Neuronal application of capsaicin modulates somatic pressor reflexes

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LeDoux, J. F., and L. B. Wilson. Neuronal application of capsaicin modulates somatic pressor reflexes. Am J Physiol Regulatory Integrative Comp Physiol 281: R868–R877, 2001.—Static contraction of skeletal muscle elicits a reflex increase in cardiovascular function. Likewise, noxious stimuli activate somatic nociceptors eliciting a reflex increase in cardiovascular function. On the basis of recent work involving spinopthalamic cells in the dorsal horn, we hypothesized that the dorsal horn cells involved in the aforementioned reflexes would be sensitized by applying capsaicin (Cap) to a peripheral nerve. If correct, then Cap would enhance the cardiovascular increases that occur when these reflexes are evoked. Cats were anesthetized, and the popliteal fossa was exposed. Static contraction was induced by electrical stimulation of the tibial nerve at an intensity that did not directly activate small-diameter muscle afferent fibers, whereas nociceptors were stimulated by high-intensity stimulation (after muscle paralysis) of either the saphenous nerve (cutaneous nociceptors) or a muscular branch of the tibial nerve (muscle nociceptors). The reflex cardiovascular responses to these perturbations (contraction or nociceptor stimulation) were determined before and after direct application of Cap (3%) onto the common peroneal nerve, using a separate group of cats for each reflex. Compared with control, application of Cap attenuated the peak change in mean arterial pressure (MAP) evoked by static contraction (ΔMAP in mmHg: 38 ± 10 before and 24 ± 8 after ipsilateral Cap; 47 ± 10 before and 33 ± 10 after contralateral Cap). On the other hand, Cap increased the peak change in MAP evoked by stimulation of the saphenous nerve from 57 ± 8 to 77 ± 9 mmHg, as well as the peak change in MAP elicited by activation of muscle nociceptors (36 ± 9 vs. 56 ± 14 mmHg). These results show that the reflex cardiovascular increases evoked by static muscle contraction and noxious input are differentially affected by Cap application to the common peroneal nerve. We hypothesize that a Cap-induced alteration in dorsal horn processing is the locus for this divergent effect on these reflexes.

muscle pressor reflex; blood pressure; dorsal horn; central sensitization; hyperalgesia

Previous work has shown that static skeletal muscle contraction elicits a reflex increase in cardiovascular function seen as a rise in mean arterial pressure (MAP) as well as heart rate (HR; 3, 15, 25, 28, 40). This increase in cardiovascular function is known as the muscle pressor reflex (MPR) and is mediated by the activation of thinly myelinated group III and unmyelinated group IV muscle afferent fibers (25). These fibers respond to mechanical as well as chemical changes that occur in the contracting muscle and are thought to synapse on spinoreticular tract neurons in the dorsal horn of the spinal cord (4, 5, 15, 17, 19, 28, 40). Ascending projections arising from these dorsal horn cells travel, at least in part, in dorsolateral regions of the spinal cord, ultimately exciting neurons in the cardiovascular control centers of the medulla (2, 11, 12, 15, 20, 28). Descending projections from the medulla then excite sympathetic preganglionic neurons located in the intermediolateral area of the spinal cord, which ultimately gives rise to the increase in cardiovascular function.

Activation of other peripheral afferent nerve fibers can elicit a pressor reflex (18, 29–31, 35). Nociceptors are primarily free nerve endings located throughout the periphery that respond to stimuli that threaten or that actually damage tissue (13, 36, 37). Activation of nociceptors causes an increase in cardiovascular function, a nociceptive pressor reflex (NPR; 30, 31). Nociceptive afferent neurons are typically thinly myelinated (group III or A-delta fibers) or unmyelinated (group IV or C fibers; 13, 36, 37). Thus the afferent fibers mediating the NPR and MPR fall within the same classification. It has been postulated that the MPR is mediated by a group of receptors known as ergoreceptors that are separate from nociceptors (14, 19). However, strong experimental evidence that ergoreceptors and nociceptors represent separate groups of receptors is lacking.

Nociceptors also project to other populations of cells in the dorsal horn of the spinal cord. One of these populations is composed of spinothalamic tract neurons (13, 36, 37). The spinothalamic tract is an important pathway in humans for the sensation of pain and temperature (13, 36, 37). Previous work has shown that spinothalamic tract neurons located in the deep dorsal horn are subject to central sensitization as evidenced by an increased responsiveness of these cells to noxious and nonnoxious stimuli (6, 22, 32). This previous work used an intradermal injection of capsaicin...
(Cap) as a model of peripheral inflammation and the subsequent afferent activation associated with inflammation because Cap is a potent stimulus for unmyelinated afferent fibers (16). This intradermal injection of Cap increased the discharge rate of spinothermal tract neurons to subthreshold as well as noxious stimuli, indicating sensitization of these cells (6, 7, 22, 32). Because spinothermal tract neurons in the dorsal horn of the spinal cord can be sensitized by peripheral administration of Cap, we looked at the possibility that the spinothalamic tract neurons responsible for pressor reflexes could also be sensitized.

