Purinergic 2X receptors play a role in evoking the exercise pressor reflex in rats with peripheral artery insufficiency

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Stone AJ, Yamauchi K, Kaufman MP. Purinergic 2X receptors play a role in evoking the exercise pressor reflex in rats with peripheral artery insufficiency. Am J Physiol Heart Circ Physiol 306: H396–H404, 2014. First published November 27, 2013; doi:10.1152/ajpheart.00762.2013.—Purinergic 2X (P2X) receptors on the endings of thin fiber afferents have been shown to play a role in evoking the exercise pressor reflex in cats. In this study, we attempted to extend this finding to decerebrated, unanesthetized rats whose femoral arteries were either freely perfused or were ligated 72 h before the start of the experiment. We first established that our dose of pyridoxal phosphate-6-azophenyl-2',4'-disulfonic acid (PPADS; 10 µg/kg), a P2X receptor antagonist, attenuated the pressor response to α,β-methylene ATP (10 µg/kg), a P2X receptor agonist. We then compared the exercise pressor reflex before and after infusing PPADS into the arterial supply of the hindlimb muscles that were statically contracted. In rats with freely perfused femoral arteries, the peak pressor responses to contraction were not significantly attenuated by PPADS (before PPADS: 19 ± 2 mmHg, 13 min after PPADS: 17 ± 2 mmHg, and 25 min after PPADS: 17 ± 3 mmHg). Likewise, the cardioacceleratory and renal sympathetic nerve responses were not significantly attenuated. In contrast, we found that in rats whose femoral arteries were ligated PPADS significantly attenuated the peak pressor responses to contraction (before PPADS: 37 ± 5 mmHg, 13 min after PPADS: 27 ± 6 mmHg, and 25 min after PPADS: 25 ± 5 mmHg; P < 0.05). Heart rate was not significantly attenuated, but renal SNA was at certain time points over the 30-s contraction period. We conclude that P2X receptors play a substantial role in evoking the exercise pressor reflex in rats whose femoral arteries were ligated but play only a minimal role in evoking the reflex in rats whose femoral arteries were freely perfused.

thin fiber muscle afferents; renal sympathetic activity; PPADS; α,β-methylene ATP; autonomic nervous system

THE EXERCISE PRESSOR REFLEX arises from contracting skeletal muscles and functions to increase mean arterial pressure, heart rate, and peripheral vascular resistance (5, 28). The sensory arm of the reflex is comprised of thinly myelinated group III afferents, which are primarily mechanosensitive, and unmyelinated group IV afferents, which are primarily metabosensitive (17, 18, 28, 31). The distinction between mechanosensitive and metabosensitive is by no means total. For example, group IV afferents can be stimulated by mechanical distortion of their receptive fields, although the force applied is usually in the noxious range (18, 31). In addition, group III afferents can be stimulated by injection of putative muscle metabolites such as ATP and bradykinin; moreover, their responses to contraction, which mechanically distort their receptive fields, can be potentiated by muscle metabolites, such as bradykinin, cyclooxygenase metabolites of arachidonic acid, and ATP (11, 17, 30, 35, 36).

Several lines of evidence indicate that purinergic compounds, especially ATP, play a role in eliciting the exercise pressor reflex. Specifically, in both rats and dogs, the concentration of ATP in the muscle interstitial space is increased by static contraction (23, 32). The source of the ATP found in the interstitial space of contracting muscle is unclear, but it most likely includes contracting myocytes, sympathetic postganglionic nerve endings, and red blood cells (7, 22). In addition, injection of ATP or its analogs into the arterial supply of skeletal muscle evokes reflex pressor, cardioaccelerator, and renal sympathetic nerve responses (9, 24). Moreover, injection of ATP or its analogs has been shown to stimulate both group III and IV muscle afferents in both cats and rats (11, 35).

When considered together, these studies suggest that P2X receptors play an important role in the generation of the exercise pressor reflex. Specifically, in both rats and dogs, the concentration of ATP in the muscle interstitial space is increased by static contraction (41). The exercise pressor reflex has been found to be greater in rats whose femoral artery was ligated for 72 h than in rats whose femoral artery was freely perfused (25). Likewise, the exercise pressor reflex on the endings of the group III and IV afferents has been shown to attenuate the pressor and cardioaccelerator responses to both injection of ATP analogs as well as to static contraction in decerebrated unanesthetized cats (10, 24). In contrast, blockade of purinergic 1 (P1) receptors had no effect on the reflex pressor and cardioaccelerator responses to either ATP injection or static contraction (10, 24).

