ATP stimulates chemically sensitive and sensitizes mechanically sensitive afferents

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Li, Jianhua, and Lawrence I. Sinoway. ATP stimulates chemically sensitive and sensitizes mechanically sensitive afferents. Am J Physiol Heart Circ Physiol 283: H2636–H2643, 2002; 10.1152/ajpheart.00395.2002.—We examined whether ATP stimulation of P2X purinoceptors would raise blood pressure in decerebrate cats. Femoral arterial injection of the P2X receptor agonist α,β-methylene ATP into the blood supply of the triceps surae muscle induced a dose-dependent increase in arterial blood pressure. The maximal increase in mean arterial pressure (MAP) evoked by 0.1, 0.2, and 0.5 mM α,β-methylene ATP (0.5 ml/min injection rate) was 6.2 ± 2.5, 22.5 ± 4.4, and 35.2 ± 3.9 mmHg, respectively. The P2X receptor antagonist pyridoxal phosphate-6-azophenyl-2′,4′-disulfonic acid (2 mM ia) attenuated, 4 respectively. The P2X receptor antagonist pyridoxal phosphate-6-azophenyl-2′,4′-disulfonic acid (2 mM ia) attenuated, 4 the increase in MAP elicited by intra-arterial α,β-methylene ATP (0.5 mM), whereas the P2Y receptor antagonist reactive blue 2 (2 mM ia) did not affect the MAP response to α,β-methylene ATP. In a second group of experiments, we tested the hypothesis that ATP acting through P2X receptors would sensitize muscle afferents and, thereby, augment the blood pressure response to muscle stretch. Two kilograms of muscle stretch evoked a 26.5 ± 4.3 mmHg increase in MAP. This MAP response was enhanced when 2 mM ATP or 0.1 mM α,β-methylene ATP (0.5 ml/min) was arterially infused 10 min before muscle stretch. Furthermore, this effect of ATP on the pressor response to stretch was attenuated by 2 mM pyridoxal phosphate-6-azophenyl-2′,4′-disulfonic acid (P < 0.05) but not by the P1 purinoceptor antagonist 8-(p-sulphophenyl)-theophylline (2 mM). These data indicate that activation of ATP-sensitive P2X receptors evokes a skeletal muscle afferent-mediated pressor response and that ATP at relatively low doses enhances the muscle pressor response to stretch via engagement of P2X receptors.

α,β-methylene ATP; muscle stretch; skeletal muscle; exercise pressor reflex; arterial blood pressure

STATIC EXERCISE REFLEXLY INCREASES renal and cardiac sympathetic nerve activity, arterial blood pressure, heart rate (HR), and myocardial contractility (11, 12, 24, 28, 31, 32, 34, 36, 37). Neural signals from exercising skeletal muscle are generated by activating metabolically and mechanically sensitive nerve endings (receptors) located in the skeletal muscle (24, 25, 36). These neural signals are subsequently carried to the central nervous system by group III and IV afferent fibers (11, 34). Together, activation of these receptors by mechanical and metabolic stimulation of skeletal muscle, along with the reflex cardiovascular responses, is termed the “exercise pressor reflex” (34, 36, 37). In this report, we focus on the potential role that ATP might play in stimulating muscle afferents that contribute to the exercise pressor reflex.

A number of issues related to ATP pharmacology are germane to this discussion. First, it is known that mechanical stimulation of epithelial and neuronal cells is a sufficient stimulus for ATP release (18, 51, 54, 55). Additionally, it has been demonstrated that touch-induced sensory nerve discharge frequency increases when ATP is injected subcutaneously in frogs. This effect was blocked by injection of a P2 purinoceptor antagonist, suramin, or an ATP-degrading enzyme, apyrase, within the receptive field (38). These data strongly suggest that ATP may play a role in sensitizing mechanically sensitive muscle afferents. If such a process were present in skeletal muscle, then muscle stretch alone could conceivably increase interstitial ATP concentrations. Second, it is known that ATP is coreleased from sympathetic nerve terminals with noradrenaline (50, 53). Therefore, the mechanical stimulation of skeletal muscle and the increased sympathetic nerve activity could cause the release of ATP into muscle interstitium, where the free nerve endings of group III and IV muscle afferents reside. Third, cellular destruction, as might be seen during extreme exercise, will lead to a large increase in interstitial ATP. This may be relevant to the study of muscle reflexes, because arterial or intra-articular injection of the selective P2X receptor agonist α,β-methylene ATP causes a rapid short-lasting excitation of a subpopulation of C and Aδ nociceptive afferent nerves innervating normal knee joints (13). This makes it logical to postulate that ATP may serve as a chemoreceptor stimulant in skeletal muscle. Fourth, ATP stimulates P2X and P2Y receptors, which are found on sensory neurons (7, 8, 10, 21, 22, 26, 38). Such afferent stimulation can evoke