The purpose of this study was to test the hypothesis that the dorsal horn cells responsible for pressor reflexes could be sensitized. Specifically, we measured the cardiovascular responses evoked by static contraction of skeletal muscle, i.e., the MPR, before and after application of Cap onto the common peroneal nerve. Using separate groups of cats, we measured the cardiovascular responses produced by activation of cutaneous or muscle nociceptors, i.e., an NPR, before and after application of Cap onto the common peroneal nerve. In other words, an NPR was evoked from two types of tissue, skin and skeletal muscle. We hypothesized that because activation of either the MPR or NPR evokes an increase in cardiovascular function, the afferent neurons mediating these reflexes project onto the same population of spinothermal tract neurons in the dorsal horn of the spinal cord, i.e., they converge in the spinal cord. We also hypothesized that activation of C fibers by applying Cap to the common peroneal nerve would sensitize these spinothermal tract cells leading to an increase in the MPR as well as the NPR.

METHODS

Experimental preparation. Adult cats of either sex with a mean body weight of 3.5 ± 0.2 kg were anesthetized by inhaling an isoflurane (5%)-oxygen (2 l/min) mixture. A catheter was inserted into the cephalic vein, and the inhalation anesthetic was removed. Further anesthesia, consisting of α-chloralose (80 mg/kg) and urethane (100 mg/kg), was given through the catheter. Polyethylene catheters were placed into an external jugular vein and a common carotid artery. The trachea was exposed, and an endotracheal tube was inserted into the airway. The cats were mechanically ventilated to maintain arterial blood gases within normal limits (pH 7.35–7.4; P\textsubscript{CO\textsubscript{2}} 35–40 mmHg; P\textsubscript{O\textsubscript{2}} >80 mmHg). Sodium bicarbonate and/or supplemental oxygen were given as needed to help maintain these limits. Body temperature was constantly measured using a rectal probe (Yellow Springs Instrument, series 400) and was kept between 36.0 and 38.0°C with a heating pad and heat lamp. We periodically checked for the presence of a corneal reflex. If this reflex was present and/or resting arterial blood pressure and/or HR increased, then additional anesthetic, α-chloralose (10 mg/kg) or pentobarbital sodium (2–4 mg/kg), was given.

The calcaneal bone was cut, and, for the first protocol, the Achilles tendon was connected to a tension transducer (Grass model FT10) for measurement of the tension developed during contraction of the triceps surae muscle. The patellar tendon was secured to a post to ensure an isometric contraction. The popliteal fossa was exposed to allow access to the tibial and common peroneal nerves, and the saphenous nerve was exposed on the medial side of the hindlimb. Pools were made around the exposed nerves and muscles by suturing skin flaps to brass bars. The pools were filled with warm (37°C) mineral oil.

Protocol 1: contraction of triceps surae muscle to evoke the MPR. Contraction of the triceps surae muscle was induced by electrically stimulating the tibial nerve in the popliteal fossa at twice motor threshold, 40 Hz, and 0.025-ms duration for 1 min. Previous work has shown that electrical stimulation of the tibial nerve using these electrical parameters does not directly activate afferent neurons that evoke cardiovascular responses (15). The resting tension was set at 1 kg (L\textsubscript{0} in this preparation) before each contraction, and 15 min was given between muscle contractions to allow the muscle to recover. After at least two contractions of the triceps surae muscle where the pressor reflex was stable, Cap (3% in a 1:1:8 solution of ethanol-Tween 80-saline) was rubbed directly onto the common peroneal nerve via a cotton-tipped applicator, either ipsilateral (n = 7) or contralateral (n = 7) to the contracting muscle. Application of Cap caused a substantial increase in MAP and HR that lasted >20 min (see RESULTS). Because of this large rise in MAP we waited until MAP returned to about the same baseline (∼20 min) before another muscle contraction was evoked. Thereafter the contractions were repeated approximately every 30 min for 1.5 h. After the MAP tests were complete, the cats were paralyzed with pancuronium bromide (0.1 mg/kg). The same electrical stimulus was repeated on the tibial nerve. This was done to verify that the MPR was due to static contraction of skeletal muscle and not the electrical stimulus used to evoke the contraction. Vehicle controls were performed on separate cats (n = 7). The vehicle (1:1:8; ethanol-Tween 80-saline) was rubbed directly onto the common peroneal nerve via a cotton-tipped applicator in the same manner as Cap, and the subsequent contractions were evoked at postapplication times that were similar to the Cap experiments.

Protocol 2: electrical stimulation of saphenous nerve to evoke a cutaneous NPR. A separate group of cats were paralyzed with pancuronium bromide after the aforementioned surgical setup. The saphenous nerve was electrically stimulated with trains of electrical pulses (1 train/s; 750-ms train duration) delivered at 20 Hz and 1-ms duration, and two different intensities were used, high intensity (∼250 μV) and low intensity (∼25 μV). The nerve was stimulated for 30 s or until the rise in MAP stabilized (usually 20–25 s). The high-intensity stimulation was performed, and then the low-intensity stimulation was evoked after a minimum of 5 min. After at least 10 min, this high-intensity/low-intensity pattern was repeated to ensure that the pressor reflex was stable. Cap was then applied in the same manner as the previous protocol, directly onto the common peroneal nerve ipsilateral (n = 5) or contralateral (n = 5) to the stimulated nerve. The electrical stimulations were repeated at different time points (∼25, 45, and 75 min postapplication of Cap), represented as Cap 1, Cap 2, and Cap 3, respectively. The order of high intensity/low intensity was counterbalanced for the post-Cap stimulations. In one cat, the low-intensity stimulation failed to produce a significant rise in MAP and HR, and thus the low-intensity data were excluded from this cat.

