7 dorsal horn.

It has been reported that passive stretch of the triceps surae muscle evokes reflex cardiovascular responses through stimulation of mechanosensitive muscle afferents. In the present study, passive stretch evoked increases in the cardiovascular and RSNA responses, which were attenuated after microdialyzing clonidine. This suggests that clonidine modulates the mechanosensitive muscle afferents through an action on the α2-adrenergic receptors in the L-7 dorsal horn region of the spinal cord. Clonidine also blunted the cardiovascular and RSNA responses to muscle contraction, which activates both mechanoreceptors and metaboreceptors. However, clonidine attenuated the pressor responses to contraction and stretch by equivalent amounts. Because there is no specific stimulus to activate the metabosensitive afferents only, it cannot be concluded from the present study that clonidine does not act on metabosensitive muscle afferents.

Microdialysis of yohimbine into the L-7 dorsal horn did not alter the baseline cardiovascular variables and RSNA. Furthermore, yohimbine had no influence on the reflex MAP, HR, and RSNA responses to contraction and stretch. Therefore, α2-adrenergic receptors in

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**Fig. 6.** Bar graphs showing peak changes in mean arterial pressure (MAP) and renal sympathetic nerve activity (RSNA) in response to static muscle contraction (A) and passive stretch (B), before yohimbine (open bar), after microdialysis of yohimbine (3 μmol/L) for 1 hour (hatched bar), 40 minutes after microdialysis of clonidine (380 μmol/L, solid bar), and 80 minutes after clonidine (stippled bar). Values are mean±SEM (n=5).

**Fig. 7.** Bar graph showing the substance P-like immunoreactivity (SP-LI) in the dialysate collected before clonidine (open bar) and after microdialysis of clonidine (380 μmol/L, solid bar). Numbers are mean±SEM (n=9). *P<.05 vs precontraction value before clonidine; tP<.05 vs corresponding precontraction value.
the L-7 dorsal horn do not appear to have a tonic influence on the neurons mediating the cardiovascular and RSNA responses to static muscle contraction or passive stretch in this preparation. Yohimbin blocked the effects of clonidine, thereby suggesting that clonidine exerted its effect via α₁-adrenergic receptors in the L-7 dorsal horn region of the spinal cord.

The effects of clonidine could have been mediated through a presynaptic and/or a postsynaptic mechanism. Iontophoretic administration of adrenergic agonists in the spinal cord produces presynaptically mediated inhibitory effects on primary afferent C fibers (ie, group IV afferents) in the dorsal horn of the cat spinal cord.25,26 Furthermore, recent reports have demonstrated that presynaptic α₁-adrenergic receptors localized on the primary afferents modulate the transmission of nociceptive information.27,28 It has also been reported that adrenergic agonists inhibit the release of SP from the primary afferents in the spinal dorsal horn through presynaptic α₁-adrenergic receptors.29 Furthermore, α₁-adrenergic receptor agonists inhibit the potassium-induced release of SP and also attenuate the spinal release of SP evoked by electrical stimulation of the sciatic nerve at intensities that activate group III and group IV afferents.30 SP is a neurotransmitter and/or a neuromodulator released in the dorsal horn of the spinal cord.31,32 and previous studies have shown that this neuropeptide plays a role in mediating the reflex increases in MAP and HR in response to static muscle contraction.15,16,24,25 It was speculated that microdialysis of clonidine attenuates the reflex cardiovascular and RSNA responses to static muscle contraction by inhibiting the release of SP from the terminals of the muscle afferents. However, the results of the present study do not support this concept, because clonidine did not attenuate the contraction-induced release of SP in the dorsal horn. Furthermore, the fact that the precontraction release of SP was not reduced by the administration of clonidine suggests that this drug does not act by inhibiting the release of this neuropeptide. Thus, clonidine may blunt the cardiovascular responses to muscle contraction through a postsynaptic mechanism. The reason for the elevated precontraction release of SP, during the microdialysis of clonidine, is unknown. It is possible that the time period between contractions was insufficient for SP to return to the control level.

Although the contraction-evoked release of SP was not decreased statistically (P=.064) by clonidine, there was a tendency for it to go down. The release of SP in response to muscle contraction during clonidine administration was 0.024 fmol/100 μL less than the preclonidine value. Although these values seem quite small, this may represent a functionally important reduction. Since microdialysis was designed to sample the interstitial fluid, and thereby quantify neurotransmitter release, a 0.024 fmol (400 000 molecules) reduction in SP may play a role in the physiological effect of clonidine. Depending on the number of synapses involved, a decrease in the release of 400 000 molecules of SP may have been the mechanism by which clonidine blunted the reflex responses to muscle contraction. However, the number of synapses from which the microdialysis probe samples is unknown. Further, the relation between the quantity of neurotransmitter release and effector response is unknown. Thus, the potential functional implication for the small decrease in the contraction-evoked release of SP by clonidine is unclear.

Although the results fail to indicate that clonidine reduces the contraction-induced release of SP, a presynaptic mediated effect of clonidine cannot be excluded, because α₁-adrenergic receptor activation may have altered the release of other neurotransmitters/neuromodulators in the dorsal horn involved in the reflex cardiovascular changes to muscle contraction. Somatostatin is a neurotransmitter and/or neuromodulator found in the afferents synapsing in the dorsal horn.33 Wilson et al.25 demonstrated that the spinal release of somatostatin plays a role in eliciting the cardiovascular responses to isometric muscle contraction. Thus, it may be possible that the attenuating effects of clonidine could have been mediated through a presynaptic inhibition of the release of somatostatin (or other neurotransmitters/neuromodulators) in the dorsal horn of the spinal cord.

In conclusion, the present study demonstrated that the reflex cardiovascular and RSNA changes evoked by both stimulation-induced muscle contraction and passive stretch were attenuated by the α₁-adrenergic receptor agonist clonidine microdialyzed into the L-7 dorsal horn of the spinal cord. By use of microdialysis, the site of action of clonidine was presumed to be localized to the L-7 dorsal horn. The attenuation produced by clonidine appears not to be mediated through a reduction in the release of SP in the dorsal horn of the spinal cord.

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