Role played by purinergic receptors on muscle afferents in evoking the exercise pressor reflex

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Submitted 4 November 2002; accepted in final form 11 December 2002

Hanna, Ramy L., and Marc P. Kaufman. Role played by purinergic receptors on muscle afferents in evoking the exercise pressor reflex. J Appl Physiol 94: 1437–1445, 2003. First published December 13, 2002; 10.1152/japplphysiol.01011.2002.—The exercise pressor reflex is believed to be evoked, in part, by multiple metabolic stimuli that are generated when blood supply to exercising muscles is inadequate to meet metabolic demand. Recently, ATP, which is a P2 receptor agonist, has been suggested to be one of the metabolic stimuli evoking this reflex. We therefore tested the hypothesis that blockade of P2 receptors within contracting skeletal muscle attenuates the exercise pressor reflex in decerebrate cats. We found that popliteal arterial injection of pyridoxal phosphate-6-azophenyl-2′,4′-disulfonic acid (PPADS; 10 mg/kg), a P2 receptor antagonist, attenuated the pressor response to static contraction of the triceps surae muscles. Specifically, the pressor response to contraction before PPADS averaged 36 ± 3 mmHg, whereas afterward it averaged 14 ± 3 mmHg (P < 0.001; n = 19). In addition, PPADS attenuated the pressor response to postcontraction circulatory occlusion (P < 0.01; n = 11). In contrast, popliteal arterial injection of CGS-15943 (250 μg/kg), a P1 receptor antagonist, had no effect on the pressor response to static contraction of the triceps surae muscles. In addition, popliteal arterial injection of PPADS but not CGS-15943 attenuated the pressor response to stretch of the calcaneal (Achilles) tendon. We conclude that P2 receptors on the endings of thin fiber muscle afferents play a role in evoking both the metabolic and mechanoreceptor components of the exercise pressor reflex.

during static exercise, arterial blood pressure, heart rate, and ventilation increase. Two neural mechanisms are believed to be responsible for these increases. The first mechanism, named the exercise pressor reflex (25), arises from the stimulation of group III and IV endings in contracting skeletal muscle (21). Both mechanical and metabolic stimuli are thought to evoke the exercise pressor reflex (12). The second mechanism is central command, which is defined as the parallel activation of somatomotor, autonomic, and ventilatory circuits in the brain and spinal cord (41). Central command does not require muscle afferent input.

Important support for the belief that the exercise pressor reflex is evoked in part by metabolic stimuli has arisen from the widely replicated finding in both humans and animals that the pressor response to exercise persisted after the end of the exercise period if the circulation to the contracting muscles was occluded (1, 6, 21, 22, 26, 30, 31, 39). In addition, the peak pressor responses either during exercise in humans (2, 17, 34) or during static contraction in animals were increased by circulatory occlusion (6, 21, 37). The persistence of the pressor response to postcontraction circulatory occlusion as well as the increase in the peak pressor response during contraction have been attributed to the stimulation of thin fiber afferents by metabolites trapped in the previously exercising muscle (12, 25).

Although the nature of the contraction-induced metabolite that evokes the exercise pressor reflex is not known, it is thought to be multifactorial (12). For example, bradykinsins, cyclooxygenase products of arachidonic acid, and lactic acid have been suggested to be candidates for this multifactorial stimulus (9, 36, 38). Other candidates that might provide part of this metabolic stimulus are the purines, ATP and adenosine. Muscle interstitial concentrations of both substances have been shown to increase during both exercise in humans (8) and static contraction in animals (27). Moreover, injection of ATP into the arterial supply of the triceps surae muscles evoked a reflex pressor response in decerebrate cats (11, 16), whereas injection of adenosine did not (11, 19).

These findings have prompted us to determine the role played by purinergic (P) receptors on thin fiber muscle afferents in evoking the exercise pressor reflex. Specifically, we measured the cardiovascular and ventilatory responses to three stimuli before and after injecting antagonists to P1 and P2 receptors into the arterial supply of the triceps surae muscles. The three stimuli were static contraction while the muscles were freely perfused, static contraction while the circulation to the muscles was occluded, and stretch of the triceps surae muscles.

METHODS

General. Cats (2.2–3.5 kg) were anesthetized with a mixture of 5% halothane and oxygen. Catheters were placed into
the right common carotid artery, the left femoral vein, and the right jugular vein. The carotid arterial catheter was connected to a Statham pressure transducer (model 23 XL) to measure arterial blood pressure. Heart rate was calculated from the arterial pressure pulse. The trachea was cannulated, and the lungs were ventilated with anesthetic gas mixture. The left triceps surae muscles and left popliteal artery were exposed and isolated. The skin covering the left triceps surae muscles was tied to brass bars to form space for a pool, which was then filled with warm (37°C) mineral oil. The calcaneal bone was severed and then attached to a force transducer (Grass FT 10), which in turn was attached to a rack and pinion.

