Responses of thoracic spinal neurons to activation and desensitization of cardiac TRPV1-containing afferents in rats

Chao Qin,1 Jay P. Farber,1 Kenneth E. Miller,2 and Robert D. Foreman1

1Department of Physiology, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma; and
2Department of Anatomy and Cell Biology, Oklahoma State University Center for Health Sciences, Tulsa, Oklahoma

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Qin, Chao, Jay P. Farber, Kenneth E. Miller, and Robert D. Foreman. Responses of thoracic spinal neurons to activation and desensitization of cardiac TRPV1-containing afferents in rats. Am J Physiol Regul Integr Comp Physiol 291: R1700–R1707, 2006. First published August 3, 2006; doi:10.1152/ajpregu.00231.2006.—The purpose of this study was to examine how upper thoracic spinal neurons responded to activation and desensitization of cardiac transient receptor potential vanilloid-1 (TRPV1)-containing afferent fibers. Extracellular potentials of single T3 spinal neurons were recorded in pentobarbital-anesthetized, paralyzed, and ventilated male rats. To activate cardiac nociceptive receptors, a catheter was placed in the pericardial sac to administer various chemicals: bradykinin (BK; 10 μg/ml, 0.2 ml), capsaicin (CAP; 10 μg/ml, 0.2 ml), or a mixture of algesic chemicals (AC; 0.2 ml) containing adenosine 10−3 M, BK, serotonin, histamine, and PGE2, 10−5 M for each. Spinal neurons that responded to intrapericardial BK and/or CAP were used in this study. Results showed that 81% (35/43) of the neurons had excitatory responses to both intrapericardial BK and CAP, and the remainder responded to either BK or CAP. Intrapericardial resiniferatoxin (RTX) (0.2 μg/ml, 0.2 ml, 1 min), which desensitizes TRPV1-containing nerve endings, abolished excitatory responses to both BK (n = 8) and CAP (n = 7), and to AC (n = 5) but not to somatic stimuli. Intrapericardial capsazepine (1 mg/ml, 0.2 ml, 3 min), a specific antagonist of TRPV1, sharply attenuated excitatory responses to CAP in 5/5 neurons, but responses to BK in 5/5 neurons was maintained. Additionally, intrapericardial capsazepine had no significant effect on excitatory responses to AC in 3/5 neurons. These data indicated that intrapericardial BK-initiated spinal neuronal responses were linked to cardiac TRPV1-containing afferent fibers, but were not dependent on TRPV1. Intraspinal signaling for cardiac nociception was mediated through CAP-sensitive afferent fibers innervating the heart.

bradykinin; capsaicin; resiniferatoxin; sympathetic afferent; vagal afferent

MYOCARDIAL ISCHEMIA RELEASES several metabolites, including bradykinin (BK) and protons, which activate cardiac chemosensitive and mechanoreceptive receptors and elicit angina pectoris and cardiovascular responses (8–14, 39). Physiological and pharmacological studies show that BK activates cardiac nociceptors and sympathoexcitatory responses via kinin B2 receptors located in thinly myelinated A6 and unmyelinated C-fiber afferent endings in the heart (1, 23, 39, 40). The afferent fibers transmitting the action potentials enter the upper thoracic spinal cord where spinal neurons, spinalthalamic tract cells, spinoreticular tract, and other ascending pathway neurons process this information (2–6, 27). However, the signaling mechanisms involved in detection of myocardial ischemia and activation of cardiac sympathetic or spinal afferent nerve endings are not fully known.

Recently, it has been suggested that the transient receptor potential vanilloid-1 (TRPV1), an important nonspecific cation channel activated by capsaicin (CAP), noxious heat, and protons, also is involved in the production of angina pectoris associated with myocardial ischemia (25). TRPV1-expressing afferent nerves are widely distributed on the epicardial surface of the rat ventricle (43). Epicardial application of CAP excites cardiac sympathetic afferent fibers and produces sympathoexcitatory reflexes (25, 34, 43). These effects can be eliminated by TRPV1 antagonists. Injections of CAP into the left atrium or pericardial sac also activate spinal neurons and spinoreticular tract neurons in cats (4). Thus TRPV1-containing sympathetic afferent fibers may function as a molecular sensor, as this receptor and receptor channel complex can detect release of BK, serotonin, and ATP, as well as changes in pH, lipid metabolites, and heat that may accompany tissue ischemia and activate cardiac nociceptors (16, 25).