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nerve impulse generation, as well as the release of sensory neurotransmitters at central and peripheral ends of afferent fibers (7, 8, 26).

On the basis of these data, we postulated that the arterial administration of ATP into the blood supply of the triceps surae muscle would raise blood pressure and that ATP would also sensitize thin-fiber muscle afferents. This, in turn, would lead to a greater pressor response for a given degree of deformation of the muscle afferent receptive field. Our data support these hypotheses.

METHODS

General Methods

Animal surgical preparation. Fourteen adult cats (4.0–5.0 kg) of either gender were anesthetized initially with ketamine (25 mg/kg im) and then by inhalation of isoflurane-oxygen. An endotracheal tube was inserted and attached to a ventilator (model 683, Harvard, South Natick, MA). Polytetrafluoroethylene (PE-90) catheters were inserted into an external jugular vein and a common carotid artery for drug administration and measurement of arterial blood pressure, respectively.

The femoral arteries and arterial collaterals of both hindlimbs were carefully isolated. The saphenous artery or descending genicular artery, arterial branches of the femoral artery, was cannulated with PE-10 catheters. This allowed for arterial injections of drugs while the arterial blood supply to the hindlimb muscles was maintained. The triceps surae muscle group was isolated, and the Achilles tendons were cut. A tie was placed around the tendon and attached to a tension transducer (model FT10, Grass Instruments). The legs were stabilized with ties around the ankles and at the top of the knees. In these experiments, the skin covering the triceps surae muscle and femoral region was surgically separated from the muscles below. This procedure should eliminate the inputs from skin afferents in the hindlimb. In some of the studies, the sciatic nerves on both legs were carefully isolated so that they could be sectioned at the end of study.

The ventilator was set with a tidal volume of 20 ml/stroke and a rate of 20–30 strokes/min. Arterial blood gases and pH were periodically checked (ABL 510 pH blood gas analyzer, Radiometer, Copenhagen, Denmark). pH was maintained at \( \sim 7.35–7.45 \), \( \text{PCO}_2 \) at \( \sim 30–40 \) mmHg, and \( \text{HCO}_3^- \) at \( \sim 20–25 \) mmol/l by adjusting the ventilator settings or by intravenously injecting a 1 M sodium bicarbonate solution. Body temperature was continuously monitored with a rectal thermometer (series 400, Yellow Springs Instruments) and maintained at 37.0–38.5°C by a water-perfused heating pad and external heat lamps.

Decerebration. Decerebration was performed because it allowed an examination of autonomic reflex responses without the need to consider the confounding effects of anesthesia (23). Before the decerebration procedure, dexamethasone (4 mg iv) was administered to help prevent procedure-induced brain stem edema. The cat’s head was fixed in a Kopf stereotaxic instrument, and decerebration was performed as anesthesia was continued. The majority of the temporal and parietal plates were removed. The two cortical hemispheres were also removed. A transverse section was made anterior to the superior colliculus and extending ventrally to the mammillary bodies. The brain rostral to the section was removed, and bleeding was controlled with cotton gauze that had been soaked in boiling saline. Gauze filled the vault, and gentle manual pressure was applied. Once the decerebration was completed, anesthesia was removed from the inhaled mixture. The general procedures employed for decerebration were performed as reported previously (30).

Measurement of arterial blood pressure and peak tension.