The cat was placed in stereotaxic instrument and spinal unit (Kopf). A micrd iccular decerebration was performed under halothane anesthesia. All neural tissue rostral to the plane of section was removed. Bleeding was controlled, and the cranial vault was filled with agar (37°C). Once the decerebration was complete, the lungs were ventilated with room air until the cat was able to breathe spontaneously. At that time, airflow was measured with a pneumotach (Fleisch), which was attached in series to the tracheal cannula. Airflow was integrated (Gould) to yield tidal volume, which was used to calculate minute volume.

All injections (see below) into the popliteal artery were accomplished by inserting a 30-gauge needle into the vessel. Before the injection, and for 15 min afterward, a ligature made of umbilical tape was tightened around the upper thigh. We have shown previously (11) that this method of injection distributed the injectate to the triceps surae and anterior tibialis muscles, but not to the hindlimb skin.

Protocols. We examined the arterial pressure, heart rate, and ventilatory responses to static contraction and to stretch of the left triceps surae muscles both before and after injecting the P2 receptor antagonist, pyridoxal phosphate-6-azo-phenyl-2′,4′-disulfonic acid (PPADS; 10 mg/kg) into the left popliteal artery (15). Previously, we found that this dose of PPADS abolished the reflex pressor and ventilatory responses to popliteal arterial injection of α,β-methylene ATP (11). A ligature made of umbilical tape was passed around the left thigh; it was tightened just before injection of PPADS and loosened 15 min later. The triceps surae muscles were perfused freely for another 15 min before they were contracted for 60 s by electrically stimulating (40 Hz; 0.025 ms; 2 times motor threshold) the tibial nerve at its junction with this muscle group. In some experiments, we contracted the triceps surae muscles while their blood supply was occluded. This was done for 3 min before contraction, during the 1-min contraction, and for 1 min after contraction. Circulatory occlusion was accomplished by clamping the popliteal artery, the left medial saphenous vein and the left popliteal vein; also the thigh ligature was tightened.

The muscles were stretched for 60 s by turning the rack and pinion attached to the calcaneal tendon. The degree of stretch was adjusted so that the tension developed approximated the tension developed by contraction. To control for recirculation of PPADS to the spinal cord and brain, we examined the arterial pressure, heart rate, and ventilatory responses to static contraction of the left triceps surae muscles before and after its injection (10 mg/kg) into the left femoral vein. We did not examine the response to tendon stretch before and after femoral venous injection of PPADS.

In another series of experiments, we examined the arterial blood pressure, heart rate, and ventilatory responses to static contraction and to stretch of the left triceps surae muscles both before and after injecting the P1 receptor antagonist CGS-15943 (250 μg/kg) into the left popliteal artery (24). A ligature made of umbilical tape was passed around the left thigh; it was tightened just before injection of the antagonist and was loosened 15 min afterward. To challenge the P1 receptor blockade induced by CGS-15943, we injected 2-chloroadenosine (25 μg/kg) into the popliteal artery and measured the depressor response before and after giving this compound.

Data analysis. We compared baseline values to the peak responses to static contraction and to tendon stretch both before and after popliteal arterial injection of PPADS and CGS-15943. Baseline values for mean arterial pressure and heart rate were taken as the steady-state values immediately preceding either static contraction or tendon stretch. Mean arterial pressure was calculated as diastolic pressure plus one-third the pulse pressure. Baseline values for ventilation were comprised of the sum of the tidal volumes for the 1-min period immediately preceding static contraction or tendon stretch. Likewise, the “peak values” for ventilation were comprised of the sum of the tidal volumes during the 1-min contraction or stretch period. The pressor response to postcontraction circulatory occlusion was defined as the peak mean arterial pressure during this time period minus the mean arterial pressure during the baseline period.

The data were analyzed statistically with a two-way repeated-measures analysis of variance. The first independent variable (i.e., main effect) was baseline vs. peak effect for either contraction or stretch. The second independent variable was the presence or absence of a P1 or P2 receptor antagonist. If either a main effect or an interaction was statistically significant, differences between individual means were tested post hoc with a Newman-Keuls test. The criterion for statistical significance was set at \( P < 0.05 \).