Protocol 3: electrical stimulation of tibial nerve to evoke a muscle NPR. In another group of cats (n = 6), a branch of the tibial nerve was isolated and placed on the stimulating electrode. Before paralyzing the cat, we visually confirmed that electrical stimulation of the nerve caused contraction of the gastrocnemius muscle. The muscles were then paralyzed, and the nerve was electrically stimulated (higher intensity: ∼250 μV) using the same stimulus parameters and time...
frame as protocol 2. Again, at least two stimulations were elicited to ensure reproducibility of the cardiovascular responses and 10 min was given between electrical stimulations to allow for recovery. Cap was then applied to the ipsilateral common peroneal nerve as described in the two previous protocols. The electrical stimulations were repeated at different time points (−25, 45, and 75 min postapplication of Cap).

Vehicle controls for the NPR were also performed on a separate group of cats. The procedure described in protocols 2 or 3 was performed, except vehicle was applied to the peroneal nerve instead of the Cap solution. For these controls, the vehicle was applied to the ipsilateral peroneal nerve and was tested for the cutaneous NPR (n = 2) or the muscle NPR (n = 2). Because the results were similar, the data were combined. Only the higher intensity (−250 μV) stimulation was performed.

Measured and calculated variables. Arterial blood pressure was measured by connecting the common carotid artery catheter to a pressure transducer (Statham model P23ID), and muscle tension was measured using the force transducer. The arterial blood pressure and tension variables were continuously monitored on a four-channel chart recorder (Astromed model 7400) and a personal computer (Biopac acquisition system). MAP and HR were obtained from the arterial blood pressure signal. Baseline values were determined by averaging at least 30 s of data before the muscle contraction or electrical stimulation. The peak values for MAP, HR, and tension represent the peak level the variable reached during the 1 min of muscle contraction or 30 s of electrical stimulation. The peak change in a given variable represents the difference between the peak and baseline values. Time course MAP data for the MPR were measured as the level of MAP at 10-s intervals from the onset to the end of the contraction. To help protect against inconsistencies associated with blood pressure oscillations, MAP values at the 10-s intervals represent the mean of the MAP signal beginning 1 s before through 1 s after the 10-s time point.

Data analysis. Data are expressed as means ± SE. The time course for the MPR was determined as the change in MAP at 10-s time points compared with baseline during the 1-min muscle contraction and was done for the contraction before application of Cap (pre-Cap), the first contraction after application of Cap, and recovery. The time course data were analyzed with a two-way repeated-measures ANOVA with the two factors being time and Cap application (pre-Cap, Cap, and recovery). Baseline and peak hemodynamic data were also analyzed using the 2-way ANOVA, with time (baseline and peak) and Cap application (pre-Cap, Cap, and recovery) being the two factors. A one-way repeated-measures ANOVA was used to compare the peak changes in hemodynamic and tension data for pre-Cap, Cap, and recovery. The one-way ANOVA was also used for analysis of the NPR experiments, except the Cap application levels were pre-Cap, Cap 1, Cap 2, and Cap 3. A Tukey’s test was used for post hoc comparisons when applicable. For all analyses, P < 0.05 was used as the level of statistical significance.

RESULTS

Cardiovascular effects of Cap. Application of Cap to the peroneal nerve caused a robust increase in MAP and HR. Within 30 s after Cap application, MAP and HR began to rise. These increases were sustained for ~15 min, and thereafter MAP and HR began a slow decline, reaching approximate pre-Cap levels after ~20 min. The mean increases in MAP and HR for all the protocols were 63 ± 5 mmHg and 19 ± 2 beats/min, respectively.

