Blockade of purinergic 2 receptors attenuates the mechanoreceptor component of the exercise pressor reflex

Angela E. Kindig, Shawn G. Hayes, and Marc P. Kaufman
Division of Cardiovascular Medicine, University of California Davis, Davis, California; and Penn State Heart & Vascular Institute, Pennsylvania State University College of Medicine, Hershey, Pennsylvania

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Kindig AE, Hayes SG, Kaufman MP. Blockade of purinergic 2 receptors attenuates the mechanoreceptor component of the exercise pressor reflex. Am J Physiol Heart Circ Physiol 293: H2995–H3000, 2007. First published August 31, 2007; doi:10.1152/ajpheart.00743.2007.—The finding that pyridoxalphosphate-6-azophenyl-2,4-disulfonic acid (PPADS), a P2 antagonist, attenuated the pressor response to calcaneal tendon stretch, a purely mechanical stimulus, raises the possibility that P2 receptors sensitize mechanoreceptors to static contraction of the triceps surae muscles. The mechanical component of the exercise pressor reflex, which is evoked by static contraction, can be assessed by measuring renal sympathetic nerve activity during the first 2–5 s of this maneuver. During this period of time, group III mechanoreceptors often discharge explosively in response to the sudden tension developed at the onset of contraction. In decerebrated cats, we, therefore, examined the effect of PPADS (10 mg/kg) injected into the popliteal artery on the renal sympathetic and pressor responses to contraction and stretch. We found that PPADS significantly attenuated the renal sympathetic response to contraction, with the effect starting 2 s after its onset and continuing throughout its 60-s period. PPADS also significantly attenuated the renal sympathetic nerve response to stretch, but did so after a latency of 10 s. Our findings lead us to conclude that P2 receptors sensitize group III muscle afferents to contraction. The difference in the onset latency between the PPADS-induced attenuation of the renal sympathetic response to contraction and the renal sympathetic response to stretch is probably due to the sensitivities of different populations of group III afferents to ATP released during contraction and stretch.

group III muscle afferents; group IV muscle afferents; ATP; cats; renal sympathetic nerve activity; neural control of the circulation

THE ROLE PLAYED BY PURINERGIC RECEPTORS on thin fiber muscle afferents in generating the exercise pressor reflex (30) has been the subject of considerable investigation and interest. Purinergic receptors are of two types, namely P1, which are stimulated by adenosine, and P2, which are stimulated by ATP (1). P1 receptors appear to play no role in evoking the exercise pressor reflex, whereas P2 receptors play a substantial role in evoking this reflex. For example, popliteal arterial injection of a P1 receptor antagonist, CGS 15943, had no effect on the exercise pressor reflex evoked by statically contracting the triceps surae muscles. In contrast, popliteal arterial injection of the P2 receptor antagonist, pyridoxalphosphate-6-azophenyl-2,4-disulfonic acid (PPADS), attenuated the reflex (10). Likewise, injection of adenosine into the arterial supply of the triceps surae muscles did not evoke a pressor reflex, whereas injection of either ATP or α,β-methylene ATP did (8, 20, 22, 23).

The afferent arm of the exercise pressor reflex is comprised of group III fibers, many of which are mechanically sensitive, and group IV fibers, many of which are metabolically sensitive (15, 26). Group IV afferents as well as group III afferents conducting impulses at <4 m/s are stimulated by P2 receptor agonists, whereas group III afferents conducting impulses at 4 m/s or more are not stimulated by these agonists (9, 35). These findings raise the possibility that P2 receptors stimulate metabolically sensitive thin fiber afferents but have no effect on the discharge of mechanically sensitive thin fiber afferents.

Recent studies, however, have shown that PPADS, a P2 receptor antagonist, attenuated the pressor response to stretch, a maneuver that stimulates mechanoreceptors but has little effect on the discharge of metaboreceptors (10). Stretch, however, has been shown to stimulate different populations of group III mechanoreceptors than did static contraction, the stimulus that evokes the exercise pressor reflex (12). Consequently, the finding that PPADS attenuates the pressor response to muscle stretch may not be applicable to the mechanoreceptor component of the exercise pressor reflex.

The mechanical component of the exercise pressor reflex is assessed during the first 2–5 s of static contraction, because group III mechanoreceptors often discharge explosively in response to the sudden tension developed during the first seconds of contraction (15). Moreover, metaboreceptors respond minimally to contraction during the first 2–5 s of its onset (15, 16). Arterial blood pressure is not a useful index of the mechanical component of the exercise pressor reflex because vascular smooth muscle has a slow time constant, making it uncertain whether mechanoreceptors were responsible for any increase in arterial pressure. In contrast, renal sympathetic nerve activity is a useful index of this component because it responds within the first 2–5 s of static contraction (17, 43). In the studies to be described, we have determined the effect of P2 receptor blockade in the triceps surae muscles on the renal sympathetic nerve response to static contraction in decerebrated cats. For purposes of comparison, we have also determined the effect of this blockade on the renal sympathetic nerve response to stretch.