RESULTS

In our first series of experiments, we examined the effect of the P2 receptor antagonist PPADS on the cardiovascular and ventilatory responses to static contraction of the triceps surae muscles in 19 decerebrate cats. We found that injection of PPADS into the arterial supply of the triceps surae muscles significantly \( P < 0.001 \); Fig. 1) decreased the pressor response to contraction. Specifically, the pressor response before PPADS averaged 36 ± 3 mmHg, whereas afterward it averaged only 14 ± 3 mmHg. In contrast to the pressor response to contraction, there were no significant \( P > 0.05 \) cardioaccelerator or ventilatory responses to static contraction. PPADS injection did not alter this finding (Fig. 1). The peak tension developed by the contracting triceps surae muscles before PPADS injection \((4.7 ± 0.3 \text{ kg})\) was not significantly different \( P > 0.05 \) from that developed after PPADS injection \((4.7 ± 0.4 \text{ kg})\).

In 11 of the 19 cats discussed above, the effect of circulatory occlusion on the cardiovascular and ventilatory responses to static contraction was examined before and after PPADS (10 mg/kg), which was injected into the left popliteal artery. The pressor response to postcontraction circulatory occlusion was abolished \( P < 0.01 \) by PPADS (Figs. 2 and 3). Specifically, the pressor response to postcontraction circulatory occlusion averaged \( 20 ± 7 \text{ mmHg} \) before PPADS injection, whereas it averaged \(-4 ± 5 \text{ mmHg} \) afterward. In addition, the peak pressor response to contraction dur-
In 12 cats, we examined the effect of PPADS on the cardiovascular and ventilatory responses to stretching the left calcaneal tendon, a maneuver that also stretched the triceps surae muscles. On average, injection of the P2 receptor antagonist into the left popliteal artery attenuated the pressor but not the cardioaccelerator responses to tendon stretch. Specifically, the pressor response to stretch before PPADS averaged
32 ± 6 mmHg, whereas the pressor response to stretch afterward averaged 15 ± 4 mmHg (P < 0.01). Ventilation was not significantly (P > 0.05) changed by tendon stretch either before or after PPADS (Fig. 4). The peak tension developed by tendon stretch before PPADS injection (4.6 ± 0.3 kg) was not significantly different (P > 0.05) from that developed by stretch afterward (4.4 ± 0.3 kg).

In the second series of experiments, we examined the effect of PPADS (10 mg/kg) injected into the left femoral vein on the cardiovascular and ventilatory responses to static contraction of the left triceps surae muscles in six cats. These experiments were done to determine whether in the first series of experiments PPADS circulated to the spinal cord or brain to exert its antagonistic action. PPADS injected intravenously had no effect on the pressor response to static contraction (Fig. 5), a finding that contrasts with that found when the P2 receptor antagonist was injected into the arterial supply of the triceps surae muscles. The peak tension developed by static contraction before PPADS (4.6 ± 0.7 kg) was not significantly different (P > 0.05) from that developed by contraction after PPADS (4.6 ± 0.7 kg).

In our third series of experiments, we examined the effects of a P1 receptor antagonist, CGS-15943, on the cardiovascular and ventilatory responses to both static contraction and tendon stretch in nine cats. We found that injection of this antagonist (250 µg/kg) into the left popliteal artery had no effect (P > 0.05) on the pressor responses to either static contraction or tendon stretch. Neither heart rate nor ventilation was changed significantly (P > 0.05) by either static contraction or tendon stretch (Figs. 6 and 7). The peak tension developed by static contraction before CGS-15943 (3.4 ± 0.4 kg) was not significantly different (P > 0.05) from that developed by contraction afterward (3.3 ± 0.4 kg). Likewise the peak tension developed by tendon stretch before CGS-15943 (3.5 ± 0.4 kg) was not significantly different (P > 0.05) from that developed by stretch afterward (3.6 ± 0.4 kg).

Because of the lack of effect of CGS-15943 on the responses to contraction and tendon stretch, we challenged the effectiveness of the blockade induced by
CGS-15943. We therefore measured the depressor response to left popliteal arterial injection of 2-chloroadenosine (25 μg/kg) before and after left popliteal arterial injection of CGS-15943. We found that the depressor response before P1 receptor blockade averaged $32 \pm 4$ mmHg, whereas after blockade it averaged $10 \pm 4$ mmHg ($P < 0.003; n = 9$).