P2X receptors have been identified on dorsal root ganglion (DRG) cells innervating hindlimb skeletal muscles of rats (25, 46). Moreover, the concentration of P2X receptors in these cells has been shown to be increased by ligating the ipsilateral femoral artery for 72 h (4, 46). In addition, the pressor response to femoral arterial injection of α,β-methylene ATP, a P2X receptor agonist, has been found to be greater in rats whose femoral artery was ligated for 72 h than in rats whose femoral artery was freely perfused (25). Likewise, the exercise pressor reflex has been found to be greater in rats whose femoral arteries were ligated 72 h before the experiment than in rats whose femoral arteries were either freely perfused or were ligated only 3 min before the contraction was initiated (41). When considered together, these findings suggest that P2X receptors play an important role in the generation of the exercise pressor reflex in rats whose femoral arteries are either freely perfused or ligated. We were prompted, therefore, to test the hypotheses that blockade of P2X receptors attenuated the exercise pressor reflex in both decerebrated unanesthetized rats whose arterial supply to their contracting hindlimb muscles was intact as well as in decerebrated unanesthetized rats whose arterial supply to their contracting muscles was ligated for 72 h before the start of the experiment.

METHODS

All procedures were reviewed and approved by the Institutional Animal Care and Use Committee of the Hershey Medical Center of Pennsylvania.
Pennsylvania State University. Adult male Sprague-Dawley rats (n = 47; average weight was 454 ± 8 g) were used in these experiments. The rats were housed in a temperature-controlled room (24 ± 1°C) with a 12:12-h light-dark cycle and fed a standard diet and tap water ad libitum. In 24 rats, we ligated the left femoral artery 72 h before the experiment. Briefly, rats were anesthetized with isoflurane (2–3%) in 100% oxygen. Under sterile procedure, the left femoral artery was surgically exposed and ligated with suture (5–0, silk) just distal to the inguinal ligament. The incision was closed with surgical staples, and the rat was monitored for at least an hour to ensure that normal awareness and behavior had returned before it was returned to animal facility. Rats were then allowed to recover for 72 h. This technique has been shown to reduce blood flow capacity to about 10–20% of its exercise-induced maximum, while having little effect on resting blood flow (33, 47).

**Surgical preparation.** On the day of the experiment, rats were anesthetized with isoflurane gas (2–3%) in oxygen. The trachea was cannulated, and the lungs were mechanically ventilated with the gas anesthetic until the decerebration was performed. Both carotid arteries and a jugular vein were cannulated (PE-50) to measure arterial blood pressure and to administer drugs and fluids, respectively. Although both carotid arteries were ligated, the aortic baroreceptors were undisturbed and still fully functional. Arterial blood gases and pH were measured using an automated blood gas analyzer (ABL 8, Radiometer). PCO2 and arterial pH were maintained within normal ranges by adjusting ventilation and oxygen through an intravenous administration of sodium bicarbonate (8.5%). Body temperature was maintained between 36.5 and 38.0°C by an isothermal heating pad and lamp. Arterial blood pressure was measured by attaching one carotid cannula to a Statham P23XL strain gauge. Heart rate was calculated beat to beat by a Gould Biotach. The left calcaneal bone was sectioned and then attached to a force transducer (FT-10, Grass) to measure tension developed by the statically contracting triceps surae muscles.

In all rats, the tip of one of the carotid arterial catheters was advanced so that it was situated in the abdominal aorta just before it bifurcated into the common iliac arteries. The location of the tip was confirmed post mortem. In all rats, including those whose femoral arteries were ligated and those whose femoral arteries were patent, we confirmed post mortem. In all rats, including those whose femoral arteries were ligated. In a second series of experiments, we followed the same protocol described above except that sterile water, the vehicle for PPADS, was slowly infused instead. During the infusion of PPADS and sterile water as well as during the injection of α,β-methylene ATP, we tightened the snare placed around the right external iliac artery and vein in an attempt to direct the infused or injectate into the arterial circulation of the left hindlimb. A CMA 402 microdialysis pump was used to perform the infusions. The snare placed around the right external iliac artery and vein was released immediately after the completion of the infusion. Injection of α,β-methylene ATP into the abdominal aorta stimulated P2X3 receptors on all hindlimb sensory nerves, including those innervating skin, muscle, joint, and bone. Our purpose of injecting α,β-methylene ATP was to challenge the blockade of P2X receptors by PPADS to demonstrate the effectiveness of PPADS as an antagonist; our purpose was not to selectively stimulate thin fiber muscle afferents.