Protocol 1: MPR. An example of the cardiovascular effects of static muscle contraction is illustrated in Fig. 1, left. Note that MAP and HR rise in response to the muscle contraction. After Cap was applied to the ipsilateral peroneal nerve and blood pressure was allowed to return toward the pre-Cap baseline, the muscle contraction was repeated. Figure 1, middle, illustrates that in this example the MPR was markedly blunted when the contraction was repeated 21 min after Cap application (even though baseline MAP is below the pre-Cap baseline). Figure 1, right, shows that when the contraction was repeated 41 min after Cap application, the MPR shows some degree of recovery. The time course data for the change in MAP produced by static contraction before and after ipsilateral application of Cap is shown in Fig. 2A. MAP rose sharply at the beginning of the muscle contraction and then reached a plateau that was maintained until the end of the contraction. After the contraction was over, MAP trended back toward baseline. However, the time course of the MAP response was reduced for the first contraction after Cap application. Statistical analysis of these data showed a significant main effect for time (P = 0.003), demonstrating that MAP rose during the muscle contraction. There was also a significant main effect for application of Cap (P = 0.04) and a significant interaction between time and Cap application (P < 0.001). This indicates that the time course for the increase in MAP to muscle contraction was reduced after Cap application. Later contractions showed a time course profile that was not statistically different compared with the control responses, i.e., the pressor response recovered. Figure 2B shows the time course of the pressor response before and after contralateral application of Cap. Similar to the ipsilateral data, Cap transiently reduced the time course of the MPR. Statistical analysis showed that there was a significant main effect for time (P < 0.001) and the interaction between time and Cap (P < 0.001) indicating that the response in MAP to muscle contraction was reduced by Cap application. There was no significant main effect for Cap (P = 0.075). Nevertheless, independent of whether Cap was applied to the ipsilateral or contralateral common peroneal nerve, the MPR was attenuated for a period of time and then recovered back toward control.

Figure 3 shows the peak increases in MAP, HR, and developed tension before and after both ipsilateral and contralateral Cap application. The peak change in MAP was significantly attenuated by Cap application (P = 0.007 ipsilateral and P = 0.005 contralateral). This peak pressor response recovered to pre-Cap levels (Fig. 3, top). Figure 3, middle, shows that Cap application tended to reduce the peak change in HR produced by static contraction, but it failed to reach statistical significance (ipsilateral: P = 0.065 for the ANOVA; contralateral: P = 0.063 for the ANOVA). The peak tensions developed during the muscle contraction before and after application of Cap as well as recovery
were not statistically different as seen in Fig. 3, bottom.

Table 1 provides the baseline and peak hemodynamic and tension data. There were no statistical differences in the baseline and peak hemodynamic data for pre-Cap vs. the first contraction after Cap. Thus the attenuation of the MPR by Cap was not due to changes in the baseline hemodynamic parameters. There was a statistically lower baseline MAP for recovery vs. Cap for the ipsilateral group. Table 1 also provides the baseline and peak hemodynamic and tension data for the vehicle controls. Vehicle was applied to the contralateral peroneal nerve for four of the experiments and to the ipsilateral peroneal nerve for the other three and the results were combined. Vehicle application to the common peroneal nerve had no effect on the MPR as static contraction increased MAP 67 ± 6 mmHg and HR 22 ± 6 beats/min before and 66 ± 6 mmHg and 22 ± 4 beats/min after vehicle application. The contraction was evoked 29 ± 2 min after vehicle, which is comparable to the time for the first contraction after Cap administration. There were no differences in the baseline and peak hemodynamic and tension data before and after vehicle application (Table 1). Vehicle application to the peroneal nerve had minimal cardiovascular effects as MAP and HR increased 7 ± 2 mmHg and 2 ± 1 beats/min, respectively.

At the end of the contraction protocol, the cats were paralyzed and the tibial nerve stimulation used to evoke the contraction was repeated. In general, this stimulation had no effect on MAP and HR. At no time did MAP and HR increase with this stimulation.

Fig. 1. Original records from 1 cat showing the changes in mean arterial pressure (MAP) and heart rate (HR) evoked by static contraction of the triceps surae muscle before application of capsaicin (Cap) to the ipsilateral peroneal nerve (Pre-Cap; left). Note that static contraction of the triceps surae evokes an increase in MAP and HR, i.e., produces a muscle pressor reflex (MPR). Middle: the magnitude of the MPR is greatly attenuated when the contraction is repeated 21 min after Cap (Post-Cap). Although the mean data show a tendency for baseline MAP to be higher for this post-Cap time point (see RESULTS), this figure illustrates that Cap effectively attenuates the MPR even when baseline MAP is lower. Right: a modest degree of recovery of the MPR when the contraction is repeated 41 min after Cap is shown. bpm, Beats/min.

Fig. 2. A: time course of the increases in MAP evoked by a 1-min static contraction (denoted by the solid bar) of the triceps surae muscle before (○) and 24 ± 3 min after (□) application of Cap to the ipsilateral peroneal nerve. *P, 0.05 compared with pre-Cap; n = 7 for both panels. Note, for clarity, error bars are not depicted for the recovery data.
ever, in two cats a slight depressor was observed (∼10 mmHg). This did not occur for the animals in which vehicle was applied to peroneal nerve.

Protocol 2: cutaneous NPR. If application of Cap attenuates the pressor response to static muscle contraction, then the same application of Cap should attenuate the pressor response from activation of cutaneous nociceptors if they follow the same pathway. Before application of Cap, high-intensity electrical stimulation of the saphenous nerve caused an increase in MAP and HR as shown in Fig. 4. Approximately 24 min after application of Cap onto the ipsilateral common peroneal nerve, the electrical stimulation evoked a significantly higher peak increase in MAP (P = 0.031), whereas the peak increase in HR also tended to be greater. At later stimulations (~45 and 72 min postapplication of Cap), the electrical stimulation continued to evoke a greater change in MAP and HR compared with preapplication data (Fig. 4; Table 2). Unlike the MPR, the NPR was enhanced after application of Cap and this accentuation was sustained even 72 min after the Cap administration, i.e., the NPR did not recover. For two cats in which it was tested, the NPR was still elevated after 2.5 h. Application of Cap to the contralateral peroneal also accentuated the NPR evoked by saphenous stimulation (Fig. 4; Table 2). Statistical analysis showed that the changes in MAP and HR were accentuated (P = 0.003 and P = 0.008, respectively) after Cap. There were no differences in baseline hemodynamic parameters at the various time points (Table 2).

