Muscle pressor reflex: potential role of vanilloid type 1 receptor and acid-sensing ion channel

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Lactic acid is produced in contracting skeletal muscle and increases blood pressure when it is injected into the hindlimb of skeletal muscle (20, 21, 23). As such, lactic acid and H+ may act as endogenous C-fiber stimulants (9, 12, 26) that reflexively increase blood pressure. It is clear that if lactic acid activates C-fibers and evokes the pulmonary chemoreflex (5, 19). Epicardial application of capsaicin stimulates cardiogenic afferent fibers, eliciting a sympathoexcitatory reflex (32). The competitive capsaicin antagonist capsazepine has been shown to reduce capsaicin-induced activation of the cloned nonselective cation channel VR1 (4).

Capsaicin also evokes cardiovascular reflexes when injected into the arterial supply of the hindlimb musculature of the dog (6, 31), cat (10), and rat (24). This reflex response is mediated via stimulation of VR1 (24). Electrophysiological studies suggest that capsaicin evokes its effect via stimulation of group IV (24 of 34 fibers tested) and group III afferents (5 of 19 tested) (10). Although the endogenous VR1 stimulant has not been determined, H+, lactic acid, histamine, serotonin, and prostaglandin E2 have been identified as potential endogenous ligands for the C-fiber “capsaicin” receptor (3, 9, 12, 26). H+ inhibits binding of the capsaicin analog resiniferatoxin (RTX) to VR1, which suggests that RTX and H+ compete for the same binding site (28).

It has recently been reported that capsaicin-stimulated VR1 evoke a pulmonary chemoreflex as well as reflex bronchoconstriction. These effects were reduced by capsazepine (18). However, similar responses induced by injection of lactic acid were unaffected by VR1 blockade. Along similar lines, it has been suggested that acid-induced activation of airway fine nerve afferent fibers (rapidly adapting low-threshold) was not mediated by VR1 (11). Instead, it was suggested that the acid-sensing ion channel (ASIC) mediated these reflex responses. This hypothesis was confirmed by Ugawa and colleagues (29), who reported that direct infusion of an acidic solution (pH >6.0) into human skin caused C-fiber-mediated nociception. This effect was blocked by the ASIC inhibitor amiloride, but not by capsazepine. Thus H+/lactate may mediate its effect by stimulating ASIC.

In the present report, we examined the role of VR1 and ASIC receptors in mediating the pressor response observed when lactic acid is injected into the rat hindlimb. The results of our studies suggest that lactic acid stimulates ASIC receptors but not VR1. Pretreatment with RTX, which destroys afferents with VR1, markedly attenuated the response to lactic acid, suggesting that ASIC and VR1 coexist on the same afferent fibers.

METHODS

All procedures outlined in this study were performed in conformance with the rules and regulations described in the National Institutes...
of Health Guide for the Care and Use of Laboratory Animals and were approved by the Animal Care Committee of the Pennsylvania State University College of Medicine. Sprague-Dawley male rats (385 ± 28 g body wt) were housed in standard rat cages and regulated on a 12:12-h light-dark schedule, with food and water available ad libitum.