The hypothesis of this study is that TRPV1-containing sympathetic afferent fibers transmit nociceptive information from the heart to activate upper thoracic spinal neurons. These fibers can be excited by capsaicin, as well as bradykinin. To test the hypothesis, the following aims were developed: 1) to examine how upper thoracic spinal neurons responded to activation TRPV1 and/or BK receptors on the heart; 2) to contrast the effects on these responses when TRPV1-containing fibers were desensitized and when TRPV1 receptors were blocked. Desensitization of fibers in the context of the present study is meant to indicate that impulses from sensory stimuli cannot be conducted by the fibers, but the mechanism is unspecified.

To interrupt transmission of information in cardiac afferent fibers (desensitization) resiniferatoxin (RTX), an ultra-potent analog of CAP was used. CAP can desensitize or destroy TRPV1-containing fibers when used as a pharmacological tool (13, 16), but these effects are achieved at much lower concentrations using RTX (17, 24, 30, 32, 38, 42). To specifically block the TRPV1 receptor, we used capsazepine. The results showed that 1) the majority of upper thoracic (T3) spinal neurons with cardiac input responded to both intrapericardial BK and CAP; 2) bilateral cervical vagotomy had no significant effect on these spinal neuronal responses; 3) RTX eliminated excitatory neuronal responses to intrapericardial BK, CAP, and a mixture of algesic chemicals; and 4) selective blockade of TRPV1 with capsazepine significantly inhibited spinal neuro-

Address for reprint requests and other correspondence: C. Qin, Dept. of Physiology, Univ. of Oklahoma Health Sciences Center, P.O. Box 26901, Oklahoma City, OK 73190 (e-mail: chao-qin@ouhsc.edu).
nal activation by CAP, but not by BK. These data suggested that cardiac CAP-sensitive sympathetic afferents play an important role in activation of upper thoracic spinal neurons by cardiac noxious stimuli. A preliminary report of this work has been published in the form of an abstract (7).

METHODS

Experiments were performed in 28 male Sprague-Dawley (Charles River, Boston, MA) rats weighing 300–480 g. The Institutional Animal Care and Use Committee of the University of Oklahoma Health Sciences Center approved the experimental protocol performed in this study. After the animals initially were anesthetized with pentobarbital sodium (50–60 mg/kg ip), catheters were inserted into the right carotid artery to monitor blood pressure and into the left jugular vein to inject saline and drugs. A continuous intravenous infusion of pentobarbital (10–20 mg·kg⁻¹·h⁻¹) was injected to maintain the appropriate level of anesthesia, which was closely monitored by mean arterial pressure (80–120 mmHg) and pupil diameter throughout the experiments. After a cannula was placed in the trachea, rats were artificially ventilated with a constant-volume pump (55–60 strokes/min, 3.0–5.0 ml stroke volume). Animals were paralyzed with pancuronium bromide (0.4 mg/kg ip) and given supplemental doses (0.2 mg/kg ip) as needed to maintain muscle relaxation. To interrupt vagal afferent pathways, the cervical vagi were isolated bilaterally and loose ligatures were placed around them. At the appropriate time during the experiment, the vagi were transected with a small scissors after pulling gently on the ligatures from each side. Throughout the experiment, a thermostatically controlled heating pad and overhead infrared lamps were used to maintain rectal temperature between 37 and 38°C.