Arterial blood pressure was measured by connecting the carotid arterial catheter to a pressure transducer (model P23ID, Statham). Mean arterial pressure (MAP) was obtained by integrating the arterial signal with a time constant of 4 s. HR was determined from the arterial pressure pulse. The Achilles tendon was connected to a force transducer (model FT10, Grass Instruments) for measurement of developed tension during muscle stretch. The pelvis was stabilized in a spinal unit (Kopf Instruments), and the knee joints were secured by attaching the patellar tendon to a steel post. All measured variables were continuously recorded on an eight-channel chart recorder. The digital signal was relayed to a Dell (Dimension P75t) computer system that employed PowerLab (AD Instruments, Castle Hill, Australia) systems software for storage and analysis of data.

Experimental Protocols

Arterial injection of \( \alpha,\beta \)-methylene ATP to activate cardiovascular responses. The purpose of this protocol was to determine whether \( \alpha,\beta \)-methylene ATP activated the muscle pressor reflex via P2X purinoceptors. The animals were surgically prepared as described in General Methods. At 40–60 min after decerebration, 0.5 ml of 0.1, 0.2, and 0.5 mM \( \alpha,\beta \)-methylene ATP (dissolved in saline; Sigma) was injected into the blood supplies of the triceps surae muscle. The duration of injections was 1 min. At least 20 min were allowed between the injections.

Effect of P2X and P2Y receptor blockade on intra-arterial \( \alpha,\beta \)-methylene ATP. To test the role of P2X and P2Y receptors, 0.5 ml of 2 M pyridoxal phosphate-6-azophenyl-2′,4′-disulfonic acid (PPADS) and 2 mM reactive blue 2 (RB-2; Sigma), respectively (both dissolved in saline), were injected. Injections were performed 5 min before injections of 0.5 mM \( \alpha,\beta \)-methylene ATP. In addition, 0.5 ml of \( \alpha,\beta \)-methylene ATP was injected by femoral artery after occlusion of the femoral vein and section of the sciatic nerve to confirm that the pressor response was due to stimulation of afferents within the hindlimb.

Effect of ATP on the cardiovascular response evoked by muscle stretch. The concentrations of \( \alpha,\beta \)-methylene ATP and ATP were chosen on the basis of previous studies (13, 52). It has been reported that intra-arterial and intra-articular injection of 60 nmol of \( \alpha,\beta \)-methylene ATP or 2,000 nmol of ATP (both in 0.1 ml volume) increased excitation of C and A\( \delta \) afferent fibers innervating knee joints (13). Additionally, intravenous administration of 100 \( \mu \)M \( \alpha,\beta \)-methylene ATP and 1 mM ATP (in 100 \( \mu \)l volume) activated bladder afferents and increased the discharge response to bladder distension (52).

The triceps surae muscle was stretched, and tension of 2 kg was produced over 5–10 s by means of a rack and pinion. Generally, −5–10 kg of tension can be generated by electrical stimulation of the L\( _2 \) and S\( _1 \) ventral roots in cats. It has been reported that significant reflex cardiovascular responses occur when the triceps surae are passively stretched (1–8 kg) (45). Muscle stretch was maintained for 1 min after tension of 2 kg was achieved. Muscle stretch was performed 10 min after femoral arterial injections of 0.5 ml of saline, 0.5 ml of 2 mM ATP, and 0.1 mM \( \alpha,\beta \)-methylene ATP (dissolved in
ethylene-ATP (α,β-Me-ATP) evoked the dose-dependent pressor response. A: maximal pressor response evoked by femoral arterial injection of 0.1, 0.2, and 0.5 mM α,β-methylene ATP. Basal mean arterial pressure (MAP) before injection of 0.1, 0.2, and 0.5 mM α,β-methylene ATP was 105.7 ± 8.6, 94.2 ± 7.7, and 105.7 ± 4.8 mmHg, respectively. Response was reduced after section of the sciatic nerve (basal MAP = 107.4 ± 8.7 mmHg). Values are means ± SE (n = 6). These basal MAPs were not different. *P < 0.05 vs. 0.1 and 0.2 mM. #P < 0.05 vs. 0.5 mM. B: representative traces of pressor response to 0.5 mM α,β-methylene ATP before (top) and after (bottom) sciatic nerve section. ABP, arterial blood pressure.