As expected, the lower intensity stimulation of the saphenous nerve caused a smaller increase in MAP and HR. Before ipsilateral Cap, low-intensity saphenous stimulation increased MAP and HR by 24 ± 5 mmHg and 9 ± 5 beats/min, respectively. These increases in MAP were unaltered after Cap application (∆MAP in mmHg: 29 ± 4 at 29 ± 2 min post-Cap; 25 ± 6 at 50 ± 3 min post-Cap; 30 ± 4 at 77 ± 4 min post-Cap; P = 0.139; n = 5). Likewise, the increases in HR were unaffected (ΔHR in beats/min: 9 ± 4, 10 ± 4, 13 ± 5 at the 3 post-Cap time points, respectively; P = 0.141; n = 5). Contralateral Cap application also failed to alter the cardiovascular increases produced by the low-intensity stimulation. The increases in MAP (in mmHg) were 19 ± 3 before Cap, 19 ± 6 at 34 ± 3 min post-Cap, 24 ± 8 at 52 ± 5 min post-Cap, and 20 ± 4 at

Table 1. Hemodynamic and tension data for protocol 1 (muscle pressor reflex) before (Pre-Cap) and after Cap or vehicle application to the peroneal nerve

<table>
<thead>
<tr>
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<th>Pre-Cap</th>
<th>Cap</th>
<th>Recovery</th>
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<tbody>
<tr>
<td></td>
<td>Baseline Peak</td>
<td>Baseline Peak</td>
<td>Baseline Peak</td>
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<tr>
<td>Ipsi Cap</td>
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<tr>
<td>MAP, mmHg</td>
<td>110 ± 10</td>
<td>150 ± 14*</td>
<td>104 ± 10†</td>
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<td></td>
<td></td>
<td>121 ± 11</td>
<td>145 ± 14*</td>
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<tr>
<td>HR, beats/min</td>
<td>185 ± 8</td>
<td>198 ± 7*</td>
<td>179 ± 9</td>
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<tr>
<td></td>
<td></td>
<td>195 ± 6*</td>
<td>189 ± 10*</td>
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<tr>
<td>Tension, kg</td>
<td>1.0</td>
<td>8.4 ± 0.7*</td>
<td>1.0</td>
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<td></td>
<td></td>
<td>8.5 ± 0.5*</td>
<td>8.1 ± 0.5*</td>
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<td>Contra Cap</td>
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<tr>
<td>MAP, mmHg</td>
<td>118 ± 6</td>
<td>165 ± 9*</td>
<td>112 ± 7</td>
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<td></td>
<td></td>
<td>118 ± 6</td>
<td>151 ± 13*</td>
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<tr>
<td>HR, beats/min</td>
<td>176 ± 5</td>
<td>191 ± 6*</td>
<td>168 ± 9</td>
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<td></td>
<td></td>
<td>173 ± 10</td>
<td>183 ± 11*</td>
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<tr>
<td>Tension, kg</td>
<td>1.0</td>
<td>8.9 ± 1.1*</td>
<td>1.0</td>
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<tr>
<td></td>
<td></td>
<td>9.3 ± 0.8*</td>
<td>9.6 ± 1.1*</td>
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<td>Vehicle</td>
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<tr>
<td>MAP, mmHg</td>
<td>120 ± 6</td>
<td>185 ± 7*</td>
<td>119 ± 4</td>
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<td></td>
<td></td>
<td>119 ± 4</td>
<td>186 ± 11*</td>
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<tr>
<td>HR, beats/min</td>
<td>176 ± 7</td>
<td>197 ± 8*</td>
<td>175 ± 7</td>
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<tr>
<td></td>
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<td>175 ± 7</td>
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<tr>
<td>Tension, kg</td>
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<td>8.6 ± 0.8*</td>
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<td></td>
<td></td>
<td>8.0 ± 0.8*</td>
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Data are means ± SE. Cap, capsaicin; Ipsi, ipsilateral; Contra, contralateral; MAP, mean arterial pressure; HR, heart rate. Cap data represent contraction evoked at 24 ± 3 min (ipsilateral) or 31 ± 5 min (contralateral) after Cap administration. Recovery represents contraction evoked at 63 ± 11 min (ipsilateral) or 57 ± 4 min (contralateral) after Cap administration. *P < 0.05 compared with corresponding baseline. †P < 0.05 compared with corresponding Cap data point.
82 ± 3 min post-Cap (P = 0.68; n = 4). The HR data for the same time points, respectively, were 11 ± 2, 8 ± 2, 10 ± 3, and 10 ± 2 beats/min (P = 0.69; n = 4). Thus Cap application failed to alter the cardiovascular responses to low-intensity saphenous stimulation regardless of whether it was applied to the ipsilateral or contralateral peroneal nerve.