Laminectomies exposed the T3 spinal segment for recording neurons in rats placed in a stereotaxic unit. A pair of clamps was attached to the T1-T2 and T7-T8 vertebrae to stabilize the spinal cord. The dura mater of the T3 spinal cord was carefully removed, and the spinal cord was covered with warm agar (3–4% in saline) to improve stability. Extracellular potentials of single spinal neurons were isolated within depths of 0.3 from the cord dorsum for superficial neurons and 0.3–1.2 mm deep for deeper neurons, respectively. Extracellular potentials of single spinal neurons were isolated within depths of 0.3 from the cord dorsum for superficial neurons and 0.3–1.2 mm deep for deeper neurons, respectively. Extracellular potentials were fed into a window discriminator, displayed on an oscilloscope, and stored in a computer with Spike 3/CED 1401 data acquisition programs (Cambridge, UK) for off-line analysis. Spinal neuronal activity during various manipulations was measured using rate histograms (1 s per bin). Spontaneous activity of neurons was determined by counting activity for 10 s and then dividing by 10 to obtain impulses per second (imp/s). Changes in neuronal activity (imp/s) were calculated by subtracting the mean of 10 s of control activity from the mean of 10 s of the greatest response to stimulation. A neuron was considered responsive to a stimulus if its discharge rate changed ≥20% of control activity. Latency to response, maximal response (imp/s), and duration of response were measured after pericardial drug administration. Statistical comparisons were made using Student’s paired or unpaired t-test and χ² analysis. Data are presented as means ± SE. Differences were considered statistically significant at P < 0.05.

To deliver chemicals to the heart and activate cardiac afferent endings, a catheter was placed in the pericardial sac using a procedure described in previous studies (5, 26). Briefly, a high midline thoracotomy (left costal cartilages of ribs 1–3) was made to expose the thymus gland and the heart. After the thymus gland was opened on the midline, a silicone tubing (0.020 ID, 0.037 OD, 14–16 cm length) with 6–8 small holes in the distal end was inserted into the pericardial sac over the left ventricle. The catheter was fixed in place by suturing together the two thymus lobes and the layers of the chest wall. Chemical solutions were injected and withdrawn via a 1-ml syringe connected to the catheter. BK (1 mg) was mixed with normal saline to create a stock solution of 0.2 mg/ml. On the day of an experiment, the stock solution of BK was diluted with saline to 10 μg/ml for intrapericardial injections. A stock solution of CAP was dissolved in a mixture of 0.5 ml Tween 80 and 0.5 ml ethanol to a concentration of 1 mg/ml. On the day of an experiment, the stock solution of CAP was diluted with saline to 10 μg/ml for administration in the pericardial sac. Vehicle solution (0.2 ml of 1% ethanol and 1% Tween 80 in normal saline) was injected in the pericardial sac as a control. A mixture of algesic chemicals (AC) containing BK, serotonin, PGE₂, histamine, and adenosine, all of which may be released during myocardial ischemia, was prepared for injections (6, 20). Drugs were individually dissolved in normal saline to a concentration of 10⁻³ M and were kept frozen. On the day of an experiment, stock solutions were warmed and further diluted in normal saline to concentrations of 10⁻² M (except adenosine, 10⁻³ M). The protocol for intrapericardial administration of BK, CAP, and AC was to inject 0.2 ml of each chemical solution, withdraw after 60 s, and to use 2–3 saline flushes (0.2 ml each) for rinsing the chemicals within the pericardial sac. At least 20 min elapsed between each application of chemicals and the order of injecting the chemicals was randomized. To desensitize cardiac afferent fibers containing TRPV1 receptors, 0.2 ml RTX (0.2 μg/ml) was injected in the pericardial sac for 1 min and then drained out without saline rinsing. A stock solution of RTX (1 mg) was dissolved in 0.5 ml ethanol and 0.5 ml Tween 80. The bottle was wrapped in foil and stored in a ~−80°C freezer. On the day of an experiment, the stock solution of RTX was diluted in saline to 0.2 μg/ml. To selectively block TRPV1 receptors in the heart, capsazepine was administered in the pericardial sac (0.2 ml, 1 mg/ml, 3 min). Capsazepine was dissolved with isotonic saline containing 1% Tween 80 and 1% ethanol. All drugs were obtained from Sigma (St. Louis, MO).