Experimental Data Analysis

All measured variables were continuously recorded on an eight-channel chart recorder (model TA 4000, Gould, Valley View, OH). These variables were also sampled by a personal computer-based Pentium computer that was equipped with analog-to-digital conversion and PowerLab data acquisition (AD Instruments). Computer-acquired data were used in post hoc analyses. Control values were determined by analyzing ≥30 s of the data immediately before femoral arterial injection or muscle stretch. The peak response of each variable was determined by the peak change from the control value.

Measured variables were analyzed by using a one-way repeated-measure analysis of variance. As appropriate, a Tukey post hoc test was utilized. Values are means ± SE. For all analyses, differences were considered significant at P < 0.05. All statistical analyses were performed by using Sigma Stat for Windows version 2.03 (SPSS, Chicago, IL).

RESULTS

Cardiovascular Responses to Arterial Injections of α,β-Methylene ATP

Femoral arterial injection of the P2X receptor agonist α,β-methylene ATP at 0.1, 0.2, and 0.5 mM (in 0.5 ml of saline) evoked the pressor response (Fig. 1). The peak responses to the three dose levels were 6.2 ± 2.5, 22.5 ± 4.4, and 35.2 ± 3.9 mmHg, respectively. The basal MAP before injection of 0.1, 0.2, and 0.5 mM α,β-methylene ATP was 105.7 ± 8.6, 94.2 ± 7.7, and 105.7 ± 4.8 mmHg (P > 0.05), respectively. After occlusion of the femoral vein, 0.5 mM α,β-methylene ATP infusions still increased MAP by 34.9 ± 8.78 mmHg (P < 0.05). After section of the sciatic nerve, arterial injection of 0.5 mM α,β-methylene ATP increased MAP by 8.4 ± 2.7 mmHg (Fig. 1). α,β-Methylene ATP at 0.5 mM also increased the HR response significantly. The HR response to α,β-methylene ATP is shown in Table 1.

Effects P2X and P2Y Receptor Blockade on the Cardiovascular Response Evoked by Arterial Injections of 0.5 mM α,β-Methylene ATP

Femoral arterial administration of 2 mM PPADS, a P2X receptor antagonist, significantly attenuated the increase of MAP elicited by 0.5 mM α,β-methylene ATP (Fig. 2). However, arterial administration of 2 mM RB-2, a P2Y receptor antagonist, did not affect the MAP response elicited by 0.5 mM α,β-methylene ATP (Fig. 2). The peak increase in MAP elicited by α,β-methylene ATP was 35.6 ± 4.1 mmHg (basal MAP = 118.7 ± 10.2 mmHg). The peak increases in MAP elicited by α,β-methylene ATP after administration of PPADS and RB-2 were 24.9 ± 3.8 and 33.7 ± 10.1 mmHg, respectively. Thus P2X blockade with 2 mM PPADS reduced the pressor response to the ATP analog by ~30%, whereas P2Y receptor blockade with 2 mM RB-2 had no effect on this response.

Table 1. Basal and reflexive HR after arterial injection of α,β-methylene ATP

<table>
<thead>
<tr>
<th>α,β-Methylene ATP Concentration</th>
<th>HR, beats/min</th>
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<tbody>
<tr>
<td></td>
<td>Basal</td>
</tr>
<tr>
<td>0.1 mM</td>
<td>175 ± 10</td>
</tr>
<tr>
<td>0.2 mM</td>
<td>177 ± 12</td>
</tr>
<tr>
<td>0.5 mM</td>
<td>185 ± 12*</td>
</tr>
<tr>
<td>0.5 mM + PPAPS</td>
<td>174 ± 15</td>
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<tr>
<td>0.5 mM + RB-2</td>
<td>175 ± 15</td>
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Values are means ± SE; n = 6. HR, heart rate; PPADS, pyridoxal phosphate-6-azoxophenyl-2',4'-disulfonic acid; RB-2, reactive blue 2. There is no significant difference among basal values. *Significant increase compared with basal.
Response to Muscle Stretch

Methylene ATP on the Cardiovascular

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DISCUSSION

Effects of Arterial Infusions of ATP and α,β-
Methylene ATP on the Cardiovascular
Response to Muscle Stretch

