P2X2/3 and P2X3 receptors contribute to the metaboreceptor component of the exercise pressor reflex

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McCord JL, Tsuchimochi H, Kaufman MP. P2X2 and P2X3 receptors contribute to the metaboreceptor component of the exercise pressor reflex. J Appl Physiol 109: 1416–1423, 2010. First published August 26, 2010; doi:10.1152/japplphysiol.00774.2010.—The exercise pressor reflex is evoked by muscle contraction and results in increased arterial pressure, heart rate, and ventilation. The sensory arm of the reflex is comprised of group III and IV muscle afferents. The former are thinly myelinated and thought to be primarily metabolically sensitive (4, 16, 27). Even though several by-products of contraction are thought to be involved in stimulating metaboreceptors, the exact mechanism of the metabolic component of the exercise pressor reflex has not been elucidated.

Several lines of evidence suggest that ATP, working through purinergic 2X (P2X) receptors, is one of the substances that evokes the metabolic component of the exercise pressor reflex. For example, contraction increased ATP concentrations in the muscle interstitium of rats and cats (23, 24). Furthermore, ATP injected into the arterial supply of skeletal muscle reflexly increased arterial pressure, heart rate, and ventilation in decerebrate cats (12); moreover ATP increased the discharge rate of group IV muscle afferents in rats (31). Last, blockade of P2 receptors with PPADS decreased the exercise pressor reflex; this blockade also decreased the responses of group III and IV muscle afferents to static contraction in decerebrate cats (10, 18–20).

There are several P2X receptor subtypes that ATP could be activating to evoke the exercise pressor reflex. PPADS has been used to show that ATP is involved in the reflex (18–20). Nevertheless, PPADS blocks all P2X receptors as well as the P2Y1 receptor (9), making it unclear which receptor subtype(s) is responsible for evoking the reflex. However, arterial injections of UTP, a selective P2Y receptor agonist, did not cause a reflex pressor response (12), a finding that prompted us to focus on the role played by P2X receptor subtypes. We became interested in P2X2/3 and P2X3 receptors as these two subtypes are located on the peripheral and central endings of group III and IV afferents, have been shown to mediate sensory neurotransmission, and are involved in transmitting pain (6, 9, 15, 17, 21). In addition, recent advances in understanding P2X2/3 and P2X3 receptor pharmacology have enabled the development of selective antagonists (9, 15).

Thus the present study is our effort to determine if P2X2/3 and P2X3 receptors on thin fiber muscle afferents play a role in evoking the metabolic component of the exercise pressor reflex. The reflex effect evoked by metaboreceptors was isolated from that evoked by mechanoreceptors by measuring the arterial pressure response during postcontraction circulatory occlusion, a maneuver in which the muscle is no longer active (i.e., absence of mechanoreceptor activation) but the metabolites released during exercise are trapped within the muscle vasculature (1).

We used two structurally different P2X2 and P2X3 receptor antagonists, A-317491 and RO-3 (9, 15), to determine the effect of P2X2/3 and P2X3 receptor blockade on the pressor responses to static contraction while the working muscle was freely perfused, static contraction while the circulation to the working muscle was occluded, and during postcontraction circulatory occlusion in decerebrate cats. We tested the hypothesis that P2X2/3 and P2X3 receptors play a role in evoking the metabolic component of the exercise pressor reflex.

METHODS

All procedures were reviewed and approved by the Institutional Animal Care and Use Committee of the Pennsylvania State University, Hershey Medical Center.

Surgical Preparation

Adult female cats (n = 27, 2.8 ± 0.2 kg, range 2.3–3.2 kg) were anesthetized with a mixture of 5% isoflurane and oxygen. The right jugular vein and common carotid artery were cannulated to deliver
drugs and fluids as well as to measure arterial blood pressure, respectively. The carotid arterial catheter was connected to a pressure transducer (model P23 XL, Statham) to monitor blood pressure. Heart rate was calculated beat-to-beat from the arterial pressure pulse (Gould Biotach). The trachea was cannulated, and the lungs were ventilated mechanically (Harvard Apparatus). Arterial blood gases and pH were measured by an automated blood gas analyzer (model ABL-700, Radiometer). PCO$_2$ and arterial pH were maintained within normal range by either adjusting ventilation or intravenous administration of sodium bicarbonate (8.5%). A temperature probe was passed through the mouth to the stomach. Temperature was continuously monitored and maintained at 37–38°C by a water-perfused heating pad and a heat lamp.

