A new function for ATP: activating cardiac sympathetic afferents during myocardial ischemia

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Submitted 20 August 2010; accepted in final form 22 September 2010

Fu LW, Longhurst JC. A new function for ATP: activating cardiac sympathetic afferents during myocardial ischemia. Am J Physiol Heart Circ Physiol 299: H1762–H1771, 2010. First published September 24, 2010; doi:10.1152/ajpheart.00822.2010.—Myocardial ischemia activates cardiac sympathetic afferents leading to chest pain and reflex cardiovascular responses. Brief myocardial ischemia leads to ATP release in the interstitial space. Furthermore, exogenous ATP and α,β-methylene ATP (α,β-meATP), a P2X receptor agonist, stimulate cutaneous group III and IV sensory nerve fibers. The present study tested the hypothesis that endogenous ATP excites cardiac afferents during ischemia through activation of P2 receptors. Nerve activity of single unit cardiac sympathetic afferents was recorded from the left sympathetic chain or rami communicates (T2-T5) in anesthetized cats. Single fields of 45 afferents (conduction velocities = 0.25–4.92 m/s) were identified in the left ventricle with a stimulating electrode. Five minutes of myocardial ischemia stimulated 39 of 45 cardiac afferents (8 Aδ, 37 C fibers). Epicardial application of ATP (1–4 μmol) stimulated six ischemically sensitive cardiac afferents in a dose-dependent manner. Additionally, epicardial ATP (2 μmol), ADP (2 μmol), a P2Y agonist, and α,β-meATP (0.5 μmol) significantly activated eight other ischemically sensitive afferents. Third, pyridoxal phosphate-6-azophenyl-2,4-disulfonic acid, a P2 receptor antagonist, abolished the responses of six afferents to epicardial ATP (2 μmol) and attenuated the ischemia-related increase in activity of seven other afferents by 37%. In the absence of P2 receptor blockade, cardiac afferents responded consistently to repeated application of ATP (n = 6) and to recurrent myocardial ischemia (n = 6). Finally, six ischemia-sensitive cardiac spinal afferents did not respond to epicardial ATP (2–4 μmol), although these afferents did respond to epicardial bradykinin. Taken together, these data indicate that, during ischemia, endogenously released ATP activates ischemia-sensitive, but not ischemia-insensitive, cardiac spinal afferents through stimulation of P2 receptors likely located on the cardiac sensory neurites.

spinal afferents; adenosine 5′-triphosphate; myocardial ischemia; pyridoxal phosphate-6-azophenyl-2,4-disulfonic acid

ACTIVATION OF THINLY MYELINATED and unmyelinated cardiac sympathetic (spinal) afferent nerve fibers during myocardial ischemia is responsible for the transmission of information from the heart to the brain that ultimately produces angina and excitatory cardiac-cardiovascular reflex responses (21, 23, 40). Many metabolites produced during myocardial ischemia, including endothernin, thromboxane A2, 5-hydroxytryptamine, histamine, reactive oxygen species, and bradykinin (BK), but not adenosine, stimulate cardiac spinal afferents during ischemia and reperfusion in an interactive and multifactorial fashion (2, 17, 19–21, 24, 25, 44, 53). However, despite almost complete inhibition of afferent action induced by the receptor agonists, specific antagonists never fully eliminate the myocardial ischemia-induced increases in cardiac afferent activity, indicating that other mediators likely contribute to excitation of these afferents during ischemia.

