Attenuation of the Reflex Pressor Response to Muscular Contraction by an Antagonist to Somatostatin

Kyle W. McCoy, Diane M. Rotto, Kenneth J. Rybicki, and Marc P. Kaufman

Although group III and IV fibers are known to compose the afferent pathway of the reflex arc causing the pressor response to static muscular contraction, little is known about the neurotransmitters released by these muscle afferents. Somatostatin might be one of these neurotransmitters because this peptide is found in the terminals of fine afferent fibers ending in the dorsal horn of the lumbar spinal cord. Therefore, in chloralose-anesthetized cats, the reflex pressor response to static contraction was examined before and after subarachnoid injections onto the lumbosacral cord of a peptide antagonist to somatostatin. We found that before giving the antagonist, the pressor response to contraction of the triceps surae muscles in 12 cats averaged $33 \pm 4$ mm Hg, while $37 \pm 7$ minutes after giving the antagonist, the pressor response averaged only $18 \pm 3$ mm Hg ($p<0.001$). In contrast, the antagonist to somatostatin had no effect on either the pressor response to electrical stimulation of the cut central end of the sciatic nerve or the pressor response to stimulation of the posterior diencephalon. Furthermore, subarachnoid injection of a peptide antagonist to luteinizing hormone-releasing hormone had no effect on the reflex pressor response to static contraction. Our findings are consistent with the hypothesis that somatostatin plays a role in the spinal transmission of the contraction-induced pressor reflex arising from hind limb skeletal muscle. (Circulation Research 1988;62:18-24)

In humans, static exercise has been firmly established to increase arterial pressure and heart rate. Part of this pressor response is believed to be a reflex arising from the exercising muscles because it was attenuated by more than half when the contracting muscles were paralyzed with either curare or local anesthetic agents. In addition, electrically induced static contractions, a maneuver that eliminated any central neural command to increase arterial pressure, has been shown to cause a pressor response in humans that was almost identical to that evoked by voluntary contraction.

In anesthetized animals, the reflex pressor response to static muscular contraction has been studied in detail (e.g., Coote et al). The afferent arm of the reflex has been shown to be comprised of group III and IV muscle afferents. There is a small spinal component to the reflex, but medullary circuits are required for its full expression.

Although the neurotransmitters released by the contraction-induced stimulation of group III and IV muscle afferents are not known, immunohistochemical evidence has shown that some thin fiber afferents terminating in the dorsal horn of the lumbar spinal cord contain substance P, while others contain somatostatin. Recent reports from this laboratory have provided evidence consistent with the hypothesis that substance P functions as a spinal neurotransmitter in the reflex pressor response to muscular contraction. Using a peptide antagonist to somatostatin, we have now tested the hypothesis that this peptide also plays a role in the spinal transmission of the reflex pressor response to static contraction of the triceps surae muscles.

Materials and Methods

Cats were anesthetized with $\alpha$-chloralose (80 mg/kg i.p.). The right common carotid artery, right jugular vein, and cervical trachea were cannulated. The lungs were ventilated with room air by a Harvard respirator (model 665). Arterial blood gases were measured periodically (Radiometer ABL-3), and arterial PO$_2$, PCO$_2$, and pH were maintained at normal levels by adjusting ventilation and by intravenous injection of sodium bicarbonate. The lumbar and sacral spinal cord were exposed, and a PE-50 catheter was passed through a hole in the dura mater into the subarachnoid space. The tip of the catheter was located approximately 1 cm rostral to the entry point into the cord of the L$_1$ dorsal root.

Arterial pressure was measured by connecting the carotid cannula to a Statham P23ID transducer. Heart rate was calculated beat-to-beat with a Gould Biotach. Tension developed by the contracting triceps surae muscles was measured by attaching the calcaneal tendon to a force transducer (Grass FT10).