**Protocol 3: muscle NPR.** The cats were paralyzed, and the tibial nerve was electrically stimulated at the same time points, respectively, were 11 ± 2, 8 ± 2, 10 ± 3, and 10 ± 2 beats/min (P = 0.69; n = 4). Thus Cap application failed to alter the cardiovascular responses to low-intensity saphenous stimulation regardless of whether it was applied to the ipsilateral or contralateral peroneal nerve.

**Table 2. Hemodynamic data for protocols 2 and 3 (nociceptive pressor reflex) before (Pre-Cap), and after Cap or vehicle application to the peroneal nerve**

<table>
<thead>
<tr>
<th></th>
<th>Pre-Cap</th>
<th>Post-Cap 1</th>
<th>Post-Cap 2</th>
<th>Post-Cap 3</th>
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<tr>
<td>Saphenous-ipsi Cap</td>
<td></td>
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</tr>
<tr>
<td>MAP, mmHg</td>
<td>93 ± 7</td>
<td>150 ± 11*</td>
<td>99 ± 12</td>
<td>168 ± 14*</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>171 ± 15</td>
<td>194 ± 17*</td>
<td>179 ± 16</td>
<td>208 ± 18*</td>
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<tr>
<td>Saphenous-contra Cap</td>
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</tr>
<tr>
<td>MAP, mmHg</td>
<td>99 ± 3</td>
<td>153 ± 8*</td>
<td>109 ± 8</td>
<td>171 ± 14*†</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>177 ± 7</td>
<td>203 ± 6*</td>
<td>190 ± 6</td>
<td>218 ± 6*</td>
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<tr>
<td>Tibial-ipsi Cap</td>
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<tr>
<td>MAP, mmHg</td>
<td>104 ± 6</td>
<td>140 ± 8*</td>
<td>109 ± 7</td>
<td>155 ± 8*†</td>
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<tr>
<td>HR, beats/min</td>
<td>178 ± 9</td>
<td>189 ± 9*</td>
<td>187 ± 11</td>
<td>200 ± 10*</td>
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<tr>
<td>Vehicle</td>
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<tr>
<td>MAP, mmHg</td>
<td>108 ± 11</td>
<td>141 ± 9*</td>
<td>106 ± 10</td>
<td>140 ± 11*</td>
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<td>HR, beats/min</td>
<td>157 ± 7</td>
<td>168 ± 7*</td>
<td>156 ± 9</td>
<td>164 ± 9*</td>
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</table>

Data are means ± SE. Times for post-Cap 1, 2, and 3 are provided in Figs 4 and 5, and the text. *P < 0.05 compared with corresponding baseline. †P < 0.05 compared with corresponding Cap data point.
a greater sensation of pain. There are two facets involved in this process. The first is a sensitization of primary afferent neurons by products of inflammation, thereby making them more excitable to noxious and nonnoxious stimuli (13, 32, 36, 37). The second involves sensitization of cells in the central nervous system (CNS), termed central sensitization, that occurs in response to the barrage of nociceptor input that occurs when tissue becomes injured and/or inflamed (6, 7, 22, 32). The purpose of the current study was to determine if cells in the CNS involved in cardiovascular regulation could be sensitized, while avoiding the potential complication of sensitizing the primary afferent neurons involved in producing these reflexes.

To prevent sensitization of the primary afferent neurons, Cap was applied to the peroneal nerve, which innervates the ventral muscles and skin of the lower portion of the hindlimb. The dorsal muscle group of the lower hindlimb, i.e., the triceps surae muscle, was contracted to evoke the MPR to ensure that the afferent neurons producing this reflex arose from a region not innervated by nerves that received the Cap insult. The same holds true for the saphenous nerve, which is a cutaneous nerve innervating the dorsal aspect of the lower hindlimb and paw. Furthermore, application of Cap to the contralateral leg still modulated both reflexes. Thus it is very unlikely that the alterations in the MPR and NPR caused by Cap application were due to alterations in the primary afferent neurons mediating the pressor reflexes. On the other hand, the fact that the NPR was accentuated in our study suggests that central cells involved in cardiovascular regulation were sensitized by Cap application. The application of Cap to the common peroneal nerve evoked an increase in MAP and HR that lasted ~20 min. This sustained increase followed a similar time course to the increased discharge rate of spinothalamic tract neurons after the intradermal injection of Cap (6, 7, 22, 32). It is also consistent with the time course of pain sensation reported in human subjects after an intradermal injection of Cap (32). In addition, this application of Cap to a peripheral nerve caused the NPR to be elevated for >1 h, indicating a relatively long-lasting enhancement. These consistencies with previous work suggest that application of Cap to the common peroneal nerve sensitized cells involved in cardiovascular regulation. Furthermore, the signal causing this sensitization, as well as the inhibition of the MPR, crosses the spinal cord as the NPR and MPR were altered by application of Cap to the contralateral peroneal nerve.