General Methods

Animal surgical preparation. The rats were anesthetized by inhalation of an isoflurane-O₂ mixture (2–5% isoflurane in 100% O₂). An endotracheal tube was inserted into the trachea and attached to a ventilator (model AWS, Hallowell EMC). Polyethylene (PE-50) catheters were inserted into an external jugular vein and the common carotid artery for drug administration and measurements of arterial blood pressure, respectively. A continuous infusion of physiological saline (0.1 ml/h) was established via the jugular vein to stabilize fluid balance and maintain basal blood pressure by using a syringe pump (Medical Industries, Western Australia). The femoral arteries and sciatic nerves of both legs were isolated (i.e., the positive component of the ventricular response), with anesthesia and decerebration (i.e., the positive component of the decerebration procedure, the rats were injected with dexamethasone (5% isoflurane in 100% O₂). An incision was made in the femoral artery. A PE-10 catheter was inserted into the femoral artery and then threaded into the popliteal artery for arterial injection of drugs into the arterial blood supply of the hindlimb musculature of each leg. The skin covering the triceps surae muscle and femoral region was surgically separated from the muscle below. The femoral and sciatic nerves of both legs were isolated carefully so that they could be sectioned at the end of the study. The animals were artificially ventilated, and respiratory parameters were monitored by connecting a pneumotachograph (Fleisch) to a respiratory gas monitor (Datex-Ohmeda, Madison, WI). PO₂ was maintained at 30–40 Torr and PO₂ at >80 Torr. Body temperature was continuously monitored with a rectal thermometer (series 400, Yellow Springs Instruments) and maintained at 37.5–38.5°C by a perfluorocarbon heat pad and external heat lamps.

Decerebration. Smith and McQueen (24) demonstrated that capsaicin injected arterially evokes cardiovascular reflexes via VR₁ located on sensory nerve fibers within the hindlimb. In their study, rats were anesthetized with urethane or pentobarbital sodium, and capsaicin induced a decrease in blood pressure. Accordingly, in a group of studies, capsaicin was injected into the rat hindlimb of isoflurane-anesthetized rats. A biphasic blood pressure response was observed. Studies were then performed in a decerebrate rat model (n = 4) in the absence of the potential confounding influences of anesthesia. In these rats, the same dose of capsaicin that induced a biphasic response in the presence of anesthesia induced only a pressor response after decerebration. The magnitude of the increase in blood pressure was similar with anesthesia and decerebration (i.e., the positive component of the biphasic response vs. the response in the decerebrate state, see RESULTS). This suggests that the depressor response seen with VR₁ stimulation by capsaicin was due to effects of the anesthetic. These findings are consistent with prior reports suggesting that stimulation of thin-fiber muscle afferent evokes a pressor response in the decerebrate rat model (25).

In the decerebration experiments, animals were first anesthetized and instrumented. They were then held in a stereotaxic head-and-spinal unit (Kopf Instrument) as anesthesia was continued. Before the decerebration procedure, the rats were injected with dexamethasone (0.2 mg iv) to help prevent decerebration-induced brain stem edema.

Briefly, a craniotomy was performed by drilling Burr holes into the parietal skull. The portion of bone superior to the central sagittal sinus was then removed. The dura was incised and reflected laterally. The majority of the temporal and parietal plates were removed, and the two cortical hemispheres were also removed. A transverse section was made anterior to the superior colliculus and extending ventrally to the mamillary bodies. The brain rostral to the section was removed, and bleeding was controlled with cotton gauze that had been soaked in boiling saline, filling the vault and applying gentle manual pressure. Small pieces of oxidized regenerated cellulose (Ethicon, Johnson & Johnson) were placed on the exposed surface of the brain. The calvarium was then packed with moist gauze, and skin covering the cranium was clamped. Once the decerebration was complete, anesthesia was removed from the inhaled mixture. A recovery period of ≥60 min after decerebration was employed to allow sufficient time for elimination of the effects of anesthesia gas from the preparation.

Measurements of cardiovascular activities. Arterial blood pressure was measured by connecting the carotid arterial catheter to a pressure transducer (model P23ID, Statham). Mean arterial pressure (MAP) was obtained by integrating the arterial signal with a time constant of 4 s. Heart rate (HR) was determined from the arterial pressure pulse. All measured variables were continuously recorded on an eight-channel chart recorder (model TA 4000, Gould, Valley View, OH) and a personal computer (Dimension P75t, DELL) that used PowerLab system (AD Instruments, Castle Hill, Australia).