The triceps surae muscles were statically contracted for 30 seconds by electrically stimulating the tibial nerve at 40 Hz (pulse duration 25 $\mu$sec, 1.5-2 times
motor threshold). The pressor response evoked by stimulating the tibial nerve at these parameters has been shown repeatedly to be a reflex caused by muscular contraction and not a reflex caused by electrical stimulation of afferent fibers in the nerve.\(^{12,13}\) The time between contractions was at least 15 minutes.

We examined the reflex pressor response to static contraction of the triceps surae muscles before and after subarachnoid injections of peptide antagonists to somatostatin and luteinizing hormone-releasing hormone (LHRH). The somatostatin antagonist used was cyclo(7-aminooctanoyl)-Phe-d-Trp-Lys-Thr[LZL],\(^{14}\) and the LHRH antagonist used was d-pGlu\(^{1},\)d-Phe\(^{2},\)d-Trp\(^{3,6}\)-LHRH.\(^{15}\) The peptide antagonist to LHRH served as a control. The dose for both antagonists was 160–200 \(\mu g\), and the injection volume was 1 ml. Both antagonists were dissolved in saline. In some experiments, increases in mean arterial pressure and heart rate were evoked by electrically stimulating the posterior diencephalon. To obtain these increases, the cats were first placed in a Kopf stereotaxic instrument. We then stimulated (80 Hz, 0.75 msec pulse duration, 240 \(\mu A\)) the posterior diencephalon with Rhodes monopolar electrodes (SNE-100) using a Grass S88 stimulator coupled to a Grass constant current unit (model PSIU-6). At the end of the experiment, anodal direct current (300 \(\mu A\)) was passed through the electrode, and the brain removed after the cat was given sodium pentobarbital (65 mg/kg i.v.). After using the Prussian blue reaction, the brains were sectioned at 50 \(\mu m\) to locate the sites of stimulation.

In other experiments, we examined the effect of placing pledgets of filter paper (1.5 cm in length) soaked in either the somatostatin antagonist (200 \(\mu g/ml\)) or lidocaine hydrochloride (1%) on L\(_5\), dorsal root action potentials evoked by single pulse (0.3 Hz, 1 msec) stimulation of the sciatic nerve. The current intensity of the pulse was adjusted so that stimulation recruited group IV fibers. The endings of the group III and IV fibers were located in the triceps surae muscles by showing that the fibers discharged bursts of impulses when their receptive fields were either gently stroked with a cotton-tipped applicator or vigorously pinched with a forceps. The conduction distance between stimulating and recording electrodes was measured by placing a thread along the conduction pathway. Conduction time between the two electrodes was measured, and conduction velocity was calculated.

All values are reported as mean \(\pm\) SEM. Analysis of variance followed by Schef\'fe post hoc tests were used to determine statistical significance.

**Results**

*Effect of Peptide Antagonist to Somatostatin on Reflex Pressor Response to Static Contraction*

In 12 cats, subarachnoid injection of the peptide antagonist to somatostatin (160–200 \(\mu g\)) attenuated by almost half the reflex pressor response to static contraction of the triceps surae muscles (Table 1 and Figure 1). Specifically, before giving the antagonist, the pressor response to contraction averaged 33 \(\pm\) 4 mm Hg, while 37 \(\pm\) 7 minutes after injecting the antagonist, the response averaged only 18 \(\pm\) 3 mm Hg (\(p<0.001\)). In addition, the reflex pressor response to contraction partially recovered. Thus, 68 \(\pm\) 10 minutes after injection of the antagonist to somatostatin, the pressor response averaged 24 \(\pm\) 3 mm Hg, an effect that was significantly greater than the pressor response to the contraction evoked 37 \(\pm\) 7 minutes after injection (\(p<0.05\)) but significantly less than the control pressor response (\(p<0.01\)). The tensions developed by the working triceps surae muscles were not significantly different for the three static contractions, averaging 3.2 \(\pm\) 0.6, 3.1 \(\pm\) 0.6, and 3.3 \(\pm\) 0.7 kg, respectively (\(p>0.05\)).