In six of these nine cats, we electrically stimulated the cut central end of the tibial nerve with the same parameters as those used to statically contract the triceps surae muscles when the nerve was intact. Stim-

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**Fig. 4.** Summary data for mean arterial pressure (A), heart rate (B), and ventilation (C) from 12 decerebrate cats showing the attenuation by PPADS injected into the popliteal artery of the pressor response to stretching the calcaneal tendon. Values are means ± SE. Solid bars, mean baseline values; open bars, mean peak responses to stretch. *Significant difference ($P < 0.05$) between adjacent bars. Horizontal bracket, pressor response to tendon stretch before PPADS was significantly greater ($P < 0.05$) than the pressor response to stretch after PPADS. Note that on average tendon stretch had no effect on either heart rate or ventilation.

**Fig. 5.** Summary data for mean arterial pressure (A), heart rate (B), and ventilation (C) from 6 decerebrate cats showing that PPADS injected intravenously had no effect on the pressor response to static contraction of the freely perfused triceps surae muscles. Note that contraction had no effect on ventilation. Values are means ± SE. Solid bars, mean baseline values; open bars, mean peak responses to contraction. *Significant difference ($P < 0.05$) between adjacent bars.
ulation of the cut tibial nerve had no effect on mean arterial pressure, heart rate, or ventilation. This finding indicated that the pressor responses evoked by tibial nerve stimulation in our experiments were caused by contraction of the triceps surae muscles and not by electrical stimulation of afferent fibers in the tibial nerve.

DISCUSSION

Purinergic receptors are of two types, namely P1 and P2. Adenosine is thought to stimulate P1 receptors, whereas ATP is thought to stimulate P2 receptors. In our experiments, blockade of P2 receptors in the triceps surae muscles attenuated the pressor reflex evoked by statically contracting this muscle group. Moreover, blockade of P2 receptors abolished, on average, the pressor reflex evoked by postcontraction circulatory occlusion. In addition, blockade of P2 receptors...
attenuated the pressor reflex evoked by stretching the calcaneal tendon. In contrast, blockade of P1 receptors did not attenuate the exercise pressor reflex evoked by contraction of the triceps surae muscles. Likewise, P1 receptor blockade did not attenuate the pressor reflex evoked by tendon stretch (35).

In our experiments, static contraction and tendon stretch evoked pressor responses that averaged between 30 and 40 mmHg but did not increase either heart rate or ventilation. Our finding that neither contraction nor stretch increased heart rate or ventilation is probably explained by the fact that the peak tension developed by the triceps surae muscles averaged only about a third of its maximum. Contractions and tendon stretches of this low magnitude have been shown previously to have minimal effects on heart rate and ventilation (42, 43). In addition, pressor responses of 30–40 mmHg would be expected to cause baroreflex-induced buffering of any cardioaccelerator and ventilatory responses to contraction.

Our rationale for using three maneuvers to evoke a reflex pressor response from the triceps surae muscles was that each stimulated different types of thin fiber afferents. The first maneuver, namely static contraction while the muscles were freely perfused, stimulated afferents that were sensitive to both mechanical and metabolic stimuli (13, 23). The second maneuver, namely static contraction while the circulation to the muscles was occluded, stimulated afferents that were sensitive to both types of stimuli, but the level of stimulation to metabolically sensitive afferents was greater than that during contraction while the muscles were freely perfused (14, 23). Moreover, during the time period when the circulation was occluded but the triceps surae muscles were no longer contracting, only metabolically sensitive afferents were stimulated (14). The third maneuver, namely tendon stretch, stimulated only mechanically sensitive afferents (13, 23).

Comparison of the pressor responses to each of the three maneuvers before purinergic receptor blockade with those after blockade allows us to reach two conclusions, each of which applies only to situations in which the muscles develop relatively low levels of tension. First, P2, but not P1, receptor blockade attenuates the pressor response to the stretch-induced stimulation of thin fiber mechanoreceptors. Most of these mechanically sensitive afferents are innervated by group III fibers, which are thinly myelinated and conduct impulses between 2.5 and 30 m/s (13, 23, 28). Second, P2, but not P1, receptor blockade attenuates the pressor response to the contraction-induced stimulation of thin fiber metaboreceptors. Most of these metaboreceptors are innervated by group IV fibers, which are unmyelinated and conduct impulses at <2.5 m/s (13, 14, 23).