Once we had established that the dose of PPADS was effective in blocking the pressor response to α,β-methylene ATP, we examined its effect on the exercise pressor reflex in a separate group of rats. The

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**Experimental protocols.** Our first task was to establish that the dose of PPADS (10 mg/kg; 200 μl) used was effective in blocking P2X receptors. To accomplish this task, we measured the pressor responses to abdominal arterial injection of α,β-methylene ATP (10 μg/kg; 100 μl) before and after PPADS. Each injection of α,β-methylene ATP was performed as a bolus and required ~2 s to complete. PPADS was slowly infused (20 μl/min) over a 10-min period into a carotid arterial catheter whose tip was placed in the abdominal aorta near its bifurcation into the external iliac arteries. α,β-Methylene ATP was first injected before PPADS and then injected again at 13 min and at 25 min after the start of PPADS infusion. This protocol was performed in seven rats whose femoral arteries were freely perfused as well as in six rats whose femoral arteries were ligated. In a second series of experiments, we followed the same protocol described above except that sterile water, the vehicle for PPADS, was slowly infused instead.

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![Fig. 1. Efficacy of the P2X receptor blockade in freely perfused rats. Pyridoxal phosphate-6-azophenyl-2,4-disulfonic acid (PPADS; 10 mg/kg; A) significantly attenuated the pressor response to α,β-methylene ATP (10 μg/kg), whereas sterile water (B), the vehicle, had no effect. MAP, mean arterial pressure. *P < 0.05, significantly smaller pressor response from the pressor response before PPADS.](http://ajpheart.physiology.org/Downloadedfrom/10.1152/ajpheart.00762.2013)
left hindlimb muscles were statically contracted for 30 s by electrically stimulating (40 Hz; 0.1 ms) the cut peripheral ends of the L4 and L5 ventral roots. After waiting 10 min, we then slowly infused PPADS (10 mg/kg) into the carotid arterial catheter whose tip was placed in the abdominal aorta near its bifurcation. The infusion period was 10 min, identical to that used by Wang et al. (44), and it was followed by a 3-min interval after which the left hindlimb muscles were contracted again. This contraction is referred to as 13 min after PPADS. Subsequently, we contracted the hindlimb muscles at 25 min after the start of the infusion, and we referred to this contraction as 25 min after PPADS. Twenty-five minutes was chosen arbitrarily to add a second time point to shed some light on the time course of the antagonistic effect of PPADS on P2 receptors. All infusions were done with a CMA microdialysis pump. In addition, the snare placed around the

Fig. 2. Peak pressor (A) and cardioaccelerator (B) responses to static contraction in freely perfused rats. C: increases in integrated renal sympathetic nerve activity (RSNA) averaged over the 30-s contraction period before and after infusion of PPADS (10 mg/kg). D: tension-time indexes (TTIs) after PPADS were not significantly different from those before PPADS. PPADS had no significant effect ($P > 0.05$) on the peak pressor, cardioaccelerator and renal nerve responses to static contraction in freely perfused rats. Likewise, PPADS had no effect on baseline mean arterial pressure or heart rate, the levels of which are shown inside the bars. Each of the increases for the pressor, cardioaccelerator, and integrated renal sympathetic nerve responses to contraction were significantly greater than their corresponding baseline values ($P < 0.05$).

Fig. 3. Time courses of the average changes in pressor and renal sympathetic nerve responses to static contraction in freely perfused rats before and 13 min after PPADS infusion ($A$ and $B$) as well as those before and 25 min after PPADS infusion ($C$ and $D$). *$P < 0.05$, significant post hoc difference at 17 s between pressor responses before PPADS and 13 min after PPADS.
right external iliac artery and vein was tightened during each infusion and was released 5 min after its completion.