Cutaneous receptive fields of spinal neurons were tested for responses to innocuous brushing with a camel-hair brush, light pressure with a blunt stick, and noxious pinching of skin and muscles with a blunt forceps and arterial clamp. Neurons were categorized as low threshold (LT), wide dynamic range (WDR), high threshold (HT), or high threshold inhibitory (HTi) based on their responses to innocuous and noxious somatic stimuli (4). LT neurons were excited by brushing the hair and light pressure but not by pinch of somatic fields. WDR neurons were excited by innocuous somatic stimuli and were excited to a greater extent by pinch. HT neurons were excited only by pinch of somatic fields. HTi neurons were excited by pinch, and the activity was reduced by hair movement. Outlines and descriptions of receptive fields were recorded manually for all neurons examined. An electrolytic lesion (50 μA DC; anodal for 20 s, cathodal for 20 s) was made at most recording sites after spinal neurons responding to cardiac chemical stimuli were studied. At the end of the experiment, the animals were euthanized with an overdose of intravenous pentobarbital sodium (200 mg/kg) or an euthanasia-S solution (1 ml). The thoracic spinal cord was removed and placed in 10% buffered formalin solution. Frozen sections (55–60 μm) were cut to examine the locations of lesions. Spinal laminae of gray matter were identified using the cytoarchitectonic scheme in rats (21).

RESULTS

Spinal neuronal responses to BK and CAP. Only upper thoracic (T3) spinal neurons with excitatory responses to intrapericardial injections of AC were selected for this study. Of the 43 spinal neurons responding to intrapericardial BK and/or CAP, 35 (81%) neurons had excitatory responses to both chemicals; 5 (12%) neurons were responsive to BK but not to CAP; 3 (7%) neurons were responsive to CAP but not to BK. The different response patterns of spinal neurons to BK and/or
CAP are shown in Fig. 1, A–C. The characteristics of neuronal excitatory responses to intrapericardial BK and CAP are presented in Table 1. No significant differences in neuronal spontaneous activity and excitatory responses to the two chemicals were found. Of 43 spinal neurons examined, five neurons were recorded from the superficial dorsal horn (depth: <0.3 mm, lamina I-III), and 38 were recorded from the deeper spinal gray matter (lamina IV, V, VII, X). All superficial neurons were excited by both intrapericardial BK and CAP. Of 38 neurons recorded in deeper laminae, 30 neurons had excitatory responses to both chemicals, whereas 8 neurons responded either to BK or CAP. Electrical lesions made at recording sites were identified histologically for 25 neurons that responded to intrapericardial chemicals (Fig. 1D).

Effects of RTX on neuronal responses to AC. To desensitize cardiac afferent nerve endings containing TRPV1, intrapericardial RTX was injected into the pericardial sac for 1 min. Spinal neuronal responses to AC were examined 20–30 min after intrapericardial RTX. Of eight neurons tested for effects of intrapericardial RTX, spontaneous activity immediately increased in five neurons, decreased in one neuron and was unchanged in two neurons. However, 20–30 min after RTX, excitatory responses of all neurons to BK, CAP, and AC were abolished (Table 2). Fig. 2, A–C shows examples of the excitatory responses of a single spinal neuron excited by BK, CAP, and AC, which were abolished by intrapericardial RTX. A summary showing the effects of intrapericardial RTX on excitatory responses to administration of the various chemicals is presented in Table 2.

Effects of RTX on neuronal responses to somatic stimuli. Somatic receptive fields were found for 37/43 (86%) upper thoracic spinal neurons tested for their responses to intrapericardial BK and CAP. The characteristics of neuronal excitatory responses to intrapericardial BK and CAP are presented in Table 1. No significant differences in neuronal spontaneous activity and excitatory responses to the two chemicals were found. Of 43 spinal neurons examined, five neurons were recorded from the superficial dorsal horn (depth: <0.3 mm, lamina I-III), and 38 were recorded from the deeper spinal gray matter (lamina IV, V, VII, X). All superficial neurons were excited by both intrapericardial BK and CAP. Of 38 neurons recorded in deeper laminae, 30 neurons had excitatory responses to both chemicals, whereas 8 neurons responded either to BK or CAP. Electrical lesions made at recording sites were identified histologically for 25 neurons that responded to intrapericardial chemicals (Fig. 1D).