METHODS

General. All procedures were reviewed and approved by both the Institutional Care and Use Committees of the University of California, Davis, and the Pennsylvania State University College of Medicine.

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Adult cats of either sex (n = 15; 3.3 ± 0.5 kg) were initially anesthetized with a mixture of 5% halothane and oxygen. The right jugular vein and common carotid artery were cannulated for the delivery of drugs and fluids and for the measurement of 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 by a Gould Biotach amplifier. 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). PCO₂ 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 throughout each experiment and maintained at 37–38°C by a water-perfused heating pad.

The right common iliac artery and vein were isolated, and snares were placed around these vessels to trap the PPADS in the leg (see below). The right popliteal artery was isolated for the injection of PPADS, the cat was placed in a Kopf stereotaxic and spinal unit. The calcaneal bone was cut, and its tendon was attached to a force transducer (model FT-10C, Grass) for measurement of the tension developed during stretch and static contraction of the right triceps surae muscles. The knee joint was sutured to a post to secure the right lower limb.

The cats were decerebrated at the mid-collicular level under halothane anesthesia. Just before the decerebration procedure, we tied off the left common carotid artery to reduce bleeding and injected dexamethasone (0.4 mg) intravenously to minimize brain edema. All neural tissue rostral to the mid-collicular section was removed, and the cranial vault was filled with agar. The lungs were then ventilated with room air.

The left renal nerve was exposed using a retroperitoneal approach while the cat was positioned in the Kopf stereotaxic frame and spinal unit. The nerve was suspended in a pool of warm (37°C) mineral oil.

| Table 1. Cardioaccelerator responses to static contraction and tendon stretch |
|-------------------------------------------------|-----------------|-----------------|-----------------|
|                                   | Baseline, | Peak, | Baseline, | Peak, |
|                                   | beats/min | beats/min | beats/min | beats/min |
| Contraction                        | 197 ± 18   | 207 ± 18* | 214 ± 15   | 219 ± 15   |
| Tendon stretch                     | 199 ± 15   | 208 ± 15* | 212 ± 13   | 219 ± 13*  |

Values are means ± SE. PPADS attenuated the pressor responses to contraction (n = 10) but not to tendon stretch (n = 15). *Peak responses to either contraction or tendon stretch were significantly different from baseline (P < 0.05).
and then cut; its central end was draped over a monopolar hook electrode attached in series with a high-impedance probe (model HIP 511, Grass) and then amplified (model P511, Grass). Renal sympathetic nerve activity (RSNA) was displayed on a storage oscilloscope (Hewlett-Packard) and made audible. The amplifier was filtered between 100 Hz and 3 kHz.

Table 2. Tension time indexes (TTI) and peak developed tensions (Dev Ten) for static contraction and tendon stretch

<table>
<thead>
<tr>
<th></th>
<th>Before PPADS</th>
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<th>After PPADS</th>
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<tr>
<td></td>
<td>TTL kg/s</td>
<td>Peak Dev Ten, kg</td>
<td>TTL kg/s</td>
<td>Peak Dev Ten, kg</td>
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<tr>
<td>Contraction</td>
<td>123±11</td>
<td>2.6±0.2</td>
<td>139±16</td>
<td>3.0±0.2</td>
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<tr>
<td>Tendon stretch</td>
<td>152±16</td>
<td>3.5±0.3</td>
<td>151±13</td>
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Values are means ± SE. Note that there were no pair wise comparisons of tension time indexes (TTIs) or peak developed tensions (Peak Dev Ten) that were significantly different from each other (P > 0.05).

RESULTS

PPADS (10 mg/ kg) injected into the popliteal artery attenuated the peak pressor responses to both static contraction and tendon stretch (Fig. 1). Likewise, PPADS attenuated the renal sympathetic nerve responses to both maneuvers when these
responses were integrated over their 60-s duration (Fig. 2). Before PPADS injection, the cardioacceleratory responses to either static contraction or tendon stretch were modest, and PPADS injection had no significant effect on them (P > 0.05; Table 1). For both static contraction and tendon stretch, the TTIIs were not significantly different from each other (P > 0.05) before and after PPADS injection (Table 2). In every experiment, RSNA was synchronous with the arterial pressure pulse and displayed an expiratory rhythm (Fig. 3). At the end of each experiment, intravenous injection of hexamethonium bromide (20 mg/kg) abolished the activity recorded from each renal nerve, confirming that the activity originated from sympathetic postganglionic fibers.