Previous work has shown that sensitization of dorsal horn cells by peripheral inflammation involves activation of spinal N-methyl-D-aspartate (NMDA) and neurokinin (NK)-1 receptors (6, 7). Activation of these receptor systems may stimulate a variety of intracellular pathways, including the production of nitric oxide (NO), which has been implicated in producing central sensitization (22, 26, 27, 41). Related to this, we recently showed that administration of L-arginine into the dorsal horn accentuates the MPR (38). It is presumed that this enhancement was due to an L-argi-
nine-induced increase in NO. Thus the L-arginine results suggest that cells in the dorsal horn involved in producing the MPR can be sensitized. However, the results of the current study suggest that applying Cap to a peripheral nerve is not a mechanism for sensitizing these cells. The implication from this is that the C fiber activation associated with acute inflammation or injury does not enhance, in fact it appears to inhibit, the MPR. It is unknown at present if enhancement of the NPR by Cap involves NO.

The basic anatomy for the MPR and NPR is thought to be very similar. Both reflexes are initiated by activating small-diameter afferent fibers that synapse in the dorsal horn of the spinal cord (15, 28, 31, 40). These dorsal horn cells in turn activate, at least in part, spinal sympathetic preganglionic neurons in the intermediolateral portion of the thoracic and upper lumbar spinal cord by stimulating medullary neurons involved in cardiovascular regulation (2, 15, 18, 28, 29, 31, 35). Furthermore, nociceptors are thinly myelinated (group III or A-delta) and unmyelinated (group IV or C fibers) afferent neurons (13, 36, 37). As stated above, the MPR is mediated by the same class of afferent fibers, i.e., groups III and IV (15, 25, 28). Activation of nociceptors causes the release of the excitatory amino acids (EAA) glutamate and aspartate, as well as substance P (SP) release into the dorsal horn of the spinal cord (8, 33, 34). Static contraction causes spinal release of the same neurochemicals (9, 39). In addition, blockade of dorsal horn receptors for EAA and SP, e.g., NMDA, non-NMDA, and NK-1, blunts the MPR (15, 40). Because of the numerous similarities between the MPR and nociceptors, we originally proposed that the afferent pathways mediating the cardiovascular responses to static contraction and nociceptor activation were the same or that they converge onto the same dorsal horn spinoreticular cells. Thus, if C fiber activation sensitizes these spinoreticular cells, both reflexes would be enhanced by Cap application. However, the results of the current study fail to support this original hypothesis.

On the other hand, it is well established that painful stimuli activate a variety of sensory modulating mechanisms including descending “diffuse noxious inhibitory controls” (21). This endogenous sensory modulating system involves descending input to the dorsal horn and various neurochemicals including the opiate peptides, serotonin (5-HT), and α2-adrenergic agonists (1, 13, 36, 37). Because application of Cap activates C fibers, many of which are nociceptors, it is likely that this endogenous sensory modulating system is engaged. Sorkin and McAdoo (34) showed that intradermal Cap increases 5-HT release in the dorsal horn of the spinal cord, supporting the concept that Cap application activates sensory modulating systems. We and others have shown that activation of dorsal horn opiate, 5-HT, or α2-adrenergic receptors attenuates the MPR (15, 40). Thus we hypothesize that activation of an endogenous sensory modulating system by Cap inhibits dorsal horn cells receiving ergoreceptive input from the contracting muscle, thereby attenuating the MPR. On the other hand, the sensory modulating system fails to inhibit the dorsal horn cells receiving nociceptive input, because they become sensitized by peripheral Cap application. Recent work indicates that sensitized spinohalamic cells are resistant to the inhibitory actions of GABA and locus ceruleus stimulation (23, 24). More work is needed to test the validity of this working hypothesis.

Although the results of the current study are consistent with hypotheses that ergoreceptors and nociceptors are distinct, an alternative possibility related to the stimulus-response properties of dorsal horn neurons is conceivable. It is likely that sensitization of dorsal horn cells steepens and/or shifts the stimulus-response curve up while activation of the endogenous sensory modulating system causes a rightward shift. Because of these shifts, a stimulus at or near the top of the curve will be augmented while a stimulus at or near the bottom of the curve will exhibit inhibition. It is possible that both reflexes converge onto the same population of spinoreticular neurons or the receptors mediating these reflexes are not distinct. Thus ergoreceptors are not distinct receptors, but “ergoreception” represents one portion of a continuum that includes nociception at a “higher” end of this continuum. In other words, the sensory input from a contracting skeletal muscle resides on a much lower point on the stimulus-response curve of dorsal horn cells (and possibly other CNS cells as well) than noxious input. Consistent with this concept is the fact that the NPR evoked by high-intensity stimulation was enhanced, whereas the lower intensity electrical stimulation was unaffected. More work is needed to determine whether the CNS processing of somatic pressor reflexes is indeed a continuum or ergoreceptors are a distinct group of receptors. Regardless, the fact that Cap either enhanced (high intensity) or failed to alter (lower intensity) the NPR, yet it attenuated the MPR, strongly suggests that the contraction-induced cardiovascular responses produced in the anesthetized cat model do not simply represent a noxious reflex response.