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cardial chemicals; 16 neurons were classified as WDR, 17 neurons were HT, and 4 neurons were HTi. No LT neuron was found. There was no correlation between the types of somatic fields and response patterns to cardiac chemical stimuli. The somatic fields of spinal neurons receiving noxious cardiac input were located on the chest, axilla, shoulder, and upper back areas. To determine whether intrapericardial RTX affects receptive sensitivity for somatic input in these viscerosomatic convergent neurons, excitatory responses to noxious pinch of somatic fields were examined before and after administration of RTX in the pericardial sac. The maximal excitatory responses to pinch, at least 20 min after intrapericardial RTX, were not significantly different from control responses before RTX (33.3 ± 7.8 vs. 27.7 ± 5.7 imp/s, n = 7). An example of an excitatory neuronal response to pinching the somatic field before and after intrapericardial RTX is shown in Fig. 2D. A summary of RTX effects on seven neuronal responses to noxious somatic input is presented in Fig. 2E. The data indicated that the local effect of intrapericardial RTX was limited to visceral afferents rather than systemically affecting somatic input.

**Effects of capsazepine on neuronal responses to BK or CAP.**

To determine whether upper thoracic spinal neurons were excited by chemical activation of cardiac CAP-sensitive sympathetic afferents, BK or CAP was injected before and after intrapericardial capsazepine, a selective blocker of TRPV1. Intrapericardial administration of capsazepine increased background activity in two neurons and did not affect background activity in three neurons. Intrapericardial capsazepine significantly reduced excitatory responses to CAP (Table 3). However, these neurons still responded to BK and to AC. One example of a neuron that had no response to CAP but still responded to BK after capsazepine was applied to the pericardial sac is shown in Fig. 2D.

**Table 2. Effects of intrapericardial pretreatment of RTX on spinal neuronal excitatory responses to intrapericardial BK, CAP, and a mixture of algesic chemicals**

<table>
<thead>
<tr>
<th>Stimuli</th>
<th>Stimuli</th>
<th>n</th>
<th>Before RTX-Treated</th>
<th>After RTX-Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Rate (imp/s)</td>
<td>Rate (imp/s)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Cell activity</td>
<td>Cell activity</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>BK</td>
<td>BK</td>
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<td></td>
<td></td>
<td></td>
<td>CAP</td>
<td>CAP</td>
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<td></td>
<td></td>
<td></td>
<td>AC</td>
<td>AC</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pi</td>
<td>Pi</td>
</tr>
</tbody>
</table>

Values are presented as means ± SE. *P < 0.01 compared to corresponding spontaneous activity (SA). MA, maximal activity during intrapericardial chemicals; AC, algesic chemicals; RTX, intrapericardial reinferatoxin.
dial sac is shown in Fig. 3, A and B. The effect of intrapericardial capsazepine on excitatory neuronal responses to noxious cardiac stimuli is summarized in Table 3.

**Effects of vagotomy on neuronal responses to BK or CAP.** To examine whether the effects of BK or CAP on upper thoracic neuronal activity were modulated via vagal afferents, BK and CAP were injected separately while recording from the same neuron before and after the cervical vagus nerves were transected. Examples of excitatory neuronal responses to intrapericardial BK and CAP are shown in Fig. 3, C and D. There were no significant differences between mean neuronal responses to BK (n = 6) and CAP (n = 5) before and after bilateral vagotomy (Table 4).