After an arterial administration of saline, muscle stretch caused MAP to rise by 26.5 ± 4.3 mmHg from a baseline of 138.8 ± 12.6 mmHg. The preadministration of 2 mM ATP into the femoral artery increased the response to stretch to 39.9 ± 5.5 mmHg (baseline = 128.9 ± 11.1 mmHg). α,β-Methylene ATP at 0.1 mM also significantly increased the pressor response to muscle stretch, with the maximal pressor response being 44.4 ± 4.2 mmHg (baseline = 122.6 ± 11.8 mmHg). α,β-Methylene ATP at 0.1 mM caused a more sustained effect than did 2 mM ATP (Fig. 3A). In a fourth trial, stretch after saline administration led to a 27.7 ± 5.9 mmHg increase in MAP from a baseline of 116.7 ± 11.4 mmHg. The effect of ATP and α,β-methylene ATP on the HR response to muscle stretch is shown in Table 2.

The sensitizing effect of ATP on the pressor response was reduced by 78% by the preadministration of 2 mM PPADS (maximal MAP response = 29.5 ± 7.8 mmHg; Figs. 4 and 5). Preadministration of 8-PT did not attenuate the ATP-sensitizing effect (maximal MAP response = 45.7 ± 7.3 mmHg; Fig. 5).

P2X RECEPTORS AND MUSCLE PRESSOR REFLEX

Fig. 2. Effects of P2X and P2Y receptors on pressor response evoked by arterial injection of α,β-methylene ATP. Maximal response was evoked by femoral arterial injection of 0.5 mM α,β-methylene ATP and α,β-methylene ATP with previous intra-arterial injection of 2 mM pyridoxal phosphate-6-azophenyl-2,4'-disulfonic acid (PPADS) and 2 mM reactive blue 2 (RB-2). Basal MAP was 118.7 ± 10.2 (control), 128.7 ± 15.4 (PPADS), and 126.1 ± 12.7 (RB-2), respectively. Values are means ± SE (n = 8). Basal MAPs were not different. *P < 0.05 vs. α,β-methylene ATP.

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Response to Muscle Stretch

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DISCUSSION

In the present studies, we have shown that arterial infusions of α,β-methylene ATP (an ATP analog) evoked a dose-dependent pressor response (Fig. 1). This effect of α,β-methylene ATP was decreased from 35.2 ± 3.9 to 8.4 ± 2.7 mmHg (76%) after section of the sciatic nerve (Fig. 1). Thus the effect of α,β-methylene ATP was due primarily to stimulation of afferents within the hindlimb. The 8.4-mmHg response after sciatic nerve section was not due to skin afferents, because they were sectioned. Moreover, the pressor response was not due to a “systemic” effect of the infused α,β-methylene ATP, because local venous occlusion that preceded the arterial infusion did not attenuate the response. Therefore, the most likely cause of the 8-mmHg response was that the arterial injections stimulated muscle afferents subserved by the femoral or obturator nerves.

The increase in blood pressure due to an arterial infusion of α,β-methylene ATP was attenuated by 30% by the P2X receptor antagonist PPADS. However, the same concentration of the P2Y receptor antagonist RB-2 had no effect on the reflex (Fig. 2). On the basis of these findings, we believe that the dose of RB-2 was sufficient to block P2Y receptors (29, 33, 49). We believe that these findings suggest that P2X (but not P2Y) receptor activation contributed to the pressor response seen with ATP administration. Recent preliminary reports also support the idea that ATP mediates the muscle pressor reflex via P2X receptors (19, 20).

We have also shown that ATP and α,β-methylene ATP, a P2X-specific analog, sensitized the pressor response evoked by mechanically sensitive skeletal muscle stretch (Figs. 3 and 5). Furthermore, PPADS reduced the ATP-sensitizing effect on the reflex by 78%, whereas the P1 receptor antagonist 8-PT produced no effect on the reflex (Figs. 4 and 5). When these results are viewed together, we believe that they suggest that ATP is a direct chemical stimulant as well as a reversible mechanoreceptor sensor.