The capability of extracellular ATP to alter somatic sensory neuronal function has been reported for over four decades (10, 13). Several sources of evidence suggest that extracellular ATP may play an important role in stimulation of cardiac spinal afferents during ischemia. For example, in living cells, the extra- and intracellular ATP concentrations range between 10 nM and 10 mM, since the cell membrane is impermeable to this nucleoside (56). During myocardial ischemia and hypoxia, a number of cell types, including myocytes, activated platelets, and nerve terminals, release large quantities of ATP in the extracellular or interstitial space through exocytosis or leakage following cell lysis (50, 57, 59). ATP concentrations are increased in the coronary effluent of hearts of experimental animals during myocardial hypoxia and ischemia (6, 57). A second piece of evidence is the observation that intradermal administration of ATP elicits pain in healthy humans and enhances inflammation-mediated pain (13, 29). ATP also activates sensory neurons, including those with cell bodies in the dorsal root (DRG) and nodose ganglia (34), and stimulates group III and IV muscle afferents in cats and pulmonary vagal afferents in dogs (30, 37, 45). Last, physiological experimental studies show that blockade of P2 receptors with pyridoxal phosphate-6-azophenyl-2,4-disulfonic acid (PPADS) abolishes the responses of muscle sensory nerve activity and the associated reflex pressor to intra-arterial injection of α,β-methylene ATP (α,β-meATP), a specific P2X receptor agonist (28, 30). Although there is no evidence for a role of extracellular ATP in activating cardiac spinal afferents during ischemia, the above evidence suggests the possibility that endogenously produced ATP might contribute to excitation of cardiac sympathetic afferent endings during myocardial ischemia.

Extracellular ATP generates its physiological actions through activation of purinergic 2 (P2) receptors, including P2X and P2Y (10). Extracellular ATP stimulates sensory neurons mainly through interaction with P2X receptors (10, 35). Most P2X receptor subtype mRNAs and proteins are found in peripheral sensory neurons, including the DRG and nodose and trigeminal ganglia (10, 11, 60). Additionally, P2Y receptor mRNAs, including P2Y1, P2Y2, P2Y4, and P2Y6 subtypes, are expressed in DRG neurons (15, 61). Application of ADP, a P2Y1 receptor agonist, to dissociated DRG neurons of rats and human embryonic kidney (HEK)-293 cells expressing TRPV1 receptors potentiates capsaicin-induced ion currents and evokes the sensation of pain in human subjects by stimulating cutaneous sensory nerves (4, 5, 51). Thus we hypothesized that extracellular ATP activates cardiac spinal afferents through...
activation of P2X and P2Y receptors. As such, the aim of the present study was to evaluate the role of extracellular ATP in activation of cardiac spinal afferents during myocardial ischemia. We employed neurophysiological approaches to test the possibility that endogenously produced ATP activates ischemia-sensitive, but not ischemia-insensitive, cardiac spinal afferents through both P2X and P2Y receptor mechanisms. Part of this study has been presented as a preliminary report (22).

METHODS

Surgical Preparation

Surgical and experimental protocols used in this study were approved by the Animal Care and Use Committee at the University of California at Irvine. The studies conformed to the American Physiological Society’s “Guiding Principles in the Care and Use of Laboratory Animals.” Adult cats of either sex (2.87 ± 0.37 kg, mean ± SD) were anesthetized by intramuscular injection of ketamine (20–30 mg/kg; Phoenix Scientific, St. Joseph, MO), followed by intravenous injection of α-chloralose (40–50 mg/kg) through the femoral vein. Additional injections of α-chloralose (5–10 mg/kg iv) were given as necessary to maintain an adequate depth of anesthesia that was assessed by observing the absence of a conjunctival reflex. The trachea of each animal was intubated, and respiration was maintained artificially (Harvard pump, model 661; Ealing, South Natick, MA). Cats were ventilated by air supplemented with 100% O2 through the respirator. The femoral vein and artery were cannulated for administration of drugs and fluid, and the measurement of blood pressure, respectively. A pressure transducer (Statham P 23 ID; Gould) was connected to the femoral arterial catheter for measuring arterial blood pressure. Arterial blood gases were assessed frequently with a blood gas analyzer (model ABL-5; Radiometer, Copenhagen, Denmark) and were maintained within physiological limits (P02 >100 mmHg, PC02 = 28–35 mmHg, pH 7.35–7.45) by adjusting the respirator rate or tidal volume, or by intravenously administering 2–3 ml of 1 M of NaHCO3 (8.4% wt/vol). Another PE-90 catheter was introduced in the high-impedance probe (model HIP511; Grass Instruments, Quincy, MA) cotton thread to the animal. The recording electrode was attached to the heart using a bipolar stimulating electrode to search for the location of the heart by gently probing the epicardial surface with a cotton swab and constricting the thoracic aorta as well as chemical stimulation with epicardial application of BK (2–3 μg) on one of the ventricles. The conduction velocity (CV) of each afferent fiber was calculated by dividing conduction distance by conduction time. The conduction time was determined by measuring the time interval from electrical stimulation to the evoked afferent’s action potential. Conduction distance was estimated by measuring the length of a wet thread between the receptive field and the recording electrode. Unmyelinated C and thinly myelinated Aδ fiber afferents were classified as those with CV of <2.5 and 2.5–30 m/s, respectively. In the present study, each afferent had a single receptive field that could be located precisely in the left ventricles. Myocardial ischemia was induced by complete occlusion of the appropriate coronary artery supplying the regional receptive field of the cardiac afferent nerve with a thread placed around the vessel. Ischemia was confirmed by observing a regional change in the color of the myocardium, which has been closely correlated with the production of lactic acid as indicated by a reduction in tissue pH (43). Afferents were considered to be ischemically sensitive if their discharge activity during 3–5 min of myocardial ischemia increased at least 50% above baseline. To determine whether an afferent was chemosensitive, BK (2–3 μg) was applied on the ventricle, and the afferent response was recorded. Mechanosensitive afferents were identified by evaluating responses to aortic constriction, which raised systolic blood pressure to 170–190 mmHg for 15 s. Aortic constriction was induced by partially occluding the aorta with an occlusion cuff that had been placed around the descending thoracic aorta at the level of Tc. Afferents were classified mechanosensitive if their activity increased at least 50% above baseline during constriction, which has been shown to increase both cardiac pressure and volume (17, 24).