Experimental Protocol

Study series 1: arterial injection of anandamide and capsaicin to activate VR₁. This study was performed in eight anesthetized rats to determine whether activation of VR₁ in the hindlimb muscle produced a pressor response. The animals were surgically prepared as previously described. At 60 min after surgery, anandamide (1 mg/kg) and capsaicin (1 μg/kg) were injected into the blood supply of the triceps surae muscle. The injection volume was 0.1–0.15 ml, and the duration of injection was 30 s. At least 20 min were allowed between successive injections. To confirm the role of VR₁, capsaicine (1 mg/kg) was injected intra-arterially 10 min before injection of anandamide and capsaicin. In addition, the same dose of anandamide and capsaicin was injected into the popliteal artery after section of the femoral and sciatic nerves to confirm that the pressor response was due to stimulation of afferents within the hindlimb.

Study series 2: effects of capsaizepine and amiloride on the pressor response induced by lactic acid. Previous reports demonstrated that lactic acid injected into the blood supply of the cat hindlimb evokes a pressor response (20, 23). The purpose of this protocol was to examine whether lactic acid causes the pressor response by stimulating VR₁ and/or ASIC receptors on thin-fiber muscle afferents in the rat hindlimb (9, 11, 12, 26, 29).

Lactic acid (4 μmol/kg) was injected into the blood supply of the triceps surae muscle, and blood pressure was monitored. Then capsaizepine (1 mg/kg) and amiloride (20, 40, and 80 μM) were injected intra-arterially 10 min before repeated injections of lactic acid. The injection volume was 0.1–0.2 ml, and the duration of the injection was 30 s. This study was performed in 14 decerebrate rats.

Study series 3: arterial injections of capsaizepine and lactic acid in rats after treatment with RTX. The rats were injected with RTX (400 μg/kg ip) to produce a prolonged desensitization of VR₁ (27, 32) 4–5 days after RTX injection. The animals were instrumented as previously described. At 60 min after surgery, capsaizepine (1 μg/kg) was injected into the arterial supply of hindlimb muscles (n = 6) of anesthetized rats. Lactic acid (4 μmol/kg) was injected into the arterial supply of six decerebrate rats. The injection volume was 0.1–0.15 ml, and duration of the injection was 30 s. Twenty minutes were allowed between the two injections.

Drugs and Solutions

Anandamide, capsaicin, capsaizepine, RTX, and amiloride were purchased from Sigma. A stock solution of anandamide (50 mg/ml) was prepared in a vehicle of 50% ethanol-50% Emulphor, and capsaicin (250 μg/ml) was prepared in 1% Tween 80-1% ethanol-98% saline. Capsazepine was dissolved in 20% Cremaphor in distilled water to make a stock solution of 10 mg/ml. RTX was dissolved in 10% Tween 80 and 10% alcohol in normal saline. Amiloride was prepared in saline to make a stock solution of 1 mM. A stock solution
Experimental Data Analysis

All measured variables were continuously recorded on an eight-channel chart recorder (model TA 4000, Gould). These variables were also sampled by a personal computer that was equipped with analog-to-digital conversion and PowerLab data acquisition. Computer-acquired data were used in post hoc analyses. Control values were determined by analysis of ±30 s of the data immediately before arterial injection. The peak response of each variable was determined by the peak change from the control value.

Experimental data (MAP and HR) were analyzed statistically using a one-way repeated-measures ANOVA. As appropriate, Tukey’s post hoc analysis was utilized to determine differences between groups. Values are ± SE. For all analyses, differences were considered significant at $P < 0.05$. All statistical analyses were performed using SPSS for Windows (version 11.5, SPSS, Chicago, IL).

