Activation of thin-fiber muscle afferents by a P2X agonist in cats

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Hanna, Ramy L., and Marc P. Kaufman. Activation of thin-fiber muscle afferents by a P2X agonist in cats. J Appl Physiol 96: 1166–1169, 2004; 10.1152/japplphysiol.01020.2003.—The responses of group III and IV triceps surae muscle afferents to intra-arterial injection of α,β-methylene ATP (50 μg/kg) was examined in decerebrate cats. We found that this P2X₁ agonist stimulated only three of 18 group III afferents but 7 of 9 group IV afferents (P < 0.004). The three group III afferents stimulated by α,β-methylene ATP conducted impulses below 4 m/s. Pyridoxal phosphate-6-azophenyl-2',4'-disulfonic acid, a P2-receptor antagonist, prevented the stimulation of these afferents by α,β-methylene ATP. We conclude that P2X₁ agonists stimulate only the slowest conducting group III muscle afferents as well as group IV afferents.

adenosine 5'-triphosphate; purines; group III afferents; group IV afferents; metaboreflex; pressor response

METABOLIC BY-PRODUCTS OF MUSCULAR contraction evoke a constellation of reflex responses that include increases in sympathetic discharge to the vasculature of skeletal muscle, increases in cardiac rate and contractility, as well as increases in ventilation (10, 22). These autonomic and ventilatory effects have been termed the muscle metaboreflex; the sensory arm of this reflex arc is composed of group III and IV muscle afferents (10, 22). The nature of the metabolic stimulus to group III and IV muscle afferents is not known, but substances such as lactic acid, bradykinin, and cyclooxygenase products of arachidonic acid have been considered candidates (4, 9, 18, 20, 21, 23, 25, 26).

Recently, the search for this metabolite has turned to adenosine triphosphate (ATP), a purine whose concentration in the muscle interstitial space has been found to increase during either exercise in humans (8) or muscular contraction in animals (13, 17). A commonly taken first step in investigating whether a by-product of muscle contraction plays a role in evoking the muscle metaboreflex is to inject the substance into the arterial supply of skeletal muscle (10). When this first step was taken in cats, α,β-methylene ATP injected into the arterial supply of the triceps surae muscles evoked a reflex pressor response (7, 14). In all likelihood, the afferent arm of the reflex arc evoking the pressor response to α,β-methylene ATP injection was composed of group III and/or group IV muscle afferents (10, 15). Nevertheless, the responses of these thin-fiber muscle afferents in cats to α,β-methylene ATP have not been characterized. Consequently, we tested the hypothesis that group III and/or IV muscle afferents responded to arterial injections of α,β-methylene ATP in doses that evoked a pressor reflex. We also tested the hypothesis that the stimulation of group III or IV afferents by α,β-methylene ATP was caused by the activation of P2 receptors.

This study was conducted in conformity with APS’s Guiding Principles in the Care and Use of Animals. Cats were anesthetized with a mixture of halothane (4%) and oxygen. The trachea, right common carotid artery, and right external jugular vein were cannulated. The cat was placed in a Kopf stereotaxic unit. While the lungs were ventilated with the halothane (4%) and oxygen mixture, the cat was decerebrated at the midsagittal level. All neural tissue rostral to the section was removed, bleeding was controlled, and the cranial vault was filled with agar. A laminectomy was performed to expose the L₄ through S₂ spinal cord, after which the gaseous anesthetic was discontinued and the lungs were ventilated with room air and oxygen. Arterial blood gases were measured and maintained at physiological levels. Mean arterial pressure was always 90 mmHg or greater. All visible branches of the left sciatic nerve, except those innervating the triceps surae muscles, were cut. Likewise, the left femoral and obturator nerves were cut. The left triceps surae muscles were exposed and then covered with gauze soaked in warm (37°C) saline.