Experimental Protocols

Dose responses of ischemically sensitive cardiac spinal afferents to ATP. This protocol examined the response of ischemically sensitive afferents (n = 6) to graded doses of ATP (0.5, 1, 2, and 4 μmol), a general P2 receptor agonist. After identifying the location of the receptive field of an afferent fiber in the ventricles, the response of the cardiac afferent was measured during 3–5 min of myocardial ischemia. If the afferent responded to ischemia, then ATP or PBS (pH 7.4, vehicle) was applied to the receptive field on the surface of the heart by 1- to 1.5-cm2 filter paper for 4 to 5 min, and the afferent activity was recorded. Dose-response curves were generated with four different doses of ATP (0.5, 1, 2, and 4 μmol) applied at least 15 min apart to avoid tachyphylaxis. ATP at different doses or the vehicle was applied randomly. The remaining ATP was washed out with 1–2 ml of saline dripped on the receptive field 5 min after each epicardial application of ATP. ATP (Sigma-Aldrich, St. Louis, MO) was dissolved in PBS to make a stock solution (pH = 7.4), adjusted with NaHCO3 (8.4% wt/vol), and stored at −20°C. On the day of experiment, the ATP stock solution was further diluted with PBS to a concentration of 20, 10, 5, and 2.5 mM. PBS served as the vehicle.
Responses of ischemically sensitive cardiac afferents to ATP, ADP, and α,β-meATP. In this protocol, we evaluated the responses of ischemically sensitive cardiac afferents to ATP, ADP, α2P2Y receptor agonist, as well as α,β-meATP, α2X receptor agonist. After identification of an ischemically sensitive cardiac afferent, we recorded the afferent response to epicardial application of ATP (2 μmol), ADP (2 μmol), and α,β-meATP (0.5 μmol) for 4–5 min, applied randomly. The doses of these agonists were chosen based on previous studies and our preliminary ATP dose-response data (28, 45). Solutions of ADP and α,β-meATP (Sigma-Aldrich) were made by dissolving the agonist in PBS and adjusting the pH of both solutions with NaHCO₃ (8.4% wt/vol) to a final value of 7.4. They were stored at −20°C and were prepared weekly. A total of eight ischemically sensitive afferents were studied in this group.