P2 receptors have two subtypes: the P2X, which is a cation channel, and the P2Y, which is G-protein coupled (4). In addition, the P2X receptor is comprised of combinations of seven different proteins (4). PPADS blocks both P2X and P2Y receptors but does not block P1 receptors (15). The available evidence leads us to speculate that PPADS antagonized the P2X3 receptor in our experiments. For example, α,β-methylene ATP, which stimulates PX1 and PX3 receptors (4), evoked a reflex pressor response when this agent was injected into the popliteal artery of decerebrate cats (11). The P2X3 receptor protein is found on the sensory endings of Aβ and C-fiber nociceptors but is not found on the endings of stretch receptors supplied by presumed thickly myelinated fibers that signal innocuous events (5). Moreover, the P2X3 receptor is found on small-diameter dorsal root ganglion cells that display markers indicating that they respond to capsaicin (33), a potent algogen that in vivo stimulates group IV muscle afferents (13). The P2X1 receptor protein is found on vascular smooth muscle cells (10) but is not found on Aβ and C-fiber endings (5).

The role played by adenosine, ATP, and their respective purinergic receptors in the generation of the exercise pressor reflex has been controversial. Frequently, the first step in investigating whether or not a metabolic by-product of contraction evokes this reflex is to inject it into the arterial supply of limb skeletal muscle to see whether it increases arterial pressure. In humans this has been done for adenosine but it has not been done for ATP. Two groups of investigators have found that injection of adenosine into the arterial supply of limb muscles increased mean arterial pressure and muscle sympathetic nerve activity, but one attributed the increases to stimulation of thin fiber afferents innervating the limb muscle (7, 8), whereas the other attributed the increases to recirculation (18). The latter group (18) found no effect if the injectate was restricted by ligature to the circulation of the limb. In animals, however, both adenosine and ATP have been injected into the arterial supply of hindlimb muscle. A consistent finding is that adenosine did not evoke a reflex pressor response (11, 19), whereas ATP did (11, 16).

In our experiments, P2 receptor blockade in the triceps surae muscles abolished the pressor reflex evoked by postcontraction circulatory occlusion. This reflex is believed to be evoked solely by metabolic by-products of contraction, which are trapped by circulatory occlusion in the previously contracting muscles. One interpretation of our data is that this metaboreflex is caused by only one substance, namely ATP. Although we cannot exclude this interpretation, we think it is unlikely. An alternative interpretation of our data is that P2 receptor blockade reduced the level of group IV muscle afferent activation below the threshold level required to evoke a metaboreflex. If this is the case, then receptor blockade of other muscle metabolites, such as bradykinin (36), or the attenuation of either prostaglandin production, such as that caused by indomethacin (38), or lactic acid production, such as that caused by dichloroacetate (9), would also abolish the metaboreflex arising from postcontraction circulatory occlusion.

We found that P2 receptor blockade in the triceps surae muscles attenuated the pressor response to tendon stretch, a finding that also has been reported
recently by Li and Sinoway (16). Tendon stretch stimulates group III mechanoreceptors (13). Nevertheless, the chemical environment surrounding these muscle afferents can influence their sensitivity to mechanical stimuli. For example, the responses of group III mechanoreceptors to contraction are attenuated by dichloroacetate, which decreases lactic acid production in skeletal muscle by increasing the activity of pyruvate dehydrogenase (32). On the basis of this prior evidence, we think it is reasonable to speculate that P2 receptor blockade also decreased the mechanical sensitivity of group III muscle afferents to both tendon stretch and static contraction in our experiments.

Although tendon stretch is a selective stimulus to mechanoreceptors, its use needs to be interpreted carefully. Specifically, it is not clear that group III mechanoreceptors respond to tendon stretch with the same number of impulses or discharge pattern as they respond to static contraction of equal tension development. An interesting parallel can be drawn from studies on Golgi tendon organs. These group Ib mechanoreceptors respond more vigorously to contraction than they do to stretch (20). If a similar finding is true for group III mechanoreceptors, then any conclusions based on tendon stretch concerning the contribution of these muscle afferents to the autonomic response to static contraction must be drawn cautiously.

Although muscle interstitial concentrations of ATP increase either during exercise in humans (8) or during electrically induced contractions in animals (27), the source of this purine is not established. Nevertheless, two possibilities are likely. The first is the release of ATP by sympathetic postganglionic endings in the skeletal muscle vasculature (3), a site containing the sensory endings of group IV afferents (40). The second is the release of ATP by contracting muscle fibers. This release might be increased when metabolic oxygen demand in the contracting muscle fibers exceeds oxygen supply. Whatever the source of ATP release, we think it is reasonable to add this purine to the list of the substances comprising the multifactorial stimuli evoking the metabolic component of the exercise pressor reflex.

We thank Angela DiStefano and Todd Heller for technical assistance. This work was supported by National Heart, Lung, and Blood Institute Grant HL-30710.

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