Afferent impulses arising from the left triceps surae muscles were recorded from thin filaments split from the left L₇ dorsal root. Afferents conducting impulses between 2.5 and 30 m/s were classified as group III fibers, and those conducting impulses below 2.5 m/s were classified as group IV fibers. Group I and II afferents were discarded. α,β-Methylene ATP was dissolved in saline and was injected into the popliteal artery in a dose (50 μg/kg) that has been shown previously to evoke a reflex pressor response (7, 14). Likewise, pyridoxal phosphate-6-azophenyl-2',4'-disulfonic acid (PPADS) was dissolved in saline and was injected into the popliteal artery in a dose (10 mg/kg) known to prevent the reflex pressor response to injection of α,β-methylene ATP (7). Immediately before injecting PPADS, we tightened a snare placed around the thigh to trap PPADS in the circulation of the triceps surae muscles. The snare was released after 15 min, and α,β-methylene ATP (50 mg/kg) was re-injected 15 min after release. All values are expressed as means ± SE. Statistical significance was determined with paired t-tests. The criterion for significance was set at P < 0.05.

We recorded the impulse activity of 18 group III afferents (conduction velocity: 9.4 ± 2.1 m/s; range: 2.6–29.5 m/s) and 9 group IV afferents (conduction velocity: 1.6 ± 0.1 m/s; range: 1.2–2.1 m/s). Popliteal arterial injection of α,β-methylene ATP (50 μg/kg) stimulated only 3 of the 18 group III but 7 of the 9 group IV afferents (Figs. 1 and 2). Fisher’s exact test revealed that α,β-methylene ATP stimulated a significantly...
higher proportion of group IV than group III afferents ($P < 0.004$). Each of the group III afferents that did respond to injection of $\alpha_\beta$-methylene ATP had conduction velocities ranging between 3.3 and 3.7 m/s. For the 10 thin-fiber afferents stimulated by $\alpha_\beta$-methylene ATP, the responses started after an average latency of $7.6 \pm 1.4$ s and lasted for an average duration of $41.4 \pm 5.0$ s. There was no apparent difference between the onset latency and duration of the three group III afferents and the seven group IV afferents stimulated by $\alpha_\beta$-methylene ATP. For these 10 thin-fiber afferents, injection of $\alpha_\beta$-methylene ATP increased baseline discharge from $0.6 \pm 0.1$ imp/s to a peak of $3.1 \pm 0.5$ imp/s ($P < 0.001$). We examined the effect of PPADS on the responses of 7 of the 10 thin-fiber afferents previously stimulated by injection of $\alpha_\beta$-methylene ATP. We found that PPADS, a P2-receptor antagonist, abolished the responses of each of the seven afferents to $\alpha_\beta$-methylene ATP after PPADS.

Two of the 10 afferents stimulated by injection of $\alpha_\beta$-methylene ATP were stimulated by stretching the calcaneal tendon; both were group IV afferents. Two of the three group III afferents and three of the seven group IV afferents were stimulated by static contraction. Similarly, two of the three group III and four of the six group IV afferents were stimulated by popliteal arterial injection of capsaicin (15 μg), a vanilloid receptor-1 (VR-1) receptor agonist. One of the seven group IV afferents stimulated by $\alpha_\beta$-methylene ATP was not challenged with capsaicin. Gentle stroking of the triceps surae muscles was not effective in discharging the 10 afferents stimulated by $\alpha_\beta$-methylene ATP. In contrast, noxious pinching of the muscles did activate these afferents.

We found that injection of $\alpha_\beta$-methylene into the popliteal artery, which perfuses the triceps surae muscles, stimulated a significantly greater proportion of group IV afferents than group III afferents. Moreover, the few group III afferents stimulated by $\alpha_\beta$-methylene ATP were slowly conducting. In contrast, the group III afferents not stimulated by injection of this P2X agonist had conduction velocities of 4 m/s or greater. These findings suggest that, within the category of thin-fiber muscle afferents, only the slowest conducting of them are stimulated by $\alpha_\beta$-methylene ATP. Consequently, these slowly conducting group III and IV afferents comprised the sensory limb of the reflex arc, causing the pressor response to injection of $\alpha_\beta$-methylene ATP into the arterial supply of the triceps surae muscles of decerebrate cats (7, 14).