Responses of ischemia-insensitive cardiac afferents to ATP, BK, and ischemia. In this protocol, we examined the responses of ischemically insensitive cardiac afferents (n = 6) to epicardial ATP (2–4 μmol), 0.2 ml of BK (10 μg/ml; Sigma-Aldrich), and ischemia. After identifying the location of the receptive field of an afferent fiber in the ventricle, the response of the cardiac afferent was measured during 3–5 min of ischemia. If the afferent did not respond to ischemia, then the afferent activity was recorded following application of ATP and BK for 4–5 min in random to the receptive field of the afferent.

Influence of P2 receptor blockade on response of afferents to ischemia. To examine whether blockade of P2 receptors with PPADS altered afferent responses to ischemia, we measured the afferent responses to repeated myocardial ischemia before and after epicardial application of PPADS (8 μmol). After locating the receptive field of an afferent in the heart, the afferent’s response to brief myocardial ischemia was evaluated. If the afferent responded to ischemia, repeated ischemia was conducted 5 min after epicardial application of PPADS and 30 min after the initial ischemia. PPADS (Sigma-Aldrich) was dissolved in PBS to a concentration of 40 mM, and the pH of the solution was adjusted with NaHCO₃ (8.4% wt/vol) to a final value of 7.4 if necessary. Previous studies have demonstrated that this dose of PPADS selectively and completely inhibits somatic sensory nerve responses by blocking P2 receptors (36, 45). BK (2 μg) was applied to the surface of the heart to establish responsiveness of the afferent after treatment with PPADS. A total of seven ischemically sensitive afferents were studied in this group.

To evaluate reproducibility of afferent responses to ischemia, six additional afferents in six animals were studied as time controls. After identifying an ischemically sensitive unit, each animal in this group was treated identically, with the exception that epicardial application of vehicle (PBS, 0.2 ml) was used in place of PPADS.

Effect of P2 receptor blockade on the afferent’s response to ATP. This protocol consisted of two groups of afferents to determine the influence of blockade of P2 receptors with PPADS on afferent responses to ATP. After the receptive field of an afferent on the heart was located, the response to ischemia was measured. If the afferent responded to ischemia, we recorded the response to epicardial application of ATP (2 μmol). Responses to repeated epicardial ATP were evaluated 5 min after epicardial application of PPADS (8 μmol) and 30 min after the initial ATP application. BK (2 μg) was applied to the surface of the heart to determine if the afferent remained responsive after treatment with PPADS. Six ischemically sensitive afferents were studied in this group.

To differentiate between variations in afferent responses to ATP and time-related effects, six additional afferents were studied as time controls. After identification, each ischemically sensitive unit was treated identically to the intervention group but was not subjected to PPADS.

Data Analysis

Discharge activity of cardiac spinal afferents was expressed in impulses per second and was averaged during the 3- to 5-min preischemia period and the 5 min of ischemia. We measured the responses of cardiac afferents to ATP, ADP, α,β-meATP, and PPADS by averaging discharge rates of the afferents during the entire period of response, defined as the time during which sustained activity exceeded baseline activity by 20%. During drug application, sampling periods varied between 50 and 150 s, depending on the responses of the afferent to the drug. Five-minute sampling periods were used to measure afferent activity during myocardial ischemia. Baseline activity was determined over the 3- to 5-min period immediately preceding ischemia.

Data are expressed as means ± SE. The effects of repeated application of ATP and repeat ischemia on the responses of the afferents were compared using a one-way repeated-measures ANOVA followed by the Tukey’s post hoc test. If the data were not normally distributed, as determined by the Kolmogorov-Smirnov test, they were compared with the Friedman repeated-measures ANOVA on Ranks and a Dunnnett’s post hoc test. We compared the effect of ATP, ADP, and α,β-meATP on the afferent discharge activity using a Student’s paired t-test. All statistical calculations were performed with SigmaStat software (Jandel Scientific Software, San Rafael, CA). Values were considered to be significantly different when P < 0.05.