It is intriguing to note that the same dose of PPADS that reduced the sensitizing effect by 78% reduced the chemical effect by only 30%. These findings may suggest that the mechanoreceptor-sensitizing effect of ATP is more specific for the P2X receptor than is its direct metaboreceptor-stimulating effect.

In the present study, we considered the possibility that ATP infused into the arterial circulation of the triceps surae muscle could have been broken down to adenosine (27). Adenosine is a P1 receptor agonist. Accordingly, the P1 receptor antagonist 8-PT was injected by femoral artery before ATP injections. The P1 receptor agonist did not alter the pressor response to the infused ATP. Thus adenosine played no role in mediating the observed ATP effect.

General Issues Regarding ATP

Electrical stimulation of the L7/S1 ventral roots in cats evokes pressor and HR responses, and the magnitude of these responses is proportional to the generated muscle tension (11). It has also been reported that significant reflex cardiovascular responses occur when the triceps surae muscle is stretched mechanically to produce a pattern and amount of tension similar to that seen during static contraction (45). Studies by McCloskey and Mitchell (34) provided the first evi-
dence that group III and IV afferents represent the afferent limb of the reflex pressor response to muscle contraction. The free nerve endings of group III and IV afferent fibers have been identified in the interstitial spaces to be in close proximity to lymphatics and blood vessels of muscle and tendon tissue. These loci seem ideal for chemotransduction. Separate populations of group III and IV fibers have been identified to be in close proximity to collagen bundles. These receptors presumably are appropriately situated to act as mechanically sensitive receptors (3).

Adreani et al. (1) used a “walking cat preparation” to investigate the discharge properties of group III and IV afferents, the receptor fields of which were in the triceps surae muscle. In these extremely demanding studies, normal gait was induced by electrical stimu-

Table 2. Basal and reflexive HR during 1-min muscle stretch after arterial injection of ATP and α,β-methylene ATP

<table>
<thead>
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<th>HR, beats/min</th>
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<tbody>
<tr>
<td></td>
<td>Basal</td>
</tr>
<tr>
<td>Control (saline)</td>
<td>180±10</td>
</tr>
<tr>
<td>ATP (2.0 mM)</td>
<td>175±11</td>
</tr>
<tr>
<td>α,β-Methylene ATP (0.1 mM)</td>
<td>176±12</td>
</tr>
<tr>
<td>8-PTA + ATP</td>
<td>178±9</td>
</tr>
<tr>
<td>PPADS + ATP</td>
<td>188±11</td>
</tr>
<tr>
<td>Recovery (saline)</td>
<td>180±16</td>
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Values are means ± SE; n = 8. 8-PTA, 8-(p-sulfophenyl)-theophylline. There is no significant difference among baselines (n = 8).

Fig. 3. Effect of ATP and α,β-methylene ATP on muscle pressor reflex evoked by activation of skeletal muscle afferents during muscle stretch of 2-kg tension. A: reflex pressor response during 1 min of induced stretch in saline (control), with arterial injection of 2 mM ATP and 0.1 mM α,β-methylene ATP, and at recovery. Basal MAPs were 138.8 ± 12.6, 128.9 ± 11.1, 122.6 ± 11.8, and 116.7 ± 11.4 mmHg, respectively (P > 0.05). Values are means ± SE (n = 8). *P < 0.05, control vs. 2 mM ATP and 0.1 mM α,β-methylene ATP. **P < 0.05, control vs. 0.1 mM α,β-methylene ATP. B: representative traces showing that 2 mM ATP and 0.1 mM α,β-methylene ATP sensitized pressor response to muscle stretch.
P2X RECEPTORS AND MUSCLE PRESSOR REFLEX

Fig. 4. Effect of P2X purinoceptors on sensitized muscle pressor reflex by ATP. Reflex pressor response was measured during 1 min of induced stretch after arterial injection of saline (control), 2 mM ATP, and ATP with previous injection of PPADS. Basal MAP was 138.8 ± 12.6, 128.9 ± 11.1, and 123.1 ± 9.6 mmHg, respectively (*P > 0.05). Values are means ± SE (n = 6). *P < 0.05, 2 mM ATP vs. 2 mM ATP + PPADS.