The left common iliac vein and abdominal aorta were isolated and snares were placed around these vessels to trap injected drugs in the circulation of the leg (see below). In addition, the sacral artery, which perfuses the tail, was ligated. A catheter with its tip pointing toward the heart was passed into the right femoral artery. When the snare placed around the abdominal aorta was tightened, fluid injected from the right femoral arterial catheter flowed into the left external iliac artery. This was checked in every cat by injecting saline into the catheter in the right femoral artery and seeing blood exit the left external iliac artery, leaving it clear. The volume of saline needed to clear the left external iliac artery, which was usually 0.15–0.2 ml, was used to flush the drugs used in this experiment.

Following placement in a Kopf stereotaxic frame, each cat was decerebrated at the midcollucular level under isoflurane anesthesia. Dexamethasone (4 mg) was injected intravenously just before the decerebration procedure to minimize brain edema. The left common carotid artery was tied off to reduce bleeding. All neural tissue rostral to the midcollucular section was removed, and the cranial vault was filled with agar.

A laminectomy was performed to expose the lower lumbar and sacral portions of the spinal cord. The skin on the back was used to form a pool that was filled with warm (37°C) mineral oil. The dura of the cord was cut and reflected allowing visual identification of the spinal roots. The left L6, L7, and S1 ventral roots were identified and cut. The peripheral cut ends were draped over a stimulating electrode. The left calcaneal bone was cut, and its tendon was attached to a force transducer (model FT-10C, Grass) for measurement of the tension developed during static contraction of the left triceps surae muscles. This was checked in every cat by injecting saline into the catheter in the right femoral artery and seeing blood exit the left external iliac artery, leaving it clear. The volume of saline needed to clear the left external iliac artery, which was usually 0.15–0.2 ml, was used to flush the drugs used in this experiment.

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Experimental Protocols

We examined the effect of two structurally different but specific P2X2/3 and P2X3 receptor antagonists [A-317491 and RO-3 (9, 15)] on four maneuvers that evoked reflex pressor and cardioaccelerator responses. To do this, we ran four separate protocols. In the 1st protocol performed on 5 decerebrate cats, we statically contracted the freely perfused left triceps surae muscles; ~10 min after the end of the contraction, we injected α,β-MeATP (50 μg/kg; dissolved in saline), a P2X receptor agonist, into the right femoral artery. Both maneuvers were performed before and after P2X2/3 and P2X3 receptor blockade with A-317491 (10 mg/kg; dissolved in 0.5 ml saline (0.9% sodium chloride); Sigma-Aldrich). In the second protocol performed on four decerebrate cats, we statically contracted the left triceps surae muscles while their circulation was occluded and maintained the occlusion for 60 s after the contraction ended. In addition, 10 min after the end of the postcontraction circulatory occlusion, we injected α,β-MeATP (50 μg/kg) into the right femoral artery. RO-3 was trapped in the hindlimb circulation for 10 min after which the hindlimb was freely perfused for 10 min. Thus the maneuvers were repeated ~20 min after injection of RO-3 into the right femoral artery. RO-3 has an in vivo plasma half-life of 25 min (8). We originally chose a dose of RO-3 to match the concentration (10 mg/kg) used by our lab for other P2X antagonists, PPADS (83.4 mM) and A-317491 (88.4 mM). The molecular weight of RO-3 is lower than that of the other two P2X antagonists; thus a dose of 5 mg/kg would have been 82.6 mM. However, in two cats, RO-3 in a dose of 5 mg/kg did not have an effect on the pressor response to α,β-MeATP injection, suggesting we needed a higher dose to block P2X2/3 and P2X3 receptors. With a dose of 10 mg/kg RO-3, we were in fact able to reduce the pressor response to α,β-MeATP.