**DISCUSSION**

The results of this study showed that 1) intrapericardial injections of BK and CAP excited spinal neurons in the

### Table 3. Effects of intrapericardial pretreatment of capsazepine on spinal neuronal excitatory responses to intrapericardial BK, CAP, and a mixture of AC

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>n</th>
<th>Capsazepine-Treated</th>
<th>Spontaneous Activity, imp/s</th>
<th>Latency, s</th>
<th>Responses, imp/s</th>
<th>Duration, s</th>
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<tbody>
<tr>
<td>BK</td>
<td>5</td>
<td>Before</td>
<td>8.0±4.8</td>
<td>6.2±1.0</td>
<td>25.5±6.9</td>
<td>142.9±65.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After</td>
<td>11.1±5.3</td>
<td>8.3±1.8</td>
<td>17.6±4.6</td>
<td>98.7±24.9</td>
</tr>
<tr>
<td>CAP</td>
<td>5</td>
<td>Before</td>
<td>13.6±5.0</td>
<td>3.8±1.1</td>
<td>36.8±11.0</td>
<td>107.2±20.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After</td>
<td>12.1±4.5</td>
<td>7.1±4.5</td>
<td>3.9±1.7*</td>
<td>15.8±8.0</td>
</tr>
<tr>
<td>AC</td>
<td>3</td>
<td>Before</td>
<td>11.0±6.0</td>
<td>3.9±0.7</td>
<td>39.9±18.4</td>
<td>92.3±37.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After</td>
<td>10.7±6.6</td>
<td>10.7±3.8</td>
<td>28.9±11.7</td>
<td>79.2±26.4</td>
</tr>
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</table>

Values are presented as means ± SE. *P < 0.05 compared to corresponding neuronal activity before capsazepine.
superficial and deeper dorsal horn of upper thoracic spinal cord in rats; 2) vagal transections did not significantly change the excitatory response characteristics of spinal neurons to BK and CAP; 3) desensitization of the CAP-sensitive afferent fibers of the heart with RTX sharply attenuated or eliminated the neuronal responses to BK, CAP, and AC, but it did not affect the noxious somatic responses of the neurons; and 4) capsazepine significantly reduced the responses of spinal neurons to CAP but not to BK or AC.

**Spinal neuronal activation by BK and CAP.** In the present study in rats, 81% of the upper thoracic (T3) spinal neurons with cardiac input had excitatory responses to both intrapericardial BK and CAP, and the remainder responded to either BK or CAP. Recordings of single sympathetic afferent units with receptive fields on the anterior surface of the left ventricle in rats show that applications of CAP and BK to the heart increase cardiac afferent activity (34). Chronic CAP administration eliminates the dose-dependent pressor effects and tachycardia to topical application of BK and CAP on the surface of heart in anesthetized guinea pigs (31). Thus the CAP-sensitive cardiac afferent sympathetic fibers most likely contribute to the excitation of upper thoracic spinal neurons when noxious chemical stimuli are applied to the heart.

A majority of spinal neurons responding to BK and/or CAP were located in deeper dorsal horn and intermediate zone of the gray matter (lamina IV, V, VII, X), whereas only a few responsive neurons were recorded in the superficial dorsal horn (lamina I–III). All superficial neurons and a majority of deeper neurons were excited by both intrapericardial BK and CAP, but a few neurons responded either to BK or CAP. The response patterns of these neurons generally agreed with the responses of spinal neurons to intracardiac injections of BK and CAP that were made in the cat (4). Laminar locations of neurons in the present study also were consistent with the observations made in cats (4).

**Interaction of TRPV1 and B2.** In the present study, desensitization with RTX of TRPV1-containing cardiac sympathetic afferent fibers abolished the excitatory responses of spinal neurons to intrapericardial BK, CAP, and even AC. These data are consistent with an observation from a previous study that examines the sympathoexcitatory reflex in rats (43). In their study, epicardial BK or CAP produces a large increase in renal sympathetic nerve activity and blood pressure, and systemic treatment with RTX four to five days before testing completely abolishes these effects. In the present study, desensitization was obtained only 20 min after the administration of RTX. Two RTX-induced effects may be relevant. First, current-clamp experiments reveal that low concentrations of RTX cause a slow and sustained depolarization beyond threshold in cultured cells while generating few action potentials (32). Second, RTX selectively induces a prolonged increase in intracellular calcium in TRPV1-containing neurons (17). Because activation of TRPV1 opens a cationic channel that strongly depolarizes afferent fibers, massive activation of these receptors using RTX would be expected to produce depolarization block. An effect on action potential generation related to Ca$^{2+}$ influx is also a possibility. In any case, BK and CAP were not able to generate action potentials, suggesting that the underlying mechanism may be fiber inactivation.