Data analysis. In all experiments, baseline as well as reflex changes in mean arterial pressure, heart rate, renal sympathetic nerve activity (RSNA), and developed tension were recorded continuously with a Spike 2 data acquisition system (CED, Cambridge) and stored on a computer hard drive (Dell). The data were analyzed in two ways. In the first, the peak pressor and cardioaccelerator responses to static contraction were compared before and after PPADS. In addition, renal sympathetic nerve activity was integrated and then summed for the entire 30-s contraction period and expressed as a percentage increase over the immediate 30-s period preceding contraction. In the second method, the time courses of the pressor and percent change in the integrated renal nerve responses were analyzed. Specifically, we plotted the pressor and integrated renal nerve responses for each second of the 30-s static contraction period and then compared them before and after PPADS. Mean arterial pressure is expressed in millimeters mercury and heart rate is in beats per minute. The tension-time index was calculated by integrating the area between the tension trace and the baseline level and is expressed in kilogram seconds.

All values are expressed as means ± SE. Statistical comparisons were performed with either a one- or two-way repeated-measures ANOVA. If the overall F value was significant, post hoc tests were performed with the Holm-Sidak’s tests between individual means. The criterion for statistical significance was set as P < 0.05.

RESULTS

Freely perfused. We first established that the dose of PPADS (10 mg/kg) used in our experiments blocked the pressor response to α,β-methylene ATP (10 μg/kg). Both substances were given through the carotid arterial catheter whose tip was placed in the abdominal aorta near its bifurcation. In eight rats whose left femoral arteries were freely perfused, we found that PPADS significantly attenuated the pressor response to α,β-methylene ATP by more than half at 13 and 25 min after the start of the infusion of the purinergic receptor antagonist (P < 0.05; Fig. 1). In addition, we found that injection of sterile water, which was the vehicle for PPADS, had no effect on the pressor response to α,β-methylene ATP (Fig. 1). The cardioaccelerator responses to α,β-methylene ATP were small, averaging no more than four beats per minute, and therefore were not analyzed.

We next determined the effect of PPADS (10 mg/kg) on the exercise pressor reflex in 11 rats whose hindlimbs were freely perfused. None of the 11 rats were used in the experiments in which we injected α,β-methylene ATP into the abdominal aorta. Using the first method of analysis, we found that PPADS had no effect on the peak pressor and cardioaccelerator responses to static contraction of the hindlimb muscles in these 11 rats; this was the case at both 13 and 25 min after the start of the PPADS infusion, time points which were identical to those used to establish the efficacy of the P2 receptor blockade (Fig. 2). In addition, PPADS had no effect on RSNA when it was summed over the 30-s contraction period (Fig. 2). This too was the case at both 13 and 25 min after the start of the infusion. When viewed on individual basis, PPADS at 13 min attenuated the peak pressor component of the exercise pressor reflex by at least 5 mmHg in 5 of the 11 rats tested; likewise PPADS at 25 min attenuated the peak pressor component of the reflex by at least 5 mmHg in 4 of the 11 rats tested.

Using the second method of analysis, we found that the main effect for PPADS 13 min after the start of its infusion had no significant effect (P = 0.39) on the time course of the pressor response to static contraction in “freely perfused rats” (Fig. 3A). Nevertheless, the overall interaction between PPADS and the contraction period of 30 s for the pressor response 13 min after PPADS infusion was significant (P = 0.003). Post hoc tests, however, revealed only one significant point in time, namely at 17 s (Fig. 3A). Neither the main effect (P = 0.82) nor the overall interaction (P = 0.78) was significant for the time course of the RSNA response to contraction 13 min after the start of PPADS infusion (Fig. 3B).

When analyzed as a main effect, PPADS, 25 min after the start of infusion, had no significant effect on the time course of either the pressor (P = 0.15) or RSNA responses to static contraction (P = 0.14) in freely perfused rats. Likewise, when analyzed for the interaction, PPADS 25 min after the start of infusion had no significant effect on the time course of either the pressor (P = 0.66) or RSNA (P = 0.90) responses to contraction in these rats (Fig. 3, C and D).

Ligated. In six rats whose femoral arteries were ligated for 72 h before the start of the experiments, we established that the dose of PPADS (10 mg/kg) used in our experiments attenuated the pressor responses to α,β-methylene ATP (10 μg/kg). Both substances were given through the carotid arterial catheter whose tip was placed in the abdominal aorta near its bifurcation. PPADS significantly attenuated the pressor response to α,β-methylene ATP by more than half at 13 and 25 min after infusion of the purinergic receptor antagonist (P < 0.05; Fig. 4). The vehicle for PPADS, namely sterile water, had no

Fig. 4. Efficacy of the P2X receptor blockade in ligated rats. PPADS (A; 10 mg/kg) significantly attenuated the pressor response to α,β-methylene ATP (10 μg/kg), whereas sterile water (B), the vehicle, had no effect. *P < 0.05, significantly smaller pressor response from the pressor response before PPADS.
effect on the pressor response to α,β-methylene ATP (Fig. 4). The cardioaccelerator responses to α,β-methylene ATP were small averaging no more than four beats per minute and therefore were not analyzed.