Before PPADS, the onset of the RSNA nerve response to either static contraction or tendon stretch was rapid, occurring within 2 s of the onset of either maneuver (Fig. 4). Moreover, the increase in RSNA was sustained throughout the 60-s period of either contraction or stretch. The effect of PPADS on the time course of the RSNA response to contraction was somewhat different from that of PPADS on the time course of the RSNA response to stretch. For contraction, the start of the attenuation was significant at the 2-s time point and continued for each time point thereafter (Fig. 4). For tendon stretch, the attenuation started at its 10-s time point and continued for each of the remaining time points except for the one at 30 s (Fig. 4).

PPADS (10 mg/kg), injected into the popliteal artery, had no effect on the pressor, cardioacceleratory, and renal sympathetic nerve responses to electrical stimulation of the tibial nerve in three paralyzed cats. Specifically, before PPADS injection, electrical stimulation increased mean arterial pressure and heart rate by 45 ± 4 mmHg and 13 ± 5 beats/min, respectively; after injection, stimulation increased arterial pressure and heart rate by 44 ± 5 mmHg and 21 ± 4 beats/min, respectively (n = 3). Likewise, before PPADS injection, stimulation increased renal sympathetic nerve activity by 62 ± 13%; after injection, it increased activity by 106 ± 21% (n = 3). In two of the three cats, we also measured the pressor, cardioaccelerator, and renal sympathetic responses to stretch before and after PPADS. We found that PPADS attenuated these responses in each of the two cats tested. Specifically, the pressor and renal sympathetic responses to stretch before PPADS averaged 28 ± 11 mmHg and 30 ± 6%, respectively; after PPADS, they averaged 19 ± 7 mmHg and 8 ± 0.3%, respectively (n = 2).

**DISCUSSION**

The most important finding from our experiments is that blockade of P2 receptors in skeletal muscle attenuated the rapid renal sympathetic nerve responses to static contraction. Specifically, the attenuation occurred after only 2 s of static contraction, and as a consequence can be attributed to a decrease in group III mechanoreceptor input. From a statistical standpoint, blockade of P2 receptors did not attenuate group III mechanoreceptor input to tendon stretch until the maneuver lasted 10 s. Nevertheless, blockade of P2 receptors did attenuate the overall RSNA response to tendon stretch as well as the responses at all but one time point after 10 s. As with contraction, these effects can only be attributed to a removal of group III mechanoreceptor input.

PPADS infused into the dorsal horn of cats has been shown to attenuate the exercise pressor reflex (6). This finding raised the possibility that, when PPADS was injected into the popliteal artery in our experiments, it traveled to the dorsal horn to exert its effects. To control for this possibility, we stimulated the tibial nerve in paralyzed cats before and after injecting PPADS into the popliteal artery. The injection procedure was identical to that used when the triceps surae muscles were contracted. We found that PPADS injection had no effect on the pressor, cardioaccelerator, or renal sympathetic nerve responses to electrical stimulation of the tibial nerve. These findings suggest that the attenuating effects of PPADS on the pressor and sympathetic responses to contraction and stretch were not caused by circulation of this purinergic antagonist to the spinal cord or brain stem.

The terminals of sympathetic postganglionic fibers (2) and the skeletal muscle cells (19) are the two most likely sources of ATP release in contracting skeletal muscle. Recently, Li et al. (19) found that neuromuscular blockade prevented ATP release into the skeletal muscle interstitium during stimulation of the ventral roots. In contrast, section of the dorsal roots, which

**Fig. 4.** Time course of the renal sympathetic nerve response to static contraction (top) and tendon stretch (bottom). Note that each maneuver lasted for 60 s. ○, Mean responses before PPADS (10 mg/kg) was injected into the popliteal artery; ●, mean responses after PPADS was injected. Vertical brackets are standard errors, and asterisk indicates a significant difference (P < 0.05) between before PPADS and after PPADS. Also note that RSNA is expressed as the percent change from its baseline value. Value at time 0 represents baseline and is expressed as 0% change in RSNA. Note that the first symbol after time 0 represents mean activity 2 s after the start of either static contraction or stretch. Subsequent symbols represent mean activity at 5-s intervals.
Prevented the contraction-induced reflex stimulation of sympathetic postganglionic fibers, had no effect on ATP release (19). In addition, Li et al. found that the interstitial concentrations of ATP increased linearly with developed tension in the contracting skeletal muscle.