The selective blockade of TRPV1 with capsazepine attenuated the activation of spinal neurons by CAP and did not have a significant effect on neuronal responses to BK. This result agrees with a previous study that showed that administration of capsazepine does not affect baseline activity of either vagal or sympathetic cardiac afferents, and, at sufficient concentration, abolishes the response of afferent fibers to CAP. However, there is no effect on afferent activation by BK (34). Other investigators show that iodo-RTX, a novel and potent TRPV1 receptor antagonist attenuates the CAP-elicited cardiogenic reflex but has no significant effect on BK-elicited sympathoexcitatory responses (43). BK is known to activate cardiac sympathetic afferent fibers by stimulating the B2 receptor (39). The B2 receptor is typically a Gq-protein-coupled receptor that when stimulated opens Ca$^{2+}$-activated Cl$^{-}$ channels (19, 22, 41). Opening the channels produces a generator potential that leads to action potential discharge. However, while BK signaling through PLC and PKC alters the likelihood of TRPV1 channel gating, its role is modulatory and not obligatory (18, 19, 35, 37). This is based on the evidence that BK produced a significantly less persistent action potential discharge response in TRPV1$^{-/-}$ mice compared with the wild type (18). Also, the TRPV1 inhibitor, iodo-RTX, partially reduced the BK-induced discharge of action potentials of vagal afferent C-fibers (19). The small sample size in the present study, which showed no effect of CAP on BK receptors, may have masked a relatively small change in cell activity.

**Somatic and visceral input to neurons.** Intrapericardial injections of RTX desensitized cardiac sympathetic afferent fibers but not the somatic afferent fibers that converged onto the same spinal neurons. Investigators in previous studies administered large systemic doses of CAP or RTX to neonatal rats (15, 33) or adult rats (24, 36) to permanently deplete TRPV1-expressing afferent neurons and fibers. This protocol destroys both somatic and visceral CAP-sensitive afferent fibers. This nonselective destruction of both somatic and visceral afferent fibers would have hampered our ability to interpret whether the changes observed in spinal neurons were caused by afferent input from both groups of fibers. To selectively interrupt visceral afferents in the present study, intrapericardial injections of lower doses of RTX were used to eliminate only the cardiac sympathetic afferent fibers. The data showed that intrapericardial RTX eliminated excitatory neuro-

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Table 4. Effects of bilateral cervical vagotomy on spinal neuronal excitatory responses to intrapericardial BK and CAP

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>n</th>
<th>Vagotomy</th>
<th>Spontaneous Activity, imp/s</th>
<th>Latency, s</th>
<th>Responses, imp/s</th>
<th>Duration, s</th>
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</thead>
<tbody>
<tr>
<td>BK</td>
<td>6</td>
<td>Before</td>
<td>3.3 ± 1.0</td>
<td>3.8 ± 1.0</td>
<td>44.2 ± 18.8</td>
<td>86.5 ± 21.1</td>
</tr>
<tr>
<td>CAP</td>
<td>5</td>
<td>Before</td>
<td>6.0 ± 4.2</td>
<td>5.9 ± 1.9</td>
<td>40.1 ± 16.8</td>
<td>107.7 ± 42.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After</td>
<td>6.1 ± 2.3</td>
<td>2.2 ± 0.5</td>
<td>33.5 ± 12.3</td>
<td>155.7 ± 33.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>After</td>
<td>11.8 ± 3.0</td>
<td>4.4 ± 1.5</td>
<td>38.0 ± 17.8</td>
<td>132.3 ± 34.2</td>
</tr>
</tbody>
</table>

Values are presented as means ± SE.
nial responses to intrapericardial BK, CAP, and AC, but excitatory responses to pinching somatic fields were not significantly different before and after administration of RTX in the pericardial sac. These results showed that RTX on the heart selectively affected cardiac afferent nerve endings but not noxious somatic afferent information.