We then determined the effect of PPADS (10 mg/kg) on the exercise pressor reflex in 10 rats whose femoral arteries were ligated 72 h before the start of the experiment. None of the 10 rats were used in the experiments in which we injected α,β-methylene ATP into the abdominal aorta. Using the first method of analysis, we found that PPADS significantly attenuated (P < 0.05) the peak pressor responses to static contraction of the hindlimb muscles. This was the case both at 13 and 25 min after PPADS infusion, time points that were identical to those used to establish the efficacy of the P2 receptor blockade (Fig. 5). PPADS had no significant effect on the peak cardioaccelerator response or the renal sympathetic nerve response to static contraction when it was summed over the 30-s contraction period. When viewed on individual basis, PPADS at 13 min attenuated the peak pressor component of the exercise pressor reflex by at least 5 mmHg in 7 of the 10 ligated rats tested; likewise PPADS at 25 min attenuated the peak pressor component of the reflex by at least 5 mmHg in 8 of the 10 ligated rats tested.

The possibility existed that PPADS, infused into the abdominal aorta over a 10-min period, circulated to the spinal cord or brainstem to exert its attenuating effect on the exercise pressor reflex. To control for this possibility, we infused PPADS (10 mg/kg) into the vena cava in four rats whose femoral arteries were ligated. We found that intravenous infusion of PPADS had no effect on the exercise pressor reflex (Fig. 6).

Using the second method of analysis and when analyzed as a main effect, we found that PPADS, 13 min after the start of its infusion, significantly attenuated the time course of the pressor response to static contraction over the 30-s contraction period (P = 0.02; Fig. 7A). Likewise, the overall interaction was significant (P = 0.04). Post hoc analysis of the interaction revealed that PPADS had an attenuating effect on the pressor response during 16 of the 30 s of static contraction (Fig. 7A). We also found that neither the main effect (P = 0.15) for PPADS nor its interaction (P = 0.60) over time was significant for RSNA response to contraction 13 min after the start of infusion of the P2X receptor antagonist (Fig. 7C).

When analyzed as a main effect, PPADS, 25 min after the start of infusion, significantly attenuated the time course of the pressor response to static contraction in the “ligated rats” (P = 0.01; Fig. 7B). The overall interaction between PPADS and the 30-s contraction period for the pressor response was not significant (P = 0.38; Fig. 7D). The main effect for the RSNA response to static contraction in the “ligated rats” was not significant (P = 0.45), but the interaction between PPADS and the 30-s contraction period was (P < 0.004; Fig. 7D). Post hoc analysis of the overall interaction revealed only one significant time point, namely at 2 s (Fig. 7D).

**Difference in exercise pressor reflex between freely perfused and ligated rats.** Before infusing PPADS, a P2 receptor antagonist, we found that the pressor, cardioaccelerator, and renal sympathetic nerve responses to static contraction of the left hindlimb muscles were significantly greater in rats with ligated femoral arteries (n = 10) than in rats with patent (i.e., freely perfused) femoral arteries (n = 11; Figs. 2, 3, 5, and 6).

*An index of mechanoreceptor activity.* PPADS had a significant effect on RSNA activity during the first 5 s of the contraction period in rats with ligated femoral arteries. In contrast, the antagonist had no significant effect on RSNA during the first 5 s of the contraction period in the rats with freely perfused arteries (Fig. 8).
DISCUSSION

There are two types of purinergic receptors. The first, termed P1, is stimulated by adenosine and plays no role in eliciting the exercise pressor reflex in either cats (10, 27) or humans (26). The second, termed P2, is stimulated by ATP, and its blockade by PPADS in cats (10, 19, 20) and by pyridoxine in humans (6) attenuated the exercise pressor reflex in either cats (10, 27) or humans (26). P2 receptors have been further classified into X and Y, with the former being ligand gated and the latter being G-protein gated (3). Chemical stimulation of somatic afferent P2X receptors in vivo evokes a reflex pressor response, whereas chemical stimulation of P2Y receptors does not (14). Consequently, P2X receptors are believed to be responsible for evoking the component of the exercise pressor reflex attributable to increases in the concentration of ATP in the interstitium of exercising muscle (9, 10, 14).