Control Protocols

Vehicle control protocol. We ran the protocol described above for freely perfused static contraction of the triceps surae muscles and α,β-MeATP injection, but instead of injecting a P2X2/3 and P2X3 receptor antagonist, we injected DMSO (1 ml; Fischer Scientific) into the right femoral artery in five cats. We injected DMSO, the vehicle for A-317491, has no effect on the pressor and cardioaccelerator responses to our maneuvers were repeatable and that any attenuation chose a dose of RO-3 to match the concentration (10 mg/kg) used by our lab for other P2X antagonists, PPADS (83.4 mM) and A-317491 (88.4 mM). The molecular weight of RO-3 is lower than that of the other two P2X antagonists; thus a dose of 5 mg/kg would have been 82.6 mM. However, in two cats, RO-3 in a dose of 5 mg/kg did not have an effect on the pressor response to α,β-MeATP injection, suggesting we needed a higher dose to block P2X2/3 and P2X3 receptors. With a dose of 10 mg/kg RO-3, we were in fact able to reduce the pressor response to α,β-MeATP.

Intravenous controls. In three cats, we injected A-317491 (10 mg/kg; dissolved in 0.5 ml 0.9% sodium chloride) and RO-3 (10 mg/kg; dissolved in 1 ml DMSO) into the jugular vein catheter to ensure that the attenuation we documented in the pressor and cardioaccelerator responses to our maneuvers was caused by blocking
Peripheral P2X2/3 and P2X3 receptors in the hindlimb and were not caused by blocking these receptors in the spinal cord or brain stem.

**Capsaicin control.** In six cats, we injected capsaicin (1–2 μg) into the right femoral artery before and after either A-317491 or RO-3 injection. Our purpose was to ensure that any attenuation of the pressor responses to the maneuvers performed in our experiment were due to P2X2/3 and P2X3 receptor blockade and were not due to a deterioration in our preparation. Capsaicin activates TRPV1 channels, which are located on the endings of thin fiber afferents. Thus blocking P2X2/3 and P2X3 receptors should have no effect on the pressor response evoked by capsaicin injection.

**Data analysis**

There was no difference between the magnitude of the attenuated pressor responses from RO-3 and A-317491 in response to any maneuver (Table 1); therefore, we pooled the data for each maneuver. Mean arterial blood pressure and heart rate values were expressed as means ± SE. Baseline mean arterial blood pressure and heart rate were measured immediately before a maneuver, and peak mean arterial blood pressure and heart rate were measured during injection of α,β-MeATP as well as during the 60-s period of static contraction or postcontraction circulatory occlusion. The tension-time index was calculated by integrating the area between the tension trace and the baseline level (Spike 2) and is expressed in kilogram seconds (kg·s) (30). Statistical comparisons were performed with either a one- or two-way repeated-measures ANOVA. If significant main effects were found with an ANOVA, post hoc tests were performed with the Tukey two-way repeated-measures ANOVA. If significant main effects were found with an ANOVA, post hoc tests were performed with the Tukey test between individual means. The criterion for statistical significance was P < 0.05.

**RESULTS**

**Freely Perfused Contraction**

In 10 cats, contraction while the muscles were freely perfused increased mean arterial pressure above baseline levels both before and after P2X2/3 and P2X3 receptor blockade (P < 0.05; Fig. 1). The magnitude of the pressor response to contraction, however, was attenuated by P2X2/3 and P2X3 receptor blockade (P < 0.05; Fig. 1). The tension-time index was not different before and after P2X2/3 and P2X3 receptor blockade (P = 0.48; Table 2). Likewise, the pressor response to α,β-MeATP injection in these 10 cats was reduced by P2X2/3 and P2X3 receptor blockade (P < 0.05; Fig. 1). In two cats, A-317491 was ineffective at reducing the pressor response to α,β-MeATP injection and static contraction; however, in these two cats, the nonspecific P2X antagonist, PPADS, reduced the pressor to α,β-MeATP injection and static contraction.

**Circulatory Occluded Contraction**

We found that the pressor and cardioaccelerator responses to contraction were greater when the circulation was occluded than those to contraction when the muscles were freely perfused (P < 0.05; Figs. 1 and 2 and Table 3). In nine cats, circulatory occluded contraction increased mean arterial pressure above baseline levels both before and after P2X2/3 and P2X3 receptor blockade (P < 0.05; Fig. 2). P2X2/3 and P2X3 receptor antagonists attenuated the pressor response to static contraction while the circulation was occluded (P < 0.05; Figs. 2 and 3). To isolate metaboreceptors from mechanoreceptors, we examined the pressor response to postcontraction circulatory occlusion before and after P2X2/3 and P2X3 receptor blockade. Before blockade, the peak pressor response during postcontraction circulatory occlusion was elevated above baseline (P < 0.05; Fig. 2). After blockade, the pressor response during postcontraction circulatory occlusion was attenuated compared with that before blockade (P < 0.05; Figs. 2 and 3). Heart rate was increased during circulatory occluded contraction (P < 0.05) but was not elevated above baseline during postcontraction circulatory occlusion (P = 0.17; Table 3). The tension-time index before P2X2/3 and P2X3 receptor antagonist injection for the static contraction with the circulation occluded was not different from that after injection (P = 0.86; Table 2). In addition, the pressor response to α,β-MeATP injection in these 10 cats was reduced by P2X2/3 and P2X3 receptor blockade (P < 0.05; Fig. 2).