Thin-fiber muscle afferents are stimulated by a variety of chemicals, many of which are metabolic by-products of muscular contraction. Three important examples are lactic acid (20, 24, 27), bradykinin (12, 16), and cyclooxygenase products of arachidonic acid (20). Each of these substances appears to

![Image](https://www.jap.org)
stimulate approximately equal percentages of group III and IV muscle afferents. In contrast, \(\alpha,\beta\)-methylenne ATP stimulated in our experiments mostly group IV afferents, a finding that parallels that reported for capsaicin, a VR-1 receptor agonist (11, 12). This parallel might be more than coincidence because P2X\(_3\) receptors, which are stimulated by \(\alpha,\beta\)-methylenne ATP, and VR-1 receptors appear to be located on the same cell bodies in the dorsal root ganglion (5). Moreover, cell bodies expressing P2X\(_3\) receptors have been reported to contain less substance P and calcitonin gene-related peptide than cell bodies not expressing P2X\(_3\) receptors (2, 5, 28).

Recently, Reinohl et al. (19) reported that ATP injected directly into the triceps surae muscles of rats stimulated 67% of the group IV afferents tested. The effects of ATP on the discharge of group III afferents were not reported in this study (19). Although our sample was small, the percentage of group IV afferents stimulated by \(\alpha,\beta\)-methylenne ATP in cats was almost identical to that stimulated by ATP in rats (19). It is difficult to compare dosages used in the two studies because the route of administration, the substance itself, and the species differed. Nevertheless, we calculate that Reinohl et al. injected \(\sim 95 \mu g\) of ATP into the substance of the triceps surae muscles of rats, whereas we injected \(\sim 150 \mu g\) of \(\alpha,\beta\)-methylenne ATP into the arterial supply of the triceps surae muscles of cats. We have extended these previous findings (19) by showing that most group III muscle afferents are not stimulated by \(\alpha,\beta\)-methylenne ATP. We have also extended these findings by showing that PPADS, a P2-receptor antagonist, prevented the stimulation of the afferents by \(\alpha,\beta\)-methylenne ATP. Consequently, this stimulation is not due to the breakdown of ATP to adenosine, which stimulates P1 receptors.

The nociceptive function of thin-fiber somatic afferents stimulated by P2X agonists in rats is not clear. Specifically, in skin, nociceptive A\(\delta\) and C-fiber afferents were stimulated by P2X agonists, whereas nonnociceptive A\(\delta\) and C-fiber afferents were not (6). In contrast, in skeletal muscle, no evidence was found that nociceptive C-fiber afferents were more responsive to P2X agonists than were nonnociceptive C fibers (19). In joints, both A\(\delta\) and C fibers appeared equally responsive to \(\alpha,\beta\)-methylenne ATP (3), and both were deemed to have a nociceptive function.

Similarly, the nociceptive function of the thin-fiber muscle afferents stimulated by \(\alpha,\beta\)-methylenne ATP in the cats of the present study is not clear. Mechanosensitive nociceptors seem to be defined by their lack of responsiveness to nonnoxious, noninjurious mechanical stimulation, as well as by their responsiveness to noxious levels of this stimulus. In our experiments, \(\alpha,\beta\)-methylenne ATP stimulated, for the most part, thin-fiber muscle afferents that were not responsive to innocuous mechanical stimuli but were responsive to noxious mechanical stimuli. Consequently, one might classify them as nociceptors, but the fact that one-half were stimulated by static contraction under freely perfused conditions makes us reluctant to use this classification at this point in time. This reluctance is buttressed by our previous finding that group IV afferents with discharge properties similar to those stimulated by \(\alpha,\beta\)-methylenne ATP in the present study responded to mild to moderate levels of dynamic exercise (1), which is also likely to be a nonnoxious stimulus.