RESULTS

Profile of Cardiac Afferents

The activities of 39 ischemia-sensitive and 6 ischemia-insensitive cardiac afferents were examined in the present study. Approximately 27% of these afferents were mechanosensitive, and all were chemosensitive. In the present study, we did not find any mechanosensitive units that discharged with a cardiac rhythm. Endings of all afferents were located in the anterior (n = 18) or posterior (n = 27) wall of the left ventricle (Fig. 1). The CV for these afferents ranged between 2.25 and 4.92 m/s. Eight-two percent (37 fibers) of the afferents were classified as C fibers. The remaining units (8 afferents) were Aδ fibers. No obvious association was found between CV and the responsiveness of the fibers to chemical stimulation or ischemia.

Dose-Related Responses of Ischemically Sensitive Cardiac Afferents to ATP

Epicardial application of ATP, a P2 receptor agonist, stimulated all ischemically sensitive cardiac sympathetic afferents tested. The effects of ATP on the entire group of six afferents (1 Aδ, CV = 3.51 m/s; 5 C fibers, CV = 0.82 ± 0.16 m/s) are summarized in Fig. 2. In our preliminary study, we observed that injection of 2–4 μmol or even 10 μmol of ATP in the left atrium did not stimulate any of the three afferents studied. However, all three afferents were activated by epicardial application of ATP (2–4 μmol). Thus we used the epicardial application of agonists and antagonists in the present study. We observed that epicardial application of 0.5 μmol of ATP excited only two of the six tested cardiac afferents. ATP at 1 μmol excited five of the six afferents, whereas 2–4 μmol of ATP excited all six afferents, significantly increasing their discharge activity in a dose-dependent manner (Fig. 2). In contrast, epicardial application of the vehicle did not stimulate any of the fibers tested (0.49 ± 0.09 to 0.51 ± 0.1 im/s). Epicardial application of ATP (2–4 μmol) evoked a small pressor response [increase in mean arterial pressure (MAP): 8 ± 2 mmHg] in three cats and a small depressor response (decrease in MAP: 11 ± 2 mmHg) in three other animals. The locations of each of the six afferent nerve endings that responded to ATP are shown in Fig. 1.
**Effect of ATP, ADP, and α,β-meATP on Activity of Ischemically Sensitive Afferents**

Representative tracings of a cardiac C fiber afferent that responded to epicardial application of ATP, ADP, and α,β-meATP are shown in Fig. 3. Epicardial ATP increased discharge activity of this afferent from 0.44 to 1.87 imp/s. α,β-meATP and ADP also noticeably increased afferent activity (Fig. 3, B and C).

Epicardial application of ATP, α,β-meATP, and ADP increased the discharge activity of eight ischemically sensitive afferents (2 Aδ, CV = 3.04 and 4.13 m/s; 6 C fibers, CV = 0.74 ± 0.13 m/s; Fig. 4). Similar to the effect of epicardial ATP, epicardial α,β-meATP (0.5 μmol), a selective P2X receptor agonist, stimulated all eight fibers and significantly increased their peak activity from 0.55 ± 0.09 to 2.33 ± 0.35 imp/s (Fig. 4A). Application of ADP (2–4 μmol) to the surface of the heart stimulated six of the eight fibers and increased their activity from 0.48 ± 0.11 to 1.60 ± 0.41 imp/s (P < 0.05). The locations of each of the eight afferent nerve endings that responded to the agonists are shown in Fig. 1.

Figure 4B shows the summated 2-s nerve activity during pre- and postapplication of the agonists on the receptive field of the afferent located in the epicardium. Similar to the changes in mean afferent nerve activity, the summated discharge activity dramatically increased after epicardial ATP, α,β-meATP, and ADP. Note that the response duration of the afferents to α,β-meATP (104 s) was much longer than the response to ATP (62 s) or ADP (50 s).

**Responses of Ischemically Insensitive Cardiac Afferents to ATP**

We observed that six cardiac spinal afferents, including two Aδ (CV = 3.04 and 4.13 m/s) and four C (CV = 0.96 ± 0.29 m/s) fiber afferents, did not respond to 5 min of ischemia. This group of ischemically insensitive afferents also did not respond to epicardial ATP (2–4 μmol). However, they did respond to epicardial BK (2 μg), and their activity was increased from 0.68 ± 0.12 to 2.26 ± 0.39 imp/s (Fig. 5). The locations of each of the six cardiac afferent nerve endings are shown in Fig. 1.