**Control Experiments**

In each of the five cats tested, we found that the pressor responses to freely perfused contraction and α,β-MeATP injection were not affected by DMSO injection (P > 0.85; Fig. 4). The tension-time index before DMSO injection for the freely perfused static contraction was not different from that after DMSO injection (P = 0.73; Table 2). In each of the six cats tested, P2X2/3 and P2X3 receptor blockade had no effect

| Table 1. Effect of each P2X2/3 and P2X3 receptor antagonist on the MAP responses to static contraction while the muscles were freely perfused (A-317491 n = 5; RO-3 n = 5), to static contraction while the circulation was occluded, and postcontraction circulatory occlusion (A-317491 n = 4; RO-3 n = 5) |
|-------------------------------------------------|-----------------|-----------------|-----------------|-----------------|
| Freely perfused contraction                     | A-317491        | RO-3            | A-317491        | RO-3            |
| Baseline MAP, mmHg                              | 131 ± 11        | 139 ± 10        | 152 ± 6         |
| Peak MAP, mmHg                                  | 160 ± 12*       | 179 ± 15*       | 172 ± 14        |
| MAP Δ from baseline, mmHg                       | 29 ± 5          | 40 ± 7          | 19 ± 11†        |
| Circulatory occluded contraction                | 148 ± 5         | 145 ± 9         | 149 ± 11        |
| Baseline MAP, mmHg                              | 190 ± 6*        | 204 ± 18*       | 169 ± 13*†      |
| Peak MAP, mmHg                                  | 42 ± 6          | 59 ± 12         | 20 ± 3†         |
| Baseline MAP, mmHg                              | 148 ± 5         | 145 ± 9         | 149 ± 11        |
| Peak MAP, mmHg                                  | 164 ± 4*        | 159 ± 9*        | 154 ± 10        |
| MAP Δ from baseline, mmHg                       | 16 ± 3          | 14 ± 1          | 5 ± 2†          |

Values are means ± SE. MAP, mean arterial pressure. *P < 0.05, baseline vs. peak. †P < 0.05, before vs. after drug.
on the pressor response to capsaicin injection (P = 0.24; Fig. 5). Likewise, in each of the six cats tested, we found that intravenous injection of the P2X2/3 and P2X3 receptor antagonists had no effect on the pressor response to freely perfused static contraction or to \(\text{Plex}_{2/3}\) and \(\text{Plex}_{3}\) injection into the arterial supply of the hindlimb (\(P < 0.05\); Table 4).

We also noted that arterial injection of RO-3 (baseline: 122 ± 7 mmHg; peak: 177 ± 11 mmHg; Δ: 56 ± 9 mmHg; \(P < 0.05\)) and DMSO (baseline: 111 ± 11 mmHg; peak: 159 ± 14 mmHg; Δ: 48 ± 5 mmHg; \(P < 0.05\)) evoked large but transient pressor responses. We did not see this pressor response when A-317491 was injected (baseline: 126 ± 12 mmHg; peak: 134 ± 11 mmHg; Δ: 8 ± 2 mmHg; \(P = 0.57\)).

**DISCUSSION**

We tested the hypothesis that P2X2/3 and P2X3 receptors play a role in evoking the metabolic component of the exercise pressor reflex. We blocked P2X2/3 and P2X3 receptors by injecting two structurally different P2X2/3 and P2X3 receptor antagonists, A-317491 and RO-3, into the arterial circulation of

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**Table 2.** TTI and peak tension for static contraction while the muscles were freely perfused (n = 10) and static contraction while the circulation to the muscles was occluded (n = 9) before and after P2X2/3 and P2X3 receptor blockade and DMSO injections (n = 5)

<table>
<thead>
<tr>
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<th>Before Drug</th>
<th>After Drug</th>
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<tbody>
<tr>
<td><strong>TTI, kg·s</strong></td>
<td>104 ± 10</td>
<td>109 ± 10</td>
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<tr>
<td><strong>Peak tension, kg</strong></td>
<td>3.07 ± 0.20</td>
<td>3.20 ± 0.24</td>
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<tr>
<td><strong>Ischemic contraction</strong></td>
<td>97 ± 9</td>
<td>95 ± 13</td>
</tr>
<tr>
<td><strong>TTI, kg·s</strong></td>
<td>3.16 ± 0.16</td>
<td>3.69 ± 0.27</td>
</tr>
<tr>
<td><strong>Peak tension, kg</strong></td>
<td>98 ± 23</td>
<td>93 ± 28</td>
</tr>
<tr>
<td><strong>DMSO</strong></td>
<td>3.45 ± 0.66</td>
<td>3.13 ± 0.53</td>
</tr>
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Values are means ± SE. TTI, time-tension index.
the muscles being contracted. The efficacy of the blockade was confirmed by showing that the pressor response to arterial injection of $^{92}$-MeATP was attenuated by about half. In addition, we ensured that the attenuation of the pressor response to contraction was due to the blockade of P2X2/3 and P2X3 receptors and not to a deterioration of our preparation by finding that the pressor response was matched before and after control vehicle and capsaicin injections. We found that the pressor response to postcontraction circulatory occlusion, when only metaboreceptors were stimulated, was attenuated by P2X2/3 and P2X3 receptor blockade. This finding suggests that ATP, working through P2X2/3 and P2X3 receptors, plays a role in evoking the metabolic component of the exercise pressor reflex.

The evidence concerning ATP as a substance that evokes the exercise pressor reflex through stimulation of muscle afferents is strong. Specifically, injection of P2X agonists into the arterial supply or directly into the substance of the gastrocnemius muscle stimulated over two-thirds of the group IV afferents tested in cats (11) and rats (31). In addition, P2X agonists injected into the arterial supply of muscle augmented the pressor response to stretch, suggesting that muscle mechano-receptors can be sensitized by P2X receptor activation (25). Further, blockade of P2 receptors by PPADS attenuated the responses of both group III and IV afferents to static contraction, both while the muscles were freely perfused and while they were ischemic. Likewise, PPADS attenuated the responses of group IV afferents to postcontraction circulatory occlusion (18, 20). These findings lead us to conclude that ATP activates P2X receptors on the endings of group III and IV thin fiber afferents during exercise and that these receptors contribute to the exercise pressor reflex by sensitizing group III mechanoreceptors and stimulating group IV metaboreceptors. Our present study supports this conclusion and suggests that P2X2/3 and P2X3 receptors are in part responsible for stimulating group IV metaboreceptors during exercise. We did not test whether P2X2/3 and P2X3 receptors play a role in acti-

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**Table 3. HR responses to static contraction while the muscles were freely perfused ($n = 10$), to static contraction while the circulation was occluded ($n = 9$), and postcontraction circulatory occlusion ($n = 9$) before and after P2X2/3 and P2X3 receptor antagonist injection.**

<table>
<thead>
<tr>
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<th>Before</th>
<th>P2X2/3 and P2X3 Receptor Blockade</th>
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<tbody>
<tr>
<td><strong>Freely perfused contraction</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline HR, beats/min</td>
<td>246 ± 17</td>
<td>242 ± 19</td>
</tr>
<tr>
<td>Peak HR, beats/min</td>
<td>257 ± 17</td>
<td>244 ± 20</td>
</tr>
<tr>
<td>HR Δ from baseline, beats/min</td>
<td>11 ± 2</td>
<td>2 ± 5</td>
</tr>
<tr>
<td><strong>Circulatory occluded contraction</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline HR, beats/min</td>
<td>204 ± 13</td>
<td>227 ± 20</td>
</tr>
<tr>
<td>Peak HR, beats/min</td>
<td>229 ± 13</td>
<td>238 ± 18</td>
</tr>
<tr>
<td>HR Δ from baseline, beats/min</td>
<td>25 ± 5†</td>
<td>11 ± 3*</td>
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<tr>
<td><strong>Postcontraction circulatory occlusion</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline HR, beats/min</td>
<td>204 ± 13</td>
<td>227 ± 20</td>
</tr>
<tr>
<td>Peak HR, beats/min</td>
<td>218 ± 14</td>
<td>231 ± 18</td>
</tr>
<tr>
<td>HR Δ from baseline, beats/min</td>
<td>14 ± 4</td>
<td>4 ± 4*</td>
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Values are means ± SE. HR, heart rate. *$P < 0.05$, before vs. P2X2/3 and P2X3 receptor blockade; †$P < 0.05$, freely perfused contraction vs. circulatory occluded contraction.