We proposed that the attenuation of the MPR is mediated by activation of supraspinal structures, not a self-contained spinal event. Activation of peripheral afferent neurons can diminish sensory transmission at the spinal level, but this is thought to result from activation of large, myelinated neurons, A-beta skin afferent neurons for example (13, 37). Cap primarily activates unmyelinated afferent fibers, and thus attenuation of the pressor reflex by large myelinated neurons is unlikely (16). It is also unlikely that differences in the segmental entry of the primary afferent neurons
accounts for the attenuation. Stimuli entering in one spinal segment can induce inhibitory effects in other spinal segments without relaying in the brain. For example, somatic and visceral inputs to the cervical spinal cord can inhibit spinothalamic cells in the lumbar spinal cord via a spinal circuit (10, 42). However, afferent fibers from the saphenous, tibial, and peroneal nerves enter the spinal cord in the same spinal segments, i.e., L6, L7, and S1. Furthermore, the tibial and peroneal nerves are branches of the sciatic. In addition, Cap application attenuated the MPR, but the NPR from the tibial nerve was enhanced. Thus it is likely that attenuation of the MPR involves activation of supraspinal structures associated with an endogenous sensory modulating system.

We proposed that the divergent actions of Cap occur at the level of the first synapse, i.e., the dorsal horn of the spinal cord. Either separate cells receive input from distinct pathways or the dorsal horn integration of these two reflexes is altered. The rationale for this proposal is that numerous studies (see above) have documented that dorsal horn cells are sensitized by Cap or other types of tissue inflammation. In addition, the MPR and other types of sensory input are inhibited at the level of the dorsal horn. However, because this study represents the initial test on the effects of Cap application on somatic pressor reflexes, other possible CNS sites may produce and/or be involved in causing the results observed in the current study. More work is needed to determine what CNS sites are involved.

A limitation to the current study was the manner in which the NPR was evoked. To induce the NPR, the saphenous and tibial nerves were stimulated at an intensity that directly activates all afferent neurons. Although there is no doubt this represents a noxious stimulus and the resultant cardiovascular responses are primarily the result of nociceptor activation (15, 31), it does not represent a “pure” nociceptor stimulus. This is particularly germane to the muscle NPR, because electrical stimulation of this nerve activates afferent neurons from nociceptors and ergoreceptors. This potential complication is unavoidable. Direct electrical stimulations were used because they provide robust and reproducible cardiovascular responses. Although ergoreceptor afferent neurons are activated by direct electrical stimulation, their contribution to the cardiovascular responses appears to be minimal compared with nociceptor activation, because the NPR evoked from the tibial nerve was augmented, while ergoreceptor stimulation associated with muscle contraction (i.e., the MPR) was attenuated.

In summary, the results of this study show that Cap application to a peripheral nerve attenuates the MPR but enhances the NPR. The attenuation of the MPR was not sustained, but the accentuation of the NPR was. These results are consistent with the hypothesis that the MPR is mediated by ergoreceptors. On the other hand, these results may indicate that even though ergoreceptors are not a distinct class of receptors, ergoreception represents an important sensory input for adjusting cardiovascular function that is distinctly different from nociception. In other words, ergoreception and nociception may be separable components within a continuum of sensory input that evokes autonomic adjustments. Furthermore, these data suggest that the CNS processing of ergoreception and nociception is differentially affected by a Cap-induced activation of peripheral C fibers.

**Perspectives**

Muscular work and noxious stimuli are two examples among many that evoke reflex cardiovascular changes. Because both are initiated by activation of somatic afferent neurons, they represent somatoautonomic reflexes and thus likely represent important homeostatic control mechanisms. The initial CNS transduction site for these reflexes is the dorsal horn of the spinal cord, but the CNS processing of these reflexes is poorly understood. Furthermore, the nervous system is plastic, and thus the CNS processing of somatic input is not immutable. The purpose of this study was to determine if somatoautonomic reflexes are altered by a stimulus (Cap insult) that has been well described to alter the CNS processing of nociception related to pain perception. Our results show a divergent effect of Cap on the cardiovascular responses to muscular work vs. noxious stimuli. Although this study does not prove the existence of ergoreceptors, it strongly supports the idea that ergoreception, the sensory input and processing related to muscular work, can be distinguished from nociception. It is well known that one can evoke cardiovascular and respiratory increases by engaging in physical work without experiencing pain, thus qualitatively demonstrating ergoreception. However, the current study extends this by showing a separation in the cardiovascular responses, and thus CNS processing, of muscular work vs. noxious input. Furthermore, the results of this study suggest that the reflex cardiovascular responses evoked in response to ergoreceptive or nociceptive input are modifiable and thus may be altered by injury or chronic pain states. It remains to be seen if this potential modification of these reflexes can be useful for patients who suffer from injuries or chronic pain, because we only examined an acute insult. Nevertheless, this model may be useful in attempting to discern whether ergoreceptors exist or if ergoreception and nociception represent different “domains” on a continuum of afferent input and thus CNS processing.

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