The tachycardia response to contraction before injection of the antagonist was small, averaging only 9 \(\pm\) 3 beats/min. Thirty-seven minutes after injection of the antagonist, the tachycardia response decreased, averaging 4 \(\pm\) 1 beats/min (\(p<0.05\)). However, 68 minutes after injection of the antagonist, the tachycardia response to contraction had not recovered, averaging 6 \(\pm\) 2 beats/min (\(p>0.05\)). Intrathecal injection of the antagonist to somatostatin had no effect on either mean arterial pressure or heart rate in 10 of the 12 cats studied. In the remaining two, injection caused a sudden increase in arterial pressure (50–60 mm Hg) and heart rate (12–20 beats/min). These increases lasted about 5–7 minutes.

In 11 of the 12 cats, we attempted to provide some information about the time course of the attenuation by the somatostatin antagonist of the pressor-tachycardic responses to contraction. To do this, the pressor-tachycardic responses to contraction before giving the antagonist were compared with those to contraction 17 \(\pm\) 1 minutes after giving the antagonist. We found that the pressor response before the antagonist averaged 32 \(\pm\) 4 mm Hg, while the response afterwards averaged 24 \(\pm\) 4 mm Hg (\(p<0.025\)). The tachycardic response before the antagonist averaged 8 \(\pm\) 3 beats/min, while afterward it averaged 7 \(\pm\) 2 beats/min (\(p>0.05\)).

Three of the 12 cats given the subarachnoid injection of the peptide antagonist to somatostatin were paralyzed with gallamine triethiodide (2–3 mg/kg i.v.). Electrical stimulation of the tibial nerve with the same parameters used to evoke static contractions before the cats were paralyzed had no effect on mean arterial pressure or on heart rate afterwards.

*Effect of Peptide Antagonist to Somatostatin on Reflex Pressor Response to Electrical Stimulation of Sciatic Nerve*

Of the 12 cats given the peptide antagonist to somatostatin, the central cut end of the sciatic nerve contralateral to the triceps surae muscles contracted was electrically stimulated (40 Hz, 1 msec) in 6 cats. The intensity of the current used to stimulate the sciatic nerve was adjusted so that it evoked reflex pressor effects. We found that in contrast to its attenuation of the reflex pressor response to contraction, the antagonist had no effect on the reflex pressor response to electrical stimulation of the sciatic nerve (Figure 2).
Table 1. Effect of Somatostatin Antagonist on Mean Arterial Pressure (MAP) and Heart Rate (HR) Responses to Static Contraction of Triceps Surae Muscles in 12 Cats

<table>
<thead>
<tr>
<th></th>
<th>Control contraction</th>
<th>First contraction</th>
<th>Second contraction</th>
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<tr>
<td></td>
<td>Baseline</td>
<td>Peak</td>
<td>Baseline</td>
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<tr>
<td>MAP (mm Hg)</td>
<td>108 ± 5</td>
<td>141 ± 6*</td>
<td>112 ± 6</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>162 ± 8</td>
<td>171 ± 8*</td>
<td>151 ± 9</td>
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*Peak value is statistically significant from its respective baseline value (p<0.05). Note that first contraction refers to contraction performed 37 ± 7 minutes after injecting somatostatin antagonist. Likewise, second contraction refers to contraction performed 68 ± 10 minutes after injecting antagonist.

Specifically, the reflex pressor response to sciatic nerve stimulation averaged 39 ± 8 mm Hg before injection of the somatostatin antagonist and 43 ± 12 mm Hg 38 ± 5 minutes after injection (p>0.05). Likewise, the reflex tachycardic response to sciatic nerve stimulation averaged 11 ± 8 beats/min before injection and 11 ± 8 beats/min afterwards (p>0.05).

Effect of Peptide Antagonist to Somatostatin on Pressor Response to Stimulation of Posterior Diencephalon

In 7 sites from 6 of the cats given the antagonist to somatostatin, the posterior diencephalon was electrically stimulated (80 Hz, 0.75 msec, 240 μA), and the effect of the antagonist on the well-known pressor response to this maneuver was examined. We found that the antagonist had no significant effect on either the pressor or the tachycardic response to stimulation (Table 2). Before injection of the antagonist, the pressor response to stimulation averaged 42 ± 7 mm Hg; 38 ± 6 minutes after injection, the pressor response averaged 38 ± 7 mm Hg (p>0.05). Likewise, before injection, the tachycardic response to stimulation averaged 9 ± 4 beats/min, and afterwards, 5 ± 4 beats/min (p>0.05). Histologic analysis revealed that each of the 7 sites was located in or medial to the fields of Forel. The anterior-posterior plane of the 7 sites was A8-10.16

Effect of Peptide Antagonist to LHRH on Reflex Pressor Response to Static Contraction

In 6 cats, the effect of a peptide antagonist to LHRH (200 μg) on the reflex pressor response to static contraction of the triceps surae muscles was examined. Like the antagonist to somatostatin, the antagonist to LHRH was injected into a cannula placed in the subarachnoid space. We found that the antagonist to LHRH had no effect on the pressor response to contraction, the reflex averaging 30 ± 6 mm Hg before injection and 30 ± 7 mm Hg 33 ± 6 minutes after injection. Likewise, the antagonist to LHRH had no effect on the reflex tachycardic response to contraction, heart rate increasing by 7 ± 4 beats/min before injection and 11 ± 7 beats/min afterwards (p>0.05) (Figure 3). Intrathecal injection of the LHRH antagonist had no

FIGURE 1. Reflex pressor-tachycardic responses to static contraction of the triceps surae muscles were attenuated by a peptide antagonist to somatostatin. Panel A: Control pressor-tachycardic responses to static contraction. Panel B: Thirty-five minutes after intrathecal injection of the peptide antagonist to somatostatin (200 μg). Reflex pressor-tachycardic responses to contraction were attenuated by the antagonist. Panel C: Fifty-three minutes after injection of antagonist to somatostatin. Pressor-tachycardic responses to contraction have partially recovered toward the levels seen in Panel A.
somatostatin blockade and pressor response to contraction

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**Figure 2.** Reflex pressor-tachycardic responses to electrical stimulation of cut central end of sciatic nerve were not attenuated by peptide antagonist to somatostatin. Panel A: Control pressor-tachycardic responses to stimulation of sciatic nerve. Panel B: Twenty-five minutes after injection of antagonist to somatostatin. Pressor-tachycardic responses to stimulation were unchanged from those in Panel A. Note that records shown in Figures 1 and 2 are from the same cat.

The immediate (i.e., within 5 minutes) effect on either mean arterial pressure or heart rate in any of the 6 cats studied.

**Effect of Peptide Antagonist to Somatostatin on the Dorsal Root Action Potential**

In 3 cats, we recorded from the L7 dorsal root action potentials that were evoked by single pulse electrical stimulation (0.3 Hz, 1 msec pulse duration) of the sciatic nerve. Each of the action potentials was shown to arise from either a group III or IV afferent whose ending was in the triceps surae muscles. We then placed onto the L7 dorsal root a pledget of filter paper soaked in a solution of somatostatin antagonist whose concentration was the same as that injected into the subarachnoid space. In each of the three cats, we found that the action potentials evoked by stimulation of the sciatic nerve were not affected by the pledget soaked in the somatostatin antagonist (Figure 4). In each case, the period between the application of the pledget and elicitation of the action potential was 37 minutes, the interval at which an attenuation by the somatostatin antagonist of the reflex pressor response to contraction was found. In each of the three cats, application of a pledget of filter paper soaked in 1% lidocaine hydrochloride solution abolished the action potentials evoked by sciatic nerve stimulation.

**Discussion**

We have shown that subarachnoid injection of a peptide antagonist to somatostatin attenuated by almost half the reflex pressor response to static contraction of the triceps surae muscles. We have also shown that approximately 1 hour after giving the antagonist to somatostatin, the reflex pressor response to contraction was partially restored to its original level, a finding that suggests that the attenuation of the reflex was not due to a time-dependent decrease in the ability of our preparation to respond to static muscular contraction. In addition, we found that the antagonist had no effect on the pressor response to electrical stimulation of the posterior diencephalon. This finding suggests that the attenuation of the contraction-induced pressor response by the antagonist was not due to a partial blockade of the thoracic and lumbar sympathetic outflow caused in turn by spread of the injectate throughout the spinal cord.

We needed to provide evidence that the attenuation of the reflex by the peptide antagonist to somatostatin

<table>
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<th>Table 2. Effect of Somatostatin Antagonist on Mean Arterial Pressure (MAP) and Heart Rate (HR) Responses to Electrical Stimulation of Posterior Diencephalon in 7 Sites From 6 Cats</th>
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<td>MAP (mm Hg)</td>
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<td>HR (beats/min)</td>
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*Peak value is statistically significant from its respective baseline value (p<0.05). Note that stimulation after antagonist condition refers to stimulation of posterior diencephalon 38 ± 6 minutes after injecting antagonist into subarachnoid space.
Arterial Pressure (mm Hg)

Heart Rate (bpm)

Tension (kg)

30 sec

FIGURE 3. Pressor-tachycardic responses to static contraction were not attenuated by peptide antagonist to LHRH. Panel A: Control pressor-tachycardic responses to contraction. Panel B: Thirty-eight minutes after intrathecal injection of peptide antagonist to LHRH (200 μg). Pressor-tachycardic responses to contraction were not attenuated by LHRH antagonist; in fact, in this particular cat, these responses were greater than those in Panel A. Panel C: Forty minutes after intrathecal injection of somatostatin antagonist (180 μg), pressor-tachycardic responses to contraction were attenuated from those in Panel A.

was not caused by a local anesthetic effect on the intraspinal course of group III and IV fibers, the afferents responsible for the contraction-induced pressor response.9 Peptide antagonists to substance P, for example, have been reported to block impulse conduction in an isolated rat sciatic nerve preparation.17 This evidence was provided by showing that the peptide antagonist to somatostatin had no effect on the dorsal

FIGURE 4. Somatostatin antagonist has no effect on L7 dorsal root action potentials evoked by single pulse electrical stimulation of tibial nerve. Panel A: Stimulation discharged a group III afferent (first 2 large spikes) and a group IV afferent (3rd spike), both of which had endings in triceps surae muscles. Note that first impulse from group III afferent was caused by electrical pulse, while second impulse was caused by muscle twitch. Panel B: Pledget of filter paper soaked in somatostatin antagonist (200 μg/ml) was placed onto L7 dorsal root. Thirty-seven minutes later, electrical pulse applied to tibial nerve still evoked same action potentials. Panel C: Pledget of filter paper soaked in 1% lidocaine was placed onto L7 dorsal root. Electrical pulse applied to tibial nerve no longer evoked action potentials. Conduction distance between stimulating and recording electrodes was 140 mm. Conduction velocities of group III and IV afferents were calculated to be 6.1 and 2.3 m/sec, respectively.
root actions of group III and IV fibers that were evoked by electrical stimulation of the sciatic nerve.

We can only speculate as to why the reflex pressor response to muscular contraction was attenuated by the peptide antagonist to somatostatin, while the reflex pressor response to electrical stimulation of the sciatic nerve, a maneuver that activated afferents arising from skin, joint, bone, and skeletal muscle, was not. One explanation for our findings might simply be that somatostatin is released in the dorsal horn of the spinal cord by group III and IV muscle afferents but not by skin, joint, and bone afferents. Although we cannot rule out this explanation, we think it is unlikely because there is evidence that skin afferents release somatostatin in the spinal cord.18

An alternative explanation involves the number of afferents stimulated by the two maneuvers. Contraction of the triceps surae muscles presumably activated far fewer afferents than did electrical stimulation of the sciatic nerve. Hence, one might speculate that the amount of somatostatin released in the dorsal horn of the spinal cord by stimulation of the sciatic nerve was large enough to overwhelm the blocking action of the antagonist, while the amount of somatostatin released by static contraction of the triceps surae muscles was not. We were not able to test this speculation because we used only one dose of the antagonist. Alternatively, it is possible that after somatostatin blockade, the release of other putative neurotransmitters, such as substance P19 and calcitonin-gene related peptide,20 were able to compensate for the loss of somatostatin-induced synaptic input during sciatic nerve stimulation but not during contraction.

One interpretation of our finding that somatostatin blockade attenuated the pressor response to muscular contraction but did not attenuate the pressor response to sciatic nerve stimulation is that the two maneuvers activated, at least in part, different pathways within the spinal cord and medulla. This interpretation, if true, needs to be considered when one attempts to compare the results of studies using electrical stimulation of mixed nerves (e.g., Abboud et al.,20 Clement et al.,21 and Khayutin et al.,25) with those of studies using muscular contraction. Although studies using electrical stimulation of mixed nerves have provided important information about somatosympathetic reflexes, our findings raise the possibility that these studies might have limitations in providing information about cardiovascular control during exercise.

Several lines of evidence have suggested that somatostatin plays a role in the spinal transmission of reflexes arising from the stimulation of thin-fiber somatic afferents. In rats, for example, intrathecal injection of somatostatin has been shown to increase the excitability of the hamstring flexion reflex evoked by electrical stimulation of C-fiber afferents in the sural nerve.23 Also, in rats, intrathecal injection of somatostatin has been shown to cause scratching movements23 and nociceptive responses,24 behaviors that are naturally caused by activation of thin fiber somatic afferents. Furthermore, in rabbits, noxious heat applied to the hind limb skin, a maneuver that stimulated C-fiber thermoreceptors, has been shown to cause the release of somatostatin in the dorsal horn of the lumbar spinal cord.14

Although LHRH is not found in the spinal cord,25 the antagonist to this peptide used in our experiments served as a control because its lack of effect on the reflex pressor response to contraction showed that the attenuation of the reflex response by the somatostatin antagonist was not a nonspecific action of peptide antagonists in the spinal cord. In addition, Takano et al.26 reported that this LHRH antagonist did not block substance P receptors on dorsal horn neurons receiving nociceptive input from thin fiber somatic afferents but did block substance P receptors on intermediodorsal horn neurons maintaining vasomotor tone. Although we did not test the latter finding of Takano et al.,25 we did test the former. Our data extended the report by Takano et al.26 because in our experiments, the peptide antagonist to LHRH had no effect on the contraction-induced pressor reflex whose afferent arm consisted of thin fiber muscle afferents.9

Our preparation was able to demonstrate that spinal blockade of somatostatin attenuated by almost half the reflex pressor response to static muscular contraction, but it was not able to demonstrate that this peptide was functioning as a neurotransmitter at the first synapse of the reflex arc. Moreover, our preparation was not able to determine whether the attenuation of the reflex pressor response to contraction was caused by a decrease in synaptic input arising from mechanoreceptors or metaboreceptors with group III and IV afferent fibers.26,27 Despite these limitations, our experiments have provided the first evidence that somatostatin plays a role in the spinal transmission of this cardiovascular reflex. Based on our findings, we think the speculation that somatostatin is a neurotransmitter at the first synapse of the reflex arc causing the exercise-induced pressor reflex is reasonable.

Acknowledgments

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