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**Fig. 3.** The pressor responses to static contraction while the circulation was occluded and postcontraction circulatory occlusion were attenuated by A-317491 (10 mg/kg). **A:** response to contraction before blockade. **B:** response to contraction after blockade. Thick solid black lines represent 60 s of static contraction. Thick dashed black lines represent 60 s of static contraction. Thin solid gray lines represent approximate systolic and diastolic arterial pressure baselines. BP, blood pressure.

**Fig. 4.** Change in mean arterial pressure from baseline to peak pressor response before (filled bars) and after DMSO injection (open bars) to freely perfused static contraction (**A**) and intra-arterial α,β-methylene ATP injection (50 μg/kg) (**B**). Baseline mean arterial pressure values are within vertical bars. *Significant difference ($P < 0.05$) between baseline and peak. Values are means ± SE; $n = 5$. The evidence concerning ATP as a substance that evokes the exercise pressor reflex through stimulation of muscle afferents is strong. Specifically, injection of P2X agonists into the arterial supply or directly into the substance of the gastrocnemius muscle stimulated over two-thirds of the group IV afferents tested in cats (11) and rats (31). In addition, P2X agonists injected into the arterial supply of muscle augmented the pressor response to stretch, suggesting that muscle mechanoreceptors can be sensitized by P2X receptor activation (25). Further, blockade of P2 receptors by PPADS attenuated the responses of both group III and IV afferents to static contraction, both while the muscles were freely perfused and while they were ischemic. Likewise, PPADS attenuated the responses of group IV afferents to postcontraction circulatory occlusion (18, 20). These findings lead us to conclude that ATP activates P2X receptors on the endings of group III and IV thin fiber afferents during exercise and that these receptors contribute to the exercise pressor reflex by sensitizing group III mechanoreceptors and stimulating group IV metaboreceptors. Our present study supports this conclusion and suggests that P2X2/3 and P2X3 receptors are in part responsible for stimulating group IV metaboreceptors during exercise. We did not test whether P2X2/3 and P2X3 receptors play a role in acti-
P2X2/3 receptors are more sensitive to a small pH change than are P2X3 receptors, and P2X3 receptors are quickly desensitized after activation (9, 34). Nonetheless, as described above the possibility exists that at least one other P2X receptor subtype is involved due to our finding that in two cats, A-317491 had no effect on the pressor responses to contraction or α,β-MeATP but that PPADs were in fact able to attenuate the pressor responses to the maneuvers.

ATP is not the only metabolite that has been suggested to evoke the metabolic component of the exercise pressor reflex. The pressor response to exercise can also be reduced by half by either blockade of acid sensing ion channels (ASIC), which are opened by lactic acid, or cyclooxygenase, an enzyme which results in the production of prostaglandins (28, 33). Then how is it possible for ATP, lactic acid, and prostaglandins to be responsible for over half the reflex response? An answer to this question comes from two areas of investigation that each suggest a combination of metabolites is the key to activation of group III and IV afferents during muscular exercise. The first found that lactate and ATP together greatly potentiated the effect of an acidic pH (~7.0–6.8) on the activation of ASIC3 (13). In addition, levels of ATP that are consistent with those released during ischemic contraction increased current through ASIC3 (29). Lactate has a relatively quick effect on ASICs, whereas ATP needs 15–60 s after its application to exert a peak effect on ASICs; moreover, current through the channel remains high for minutes even after ATP has been removed (29). The second area of investigation found that combinations of protons, lactic acid, and ATP were needed to activate cultured dorsal root ganglion cells (26). When the cells were exposed to just one of the metabolites only a small stimulatory effect on cells was measured, whereas when the cells were exposed to a combination of the three metabolites the effect far exceeded the summation of each one individually (26). These investigations suggest that the full expression of the exercise pressor reflex requires the synergistic interaction of at least two muscle metabolites.