Fig. 5. Peak pressor response by muscle stretch 10 min after administration of saline (control), 2 mM ATP, 0.1 mM α,β-methylene ATP, 2 mM ATP and previously injected 2 mM 8-(p-sulfophenyl)-theophylline (8-PT), 2 mM ATP and previously injected PPADS, and saline (recovery). *P < 0.05 vs. control. **P < 0.05 vs. ATP.

ulation of the mesencephalic locomotor region. Group III and IV fibers discharged synchronously with muscle contraction (1). In a separate study with the same model, Adreani and Kaufman (2) showed that ischemia increased the discharge of equal percentages of group III and IV afferent fibers.

A number of substances, including diprotonated phosphate, prostaglandin, bradykinin, and lactic acid, have been suggested as potential chemical stimulators and mechanoreceptor sensitizers of muscle afferents (40–44, 46, 47). It must be emphasized that the precise role of each of these substances in evoking the muscle reflex remains to be determined. It is with this background and for the reasons previously cited that we performed studies to examine the role of ATP in stimulating muscle afferents.

In addition to intracellular functions (5, 6), purine nucleotides play a role as extracellular neurotransmitters or modulators by engaging P1 and P2 purinoceptors. P1 receptors are important in mediating the modulatory effects of adenosine. P2 receptors mediate the actions of ATP and related substances. Two main types of P2 purinoceptors have been recognized: P2X and P2Y (5, 16). P2X receptors are ligand-gated ion channels, and P2Y receptors are linked to G proteins. Stimulation of P2X receptors appears to be the primary way in which ATP evokes its effect on sensory nerves (4, 35, 48, 52). For example, in the urinary bladder, ATP is released by smooth muscle cells during stretch. This released ATP stimulates P2X receptors on afferent nerves, evoking pain and bladder distension (9, 14).

ATP can be released from exercising muscle cells (15, 39). This effect need not involve muscle cell destruction, because it has been demonstrated that mechanical stimulation from excitable and nonexcitable cells, including epithelial, neuronal, and muscle cells, can cause the release of ATP (18, 38, 51, 54). Thus mechanical stimulation of muscle, as well as muscle cell destruction, can lead to the release of ATP by muscle. It has also been reported that ATP and norepinephrine are coreleased from sympathetically innervated smooth muscle (50, 53). Thus sympathoexcitation during exercise may release ATP, which in turn may sensitize sensory afferents in muscle. Despite the ability of muscle cells and sympathetic nerves to release ATP, it must be emphasized that muscle cells are not permeable to the relatively large ATP molecule and that ATP in the extracellular milieu is normally kept extremely low by extracellular ectonucleotidases (17).

Study Limitations

A few issues need to be addressed before it can be definitively concluded that ATP is an important stimulant of the muscle reflex during physiological circumstances. First, it must be determined that muscle contraction and/or muscle stretch increases interstitial ATP concentrations to a level necessary to stimulate and/or sensitize muscle afferents. Second, it must be demonstrated that the effects seen with injections of ATP are not due to the release of other substances from muscle or nerve into the interstitium. Third, the effects of P2X receptor blockade on the pressor response to muscle contraction must be determined. A recent preliminary report (20) has shown that the P2X receptor antagonist PPADS, injected into the femoral artery, attenuates the pressor response seen with static muscle contraction.

Conclusion

The findings of this report suggest that ATP stimulates P2X receptors located on the free nerve endings of muscle afferents in a manner similar to that whereby ATP and its analogs excite sensory afferent nerves in other organs. Arterial injection of the P2X receptor agonist α,β-methylene ATP in the blood supply of the triceps surae muscle evoked a pressor response that was a reflex localized to the cat hindlimb. The reflex response to arterial infusions of ATP was reduced by 30% by P2X receptor blockade. Furthermore, the findings of this report suggest that α,β-methylene ATP and
ATP enhance the muscle pressor response evoked by mechanically sensitive muscle stretch. The P2X receptor antagonist PPADS reduces the effect of ATP by 78%. This activation of ATP-sensitive P2X purinoceptors in skeletal muscle may play a role in mediating the autonomic adjustments to exercise.

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REFERENCES