RESULTS

Anandamide and Capsaicin Activate VR1 to Induce Cardiovascular Responses

Baseline values for MAP and HR before arterial injections are presented in Table 1. There were no significant differences in basal MAP and HR before drug injections. Anandamide and capsaicin evoked changes in blood pressure in anesthetized animals ($n = 8$). A decrease in blood pressure was immediately followed by a pressor response (Table 1, Fig. 1). The peak pressor responses evoked by arterial injections of anandamide and capsaicin were attenuated after section of the sciatic and femoral nerves, demonstrating that the pressor response was mediated by a neural reflex. After the neural section, the reflex response to anandamide was 12.6 ± 3.8 mmHg ($P < 0.05$ vs. control of 36.5 ± 7.6 mmHg, $n = 4$). The effect of the afferent nerve section on the pressor response induced by capsaicin is shown in Table 1 and Fig. 1. The decrease in blood pressure after the arterial injection of capsaicin was unaffected by nerve sectioning (Table 1, Fig. 1).

Table 1. Baseline values and peak responses of MAP and HR in anesthetized animals

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Depressor Response</th>
<th>Pressor Response</th>
<th>Recovery</th>
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<tr>
<td><strong>Anandamide</strong></td>
<td></td>
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</tr>
<tr>
<td>MAP, mmHg</td>
<td>87.2±7.5</td>
<td>63.2±5.1*</td>
<td>124.4±11.2*</td>
<td>91.6±10.5</td>
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<tr>
<td>HR, beats/min</td>
<td>375±25</td>
<td>358±28</td>
<td>395±32</td>
<td>380±24</td>
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<td><strong>Capsaicin</strong></td>
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<tr>
<td>MAP, mmHg</td>
<td>95.8±9.5</td>
<td>70.4±4.5*</td>
<td>131.6±11.3*</td>
<td>106.2±11.2</td>
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<tr>
<td>HR, beats/min</td>
<td>384±16</td>
<td>377±22</td>
<td>412±18*</td>
<td>384±15</td>
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<tr>
<td><strong>Capsazepine+</strong></td>
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<tr>
<td>Anandamide</td>
<td></td>
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</tr>
<tr>
<td>MAP, mmHg</td>
<td>92.7±10.2</td>
<td>68.5±8.5*</td>
<td>104.3±12.2</td>
<td>100.3±10.2</td>
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<td>HR, beats/min</td>
<td>400±20</td>
<td>375±24</td>
<td>415±25</td>
<td>393±16</td>
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<tr>
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<tr>
<td>MAP, mmHg</td>
<td>98.3±13.4</td>
<td>71.3±10.1*</td>
<td>105.3±17.6</td>
<td>98.5±15.4</td>
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<td>HR, beats/min</td>
<td>399±16</td>
<td>386±16</td>
<td>415±20</td>
<td>406±13</td>
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<td><strong>Capsaicin after nerves section</strong></td>
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<tr>
<td>MAP, mmHg</td>
<td>86.8±9.7</td>
<td>58.5±6.8*</td>
<td>90.8±10.6</td>
<td>92.2±9.8</td>
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<tr>
<td>HR, beats/min</td>
<td>390±30</td>
<td>372±33</td>
<td>395±31</td>
<td>391±21</td>
</tr>
</tbody>
</table>

Values are means ± SE ($n = 8$). MAP, mean arterial pressure; HR, heart rate. There is no significant difference among baseline values. *$P < 0.05$ vs. baseline.

In six rats, the effects of VR1 blockade on the anandamide- and capsaicin-induced pressor responses were examined. Arterial administration of capsazepine (a VR1 antagonist) significantly attenuated the increase of MAP elicited by anandamide or capsaicin (Table 1, Fig. 2). However, arterial administration of capsazepine did not affect the depressor response elicited by anandamide or capsaicin (Table 1, Fig. 2).