We noted a pronounced pressor response when injecting RO-3 into the hindlimb vasculature, but we did not see this response when we injected A-317491. In addition, injection of DMSO, the vehicle for RO-3, also evoked a pronounced pressor response when injected into the muscular vasculature. We speculate that the pressor response came from the vehicle itself, DMSO. DMSO has been shown to cause a rapid increase in the firing rate of afferents arising from the vating mechanoreceptors but based on the above studies, we would propose that P2X2/3 and P2X3 receptor antagonists or at the very least sensitize group III mechanoreceptors.

P2X2/3 and P2X3 receptors are not the only P2X receptors that could evoke the exercise pressor reflex. There are several P2X receptor subtypes, some of which are good candidates for investigation in the role they play in evoking the reflex. Of the P2X receptors, α,β-MeATP preferentially stimulates P2X1, P2X2/3, P2X3, P2X1/5, and P2X6 receptors (15), thus raising the possibility that any or a combination of these receptors could be involved in the exercise pressor reflex. Nevertheless, P2X1 receptors appear to be located on smooth muscle cells in the vas deferens but do not appear to be located on dorsal root ganglion cells, which were found to lack P2X1 mRNA (15, 21). P2X6 receptors are present in the central nervous system and have also been found in skeletal muscle; however, a major function for this receptor has not been found (9). Little is known about P2X1/5 receptors other than that they hold a combination of P2X1 and P2X5 receptor characteristics (9). Little is also known about P2X5 receptors except that they are primarily located on skeletal muscle cells (9). Nevertheless, recent experiments on cultured dorsal root ganglion cells using calcium imaging techniques suggested that P2X4 and P2X5 receptors are the main P2X receptor subtypes that are responsible for detecting concentrations of ATP that would be present during muscle contraction and/or pain (26). In addition, P2X4, P2X5, and P2X6 mRNAs were found in many dorsal root ganglion neurons as were P2X2/3 and P2X3 mRNAs in rats (21). P2X7 receptors are also involved in the sensation of pain, although the majority of these are thought to be located on macrophages and monocytes and are thought to be rare on dorsal root ganglion neurons (15, 21). While there are specific antagonists for P2X2 and P2X7 receptors, specific antagonists for the other receptor subtypes are not yet available. Moreover, singling out the effects of P2X3 from P2X2/3 receptors is not yet possible. However, we speculate that P2X2/3 receptors might play a bigger role in eliciting the exercise pressor reflex than do P2X3 receptors because P2X2/3 receptors are more sensitive to a small pH change.

**Table 4. MAP responses to freely perfused contraction and α,β-MeATP injection before and after intravenous injection of P2X2/3 and P2X3 receptor antagonists (n = 6)**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Baseline MAP, mmHg</th>
<th>Peak MAP, mmHg</th>
<th>MAP Δ from Baseline, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freely perfused contraction</td>
<td>119 ± 12</td>
<td>135 ± 13</td>
<td>19 ± 3</td>
</tr>
<tr>
<td>Before</td>
<td>119 ± 14</td>
<td>136 ± 15</td>
<td>20 ± 4</td>
</tr>
<tr>
<td>P2X2/3 and P2X3 receptor blockade</td>
<td>120 ± 13</td>
<td>138 ± 13</td>
<td>18 ± 2</td>
</tr>
<tr>
<td>α,β-MeATP injection</td>
<td>120 ± 16</td>
<td>130 ± 17</td>
<td>18 ± 3</td>
</tr>
</tbody>
</table>

Values are means ± SE. α,β-MeATP, α,β-methylene ATP.
pressor responses to P2X2/3 and P2X3 receptors. However, the attenuation of the afferents to respond to stimuli rather than to the blockade of afferents (7). This finding raises the possibility that our data DMSO has been shown to reduce discharge rate of these evoked by DMSO, prolonged exposure (i.e., over 30 min) to reduce pain through reducing afferent discharge. After the in the spinal cord (2, 22).

Pressure remained above baseline in 8 of the cats for 30 min. That lasted 3–4 min and was then followed by an elevated discharge rate above baseline for 30 min (5). This seems to parallel what we found when injecting RO-3 in DMSO and DMSO alone, namely a pronounced pressor response to parallel what we found when injecting RO-3 in DMSO.

In conclusion, we found that the pressor response to a freely perfused contraction was attenuated with P2X2/3 and P2X3 receptor blockade. In addition, the pressor response to capsaicin injections was not different before or after P2X2/3 and P2X3 blockade.

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DISCLOSURES
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