P2 receptors have two subtypes: the P2X, which is a cation channel, and the P2Y, which is G-protein coupled (3). The available evidence suggests that PPADS does not clearly distinguish between the two P2 receptor subtypes in its blocking activity (18). PPADS, however, does not block P1 receptors (18). We note with interest that the P2X receptor agonist a,β methylene ATP stimulated group IV muscle afferents as well as evoked a reflex pressor response when injected into the arterial supply of hind limb muscle (8, 9, 20). Presently, there is no information about the reflex effects evoked by P2Y receptor agonists when injected into the arterial supply of skeletal muscle, although there is a report that the P2Y2,4 agonist, UTP, stimulated thin-fiber cutaneous afferents (42). Consequently, the relative roles played by P2X and P2Y receptors in evoking the ATP-induced muscle chemoreflex are an unresolved issue.

ATP, lactic acid, bradykinin, and arachidonic acid are produced by contracting skeletal muscle (19, 21, 36, 41). Lactic acid, bradykinin, and cyclooxygenase metabolites of arachidonic acid have been shown to sensitize group III afferents to static contraction and tendon stretch (27, 37, 38). In contrast, information is lacking about whether ATP sensitizes these thinly myelinated muscle afferents to these stimuli. Of interest is the fact that lactic acid, bradykinin, and cyclooxygenase metabolites of arachidonic acid have been found to stimulate directly some group III afferents with conduction velocities in the range of 10–30 m/s (27, 37, 38). In contrast, the ATP analog, α,β methylene ATP, had no effect on the discharge of group III afferents with conduction velocities over 4 m/s (9). Consequently, if electrophysiological evidence was found that P2 agonists sensitized group III afferents to contraction and stretch, as the reflex evidence suggests is the case, ATP would be the first substance that has this action without also stimulating these thin-fiber afferents when administered exogenously.

The validity of tendon stretch to determine the mechanical component of the exercise pressor reflex is an important issue. Tendon stretch is easy to perform, does not stimulate metabolism in the muscles, and evokes repeatable reflex pressor responses (40) that can be attenuated by gadolinium (11, 25, 39), an agent that blocks mechanogated channels. The problem with tendon stretch is that it does not stimulate the same population of group III muscle afferents as does static contraction (12), which is the defining stimulus for the exercise pressor reflex (30). Specifically, Hayes et al. (12) found that the group III afferents stimulated by tendon stretch conducted impulses, on average, at 16.7 ± 1.5 m/s (n = 14), whereas those stimulated by static contraction conducted impulses, on average at 11.6 ± 1.6 m/s (n = 18; P = 0.03).

Some parallels can be drawn between the responses of golgi tendon organs (i.e., group Ib) and group III afferents to contraction and stretch. Like Golgi tendon organs, some group III afferents respond vigorously to contraction, but only weakly if at all to stretch. Houk and Henneman (14) suggested that contractile elements in muscle, contributing to its elasticity, decreased the tension impinging onto Golgi tendon organs during stretch. This elastic factor was not present during contraction, thereby resulting in this stimulus to be more effective than stretch in stimulating group Ib afferents. If this situation is also found to exist for group III afferents, then the specific response of a group III afferent to contraction and stretch might depend on the orientation of its ending in the contractile elements of the muscle.

The findings by Hayes et al. in cats (12) need to be considered in conjunction with those by Coote and Perez-Gonzalez (4) who, in the same species, found that electrical stimulation of gastrocnemius muscle afferents conducting impulses at 15 m/s or more reflexly decreased sympathetic discharge, whereas electrical stimulation of gastrocnemius afferents conducting impulses at <15 m/s reflexively increased sympathetic discharge (4). The report by Coote and Perez-Gonzalez (4) might provide an electrophysiological basis for the fact that tendon stretch often evokes a smaller pressor response than does static contraction. Specifically, tendon stretch stimulated a population of group III muscle afferents, whose reflex effect was to decrease sympathetic activity; it also had little effect on the discharge of group IV muscle afferents, whose reflex effect was to increase sympathetic activity (15).

Our understanding about the role played by group III mechanoreceptors in the exercise pressor reflex in humans has been evolving. Originally, this role was thought to be small, if not nonexistent, because muscle sympathetic nerve activity did not increase for the first 30 s of static handgrip (24). Subsequently, signal averaging techniques showed that static quadriceps exercise increased muscle sympathetic nerve activity with an onset of only 4–6 s (13), a latency that suggested mechanoreceptor activation. In addition, group III mechanoreceptors have been implicated in the cardioaccelerator and renal vasconstrictor responses to passive and actual exercise in healthy humans (7, 32–34). Recently, the role played by group III mechanoreceptors in evoking the renal vasconstrictor component of the exercise pressor reflex has been shown to be even more important in heart failure than in health (28, 29, 31).

In summary, we have presented evidence that ATP release during static contraction plays a role in the reflex stimulation of renal sympathetic component of the exercise pressor reflex. The specific function of the contraction-induced RSNA is not clear and must be defined by measurements of renal function. These measurements should include renal vascular resistance, sodium excretion, and renin production (5).

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GRANTS

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REFERENCES

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PPADS AND MECHANOREFLEX