We found that PPADS-induced blockade of P2X receptors had minimal effect on the exercise pressor pressor reflex in decerebrated rats with patent femoral arteries. In contrast, PPADS substantially reduced the reflex in decerebrated rats whose femoral arteries were ligated 72 h before the start of the experiment. The attenuating effect of PPADS on the exercise pressor reflex in the ligated rats was not caused by its circulation to either the spinal cord or brainstem because intravenous injection of the P2X receptor antagonist had no effect on the pressor responses to static contraction of the hindlimb muscles. The most likely explanation for our findings is that PPADS attenuated the exercise pressor reflex by blocking P2X receptors on the endings of group III and IV muscle afferents in rats whose femoral arteries were ligated.

Previously we reported that PPADS attenuated the exercise pressor reflex in decerebrated cats with freely perfused hindlimbs, whereas in the present study we report that the antagonist had little if any effect on the reflex in decerebrated rats with freely perfused hindlimbs (10, 20). One explanation for this difference may involve the number of purinergic receptors found on thin fiber afferents innervating the hindlimb muscles in rats and cats. Immunocytochemical methods have shown that there are few P2X3 receptors on the DRG cells innervating the hindlimb muscles of rats (2). Unfortunately, the number of P2X3 receptors on DRG cells innervating the hindlimb muscles of cats is not known and, as a consequence, our explanation must be considered to be speculation.

Blockade of P2X receptors with PPADS in our experiments was much more effective in attenuating the exercise pressor reflex in rats with ligated femoral arteries than it was in attenuating the reflex in rats with freely perfused arteries. This may have been caused by an occlusion-induced increase in the number of P2X receptors on the thin fiber muscle afferents evoking the reflex. For example, using immunocytochemistry, Xing et al. (46) found that occluding the femoral artery for 24 h increased the number of P2X3 receptors in DRG cells innervating the hindlimb muscles of rats (2). Unfortunately, the number of P2X3 receptors on DRG cells innervating the hindlimb muscles of cats is not known and, as a consequence, our explanation must be considered to be speculation.

Group III mechanoreceptors contribute to the afferent arm of the exercise pressor reflex (12, 15, 16, 38). One can determine if a muscle metabolite, such as ATP, sensitized group III mechanoreceptors by measuring the muscle mechanoreflex (40), which is evoked by stretching the calcaneal tendon, before and after giving a purinergic antagonist, such as PPADS. However, calcaneal tendon stretch activates group III mechanoreceptors that are not activated by static contraction (13, 44). Consequently, the usefulness of tendon stretch as a maneuver to stimulate the same group III mechanoreceptors that are stimulated by static contraction is limited. A superior method of determining if ATP sensitized the group III mechanoreceptors participating in the exercise pressor reflex involves the recording of the renal sympathetic nerve component of the reflex before and after giving PPADS. Static contraction in-
increases RSNA within 200 ms of its onset (43), and although speculative we think it is reasonable to attribute any renal sympathetic response to contraction that occurs within the first 2 s of contraction to the stimulation of group III mechanoreceptors (19). The first 2 s of contraction seems to be too short for ischemic metabolites, such as lactic acid and prostaglandins, to be either produced by the working muscles or, if they are produced, for their concentration to be high enough to stimulate group IV metaboreceptors, which require at least 5 and sometimes 30 s to respond to this stimulus (17, 18, 44). In contrast, ATP is released almost instantaneously at the onset of exercise with one possible source being red blood cells, which are mechanically distorted by the contractile process (39). The immediate release of ATP could contribute to the immediate sensitization of mechanoreceptors.

Our findings concerning the effects of PPADS on the first 2 s of the renal sympathetic nerve responses to static contraction in both rats with ligated femoral arteries and in rats with freely perfused femoral arteries can be interpreted with the above rationale in mind. Thus our finding that PPADS did not

Fig. 7. Time courses of the average changes in pressor and renal sympathetic nerve responses to static contraction in ligated rats before and 13 min after PPADS infusion (A and B) as well as those before and 25 min after PPADS infusion (C and D). *P < 0.05, significant post hoc differences between pressor responses before PPADS and 13 min after PPADS.