A previous study from this laboratory showed that effects of intracardiac BK and CAP on spinal neurons depended on somatic classification. BK-sensitive cardiac afferents excite HT/HTi and WDR spinal or spinoreticular tract neurons, whereas CAP-sensitive cardiac afferent fibers are more likely to excite HT/HTi than WDR spinal neurons when intracardiac injections of BK and CAP are performed in cats (4). This observation suggests that certain subpopulations of cardiac afferent fibers can elicit specific responses among spinal neurons with different types of somatic receptive fields. In the present study, there was no correlation between receptive field properties of somatic fields and response patterns of spinal neurons to cardiac chemical stimuli. The differences between the two studies could be due to observations in selective populations of spinal neurons. Only spinal neurons with excitatory responses to cardiac stimuli were selected in the present study, and our observations were made in a general population of spinal neurons that may include projecting neurons and interneurons. In addition, the different animal species in which these studies were performed may be an important factor.

Vagal afferent pathway. In the present study, no significant differences in excitatory neuronal responses were observed for either BK or CAP before and after bilateral cervical vagotomy. This result is consistent with a previous study in vagotomized cats showing that application of BK and CAP to the heart still excites most spinoreticular tract neurons and many spinal neurons (4). Bilateral vagotomy and systemic administration of atropine do not attenuate pressor responses and tachycardia when BK is applied to the heart (31). In a study conducted in dogs, bilateral sectioning of the upper thoracic (T1-T4) white rami communicantes and stellategery, but not vagotomy, eliminates the dose-related pressor effects and tachycardia to local application of CAP to the parietal pericardium (36). These results suggest that the excitatory afferent effects on thoracic neurons of epicardial BK and CAP are likely to result from the stimulation of cardiac sympathetic, CAP-sensitive, primary afferents. Thus it is presumed that vagal afferent tonic activity may modulate to some degree the activity of spinal neurons involving different local transmission circuits and autonomic reflexes but generally do not play an important role in thoracic spinorecticular processing of cardiac nociception.

Limitations of the study. The study does not directly address the physiological or pathophysiological condition because coronary occlusions were not used to generate myocardial ischemia. Nevertheless, the chemicals used in this study to activate cardiac receptors are released during myocardial ischemia and provide an indirect method for determining the characteristics of the sympathetic afferent fibers that transmit nociceptive information from the heart to spinal neurons. We chose to use intrapericardial injections, because chemicals could be injected through a catheter that was placed in the pericardial sac without affecting the somatic fields of the chest overlying the heart. The preservation of the somatic fields was important for this study because viscerosomatic thoracic spinal neurons were being investigated. Incisions of the chest, which were required for occluding the coronary arteries to produce ischemia, would have influenced somatic fields. The intrapericardial doses of BK and AC used in this study were based on previously obtained data that showed responses could be modulated by a variety of inputs (28, 29). We produced a dose-response curve for BK to aid in our selection of the dose. Similarly, in the present study, responses after intrapericardial injections of chemicals could be strongly modulated.

In summary, the present study found that the majority of upper thoracic spinal neurons with cardiac input responded to intrapericardial administration of both BK and CAP. Vagal afferent tonic activity did not significantly affect the excitatory responses of these spinal neurons to noxious cardiac stimuli. The desensitization of cardiac sympathetic afferent fibers containing TRPV1 with intrapericardial RTX eliminated excitatory neuronal responses to BK, CAP, and also to AC. However, the selective blockade of TRPV1 with intrapericardial capsazepine significantly attenuated the activation of spinal neurons by CAP but did not affect neuronal responses to BK. These data suggested that CAP-sensitive sympathetic afferents play an important role in the activation of the upper thoracic spinal neurons by cardiac noxious stimuli. However, the BK-elicted spinal neuronal responses are not dependent upon TRPV1 receptors at the nerve endings of cardiac afferents. These new findings are important for our understanding of the sensory mechanisms of cardiac pain and the phenotypes of afferent neurons involved in the sympathetic reflex responses to myocardial ischemia.

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