In another group of animals ($n = 4$), the same amount of capsaicin was injected before and after decerebration. Capsaicin induced an increase in blood pressure only in decerebrate rats (34.5 ± 5.2 mmHg). This finding suggests that isoflurane was responsible for the depressor part of the response when this anesthetic was used.
Effects of Capsazepine and Amiloride on Lactic Acid-Induced Pressor Response

Baseline values of MAP and HR were 95.2 ± 12.5 mmHg and 395 ± 35 beats/min, respectively. There were no significant differences in basal values under all experimental paradigms. Lactic acid caused an increase in blood pressure in decerebrate animals (n = 14). The peak pressor response to arterial injection of lactic acid is shown in Fig. 3. The response was unchanged after VR1 blockade with a dose of capsazepine that was shown to attenuate the capsaicin-induced pressor response (n = 8). However, blockade of ASIC with injection of amiloride (80 µM but not 20 and 40 µM) attenuated the pressor response induced by lactic acid (Fig. 3). Finally, section of the sciatic and femoral nerves attenuated lactic acid-induced reflex (25.8 ± 5.6 and 3.5 ± 1.1 mmHg in control and after nerve section, respectively, P < 0.05).

Cardiovascular Responses to Capsaicin and Lactic Acid in RTX-Treated Rats

There were no significant differences in basal MAP before injections between control and RTX-treated rats (95.8 ± 9.5 and 98.6 ± 10.2 mmHg). Compared with the elevation in MAP evoked by capsaicin in control animals, arterial injection of capsaicin into the blood supply of the hindlimb muscle produced negligible changes in the MAP increase in RTX-pre-treated rats (n = 6; Fig. 4). A similar depressor response pattern was still elicited by capsaicin in these RTX-treated rats (from 95.8 ± 9.5 to 70.4 ± 4.5 mmHg in control and from 98.6 ± 10.2 to 75.5 ± 7.6 mmHg in RTX group, P > 0.05). In addition, the pressor response to lactic acid was attenuated in the RTX-treated rats (n = 6) compared with controls (Fig. 4). Original traces in Fig. 4 also show that arterial administration of capsaicin produced the blunted pressor reflex and that the acid-induced response is smaller in the RTX-treated animals.

DISCUSSION

Static exercise evokes increases in blood pressure and HR that are mediated in part by the reflex activation of sensory afferents in contracting muscle (2, 15–17). A population of these sensory nerves, termed metaboreceptor muscle afferents, is activated by metabolic products of muscle contraction (16, 17). However, receptor mechanisms by which metabolic products stimulate muscle afferent fibers remain unclear. In this study, we used hindlimb arterial injections to stimulate muscle...
afferents in an effort to learn more about the receptor types that are engaged when blood pressure is raised by lactic acid and capsaicin. We found that a pressor response elicited when capsaicin was injected into the muscles’ arterial supply was significantly attenuated when VR1 were blocked with capsazepine. Additionally, the response to capsaicin was abolished in the RTX-treated rats. We then determined whether the pressor response seen with H+/lactic acid was due to stimulation of VR1 by examining pressor responses to lactic acid before and after infusion of the competitive VR1 antagonist capsazepine. Blockade of VR1 did not attenuate acid-induced responses, suggesting that lactic acid does not evoke a response by stimulating VR1. We then demonstrated that a blockade of ASIC significantly attenuated the pressor response observed when lactic acid was infused. This suggests that H+ stimulates ASIC and evokes a reflex response. Interestingly, lactic acid-induced responses were attenuated in the RTX-treated rats. Because RTX causes degeneration of VR1-expressing afferent neurons (27, 32), lactic acid must be stimulating ASIC on afferent fibers that also contain VR1.

The study of lactic acid is important, because lactic acid is produced in contracting skeletal muscle (14), and injection of lactic acid into the hindlimb of skeletal muscle increases blood pressure (20, 23). Importantly, use of dichloroacetate to reduce lactate led to a decrease in muscle afferent discharge in cats (22) and reduced sympathetic nerve activity in humans (7). Our findings are at odds with studies that showed that the competitive VR antagonist capsazepine attenuates a number of acid-induced physiological processes (8, 13, 30). However, Nault et al. (22) and reduced sympathetic nerve activity in humans, possibly due to stimulation of VR1. These findings may have important implications for VR1 and ASIC in the processing of muscle afferent signals that evoke the exercise pressor reflex.

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