Fig. 8. Increase in integrated RSNA during the first five s of static contraction in freely perfused rats (A and C) and in ligated rats (B and D) before and 13 min after PPADS infusion (A and B), as well as before and 25 min after PPADS infusion (C and D). *P < 0.05, significant decrease in activity after PPADS at its corresponding time point.
attenuate the renal sympathetic nerve responses to the first 2 s of static contraction in “freely perfused rats” is consistent with the possibility that the number of P2X receptors on the endings of group III mechanoreceptors was not sufficient to allow these thinly myelinated afferents to be sensitized by ATP released into the muscle interstitium during static contraction. In contrast, our finding that PPADS attenuated the renal sympathetic nerve responses to the first 2 s of contraction in “ligated” rats is consistent with the possibility that 72 h of femoral artery occlusion increased the number of P2X receptors on the endings of group III mechanoreceptors to a level that was sufficient to allow them to be sensitized by ATP during contraction. With regard to our latter finding, ligation of a femoral artery for only 24 h has been shown to increase the number of P2X3 receptors on the DRG cells that innervate the hindlimbs of rats (25). Moreover, ligation potentiated the pressor and renal sympathetic nerve responses to femoral arterial injection of α,β-methylene ATP, a P2X3 receptor antagonist (25).

Our finding that PPADS had no effect on the first 15 s of the static contraction period might be viewed as surprising in light of the recent report by Wang et al. (44) that PPADS significantly attenuated the responses of group III mechanoreceptors to static contraction in “sham” rats whose hindlimbs were freely perfused. Our method of infusing PPADS was identical to that used by Wang et al. (44) and therefore cannot serve as an explanation for the lack of consistency between our findings and those of Wang et al. One explanation may be that the PPADS-induced attenuation of group III afferent responses to static contraction reported by Wang et al. (44) was not sufficient to have an effect on the exercise pressor reflex.

The mechanism responsible for ligation-induced augmentation of the exercise pressor reflex remains to be determined. Nevertheless, there is evidence suggesting that growth factors may play an important role. For example, infusion of nerve growth factor (NGF) for 72 h into the femoral triangle of rats increased the number of P2X3 positive cells (45). Furthermore, intrathecal infusion of NGF increased the number of P2X3 positive cells in the L4–6 DRG (45). Additionally, intrathecal infusion of GDNF increased the number of P2X3 positive cells in the lumbar dorsal ganglia of rats (34). Last, intrathecal infusion of GDNF completely reversed the decrease in P2X3 receptor protein expression induced by dorsal root section in primary afferent fibers synapsing in the L4–6 dorsal horns of rats (2). Although these lines of evidence are suggestive of growth factors playing a role in the femoral arterial ligation-induced exaggeration of the exercise pressor reflex in our experiments, it remains to be shown that blockade of NGF or GDNF receptors prevents this exaggeration.

Interpretation of our findings should be tempered with three limitations in mind. First, PPADS displays in vitro some antagonistic activity towards the P2Y receptor (21), a finding that has minimal impact on our conclusions because the P2Y receptor plays little role in evoking the exercise pressor reflex (14). Second, PPADS blocks all P2X receptors, and therefore our conclusions should not be limited to the P2X3 receptor, although this receptor appears to be prevalent on thin fiber somatic afferents (8), and its specific blockade on the peripheral endings of thin fiber afferents in cats has already been shown to attenuate the exercise pressor reflex (29). Third, our findings shed no light on the pressor and MSNA responses evoked by postcontraction circulatory occlusion. These responses, although a useful index, can be viewed as artificial because they do not simulate a mismatch between blood supply and demand during contraction, which is the relevant variable with regard to cardiovascular control by the nervous system during exercise.

In conclusion, we have shown that blockade of P2X receptors in rats whose femoral arteries were ligated for 72 h attenuated the exercise pressor reflex. In contrast, blockade of P2X receptors in rats whose femoral arteries were freely perfused had little effect on this reflex. Our findings in rats may be relevant to patients with peripheral artery disease. We note with interest that the exercise pressor reflex in patients with peripheral artery disease is exaggerated (1) just as it is in rats with ligated femoral arteries (41). Ligation of the femoral artery in rats is an established method of simulating the blood flow patterns found in exercising muscles of humans with peripheral artery disease (33, 47). We speculate that excessive stimulation by ATP of P2X receptors on the endings of group III and IV muscle afferents is responsible for the reports of pain and the exaggerated pressor response to exercise seen in patients with peripheral artery disease.

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Disclosures

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Author Contributions


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