**Effect of Blockade of P2 Receptors on Activity of Cardiac Afferents During Myocardial Ischemia**

Representative tracings of a cardiac C fiber afferent that responded to myocardial ischemia in the absence and presence of PPADS are shown in Fig. 6. Ischemia increased discharge activity of this afferent from 1.41 to 5.32 imp/s (Fig. 6A). Antagonism of P2 receptors with epicardial application of PPADS (8 μmol) attenuated the ischemia-induced increases in cardiac afferent activity by 37% when their activity was averaged over a 5-min period (3.32 to 3.35 imp/s) (Fig. 6B).

The responses of two groups of cardiac afferents to brief myocardial ischemia are displayed in Fig. 7. In the first group, 5 min of ischemia significantly increased discharge activity of seven afferents (1 Aδ, CV = 3.23 m/s; 6 C fibers, CV = 0.66 ± 0.12 m/s) from 0.63 ± 0.18 to 2.49 ± 0.66 imp/s (Fig. 6A). After blockade of P2 receptors with PPADS (8 μmol), however, the ischemia-induced increase in the activity of these
cardiac afferents was significantly attenuated (2.49 ± 0.66 to 1.34 ± 0.47 imp/s) compared with their responses during the initial period of ischemia (Fig. 7A). However, these afferents still responded to epicardial BK application (2 μg, 0.55 ± 0.14 to 3.34 ± 0.46 imp/s) after PPADS. In another group, six additional afferents (1 Aδ, CV = 3.67 m/s; 5 C fibers, CV = 0.48 ± 0.07 m/s) responded consistently to 5 min of repeated myocardial ischemia in the presence of the vehicle (Fig. 7B). Locations of the 13 afferent nerve endings tested during ischemia are provided in Fig. 1.

Figure 7C shows the summed 2-s nerve activity during 5 min of myocardial ischemia in all seven cardiac afferents before and after administration of PPADS. Similar to the changes in mean afferent nerve activity, the summed discharge activity during the entire 5-min period of ischemia (including both the early and late periods of stimulation) was attenuated by ~36% after blockade of P2 receptors with PPADS, indicating that PPADS blocks predominantly the early and to a lesser extent the later afferent responses to myocardial ischemia.

Fig. 3. Neurohistograms in A–C show responses of ischemically sensitive cardiac sympathetic afferents [conduction velocity (CV) = 0.88 m/s, innervating posterior LV] to epicardial application of ATP (2 μmol, A), P2X receptor agonist αβ-meATP (0.5 μmol, B), and P2Y receptor agonist ADP (2 μmol, C). Panels 1–3 display representative tracings of discharge activity at times indicated by the lines above histograms. Each arrow in panels 1–3 indicates when the agonist was applied on the epicardium. Activity of this afferent increased from 0.89 to 3.26 imp/s during 5 min of myocardial ischemia. The afferent responses to epicardial ATP (1.87 imp/s), αβ-meATP (3.19 imp/s), and ADP (1.06 imp/s) were significantly higher than baseline activity.

Fig. 4. Bar graph displaying responses of 8 ischemia-sensitive cardiac afferents to epicardial application of ATP (2 μmol), αβ-meATP (0.5 μmol), and ADP (2 μmol). B: neurohistograms showing the summed 2-s discharge activity of all 8 cardiac afferents during application of ATP (B1), αβ-meATP (B2), and ADP (B3). Data are represented as means ± SE. *P < 0.05 compared with control.
Effect of Blockade of P2 Receptors on Cardiac Afferent Responses to ATP

The responses of two groups of cardiac afferents to ATP are displayed in Fig. 8. In the first group, ATP (2 μmol) significantly increased the discharge activity of six afferents (1 Aδ fiber, CV = 3.45 m/s; 5 C fibers, CV = 0.67 ± 0.11 m/s) from 0.42 ± 0.07 to 2.43 ± 0.39 imp/s (Fig. 8A). However, the responses of the afferents to ATP were abolished by blockade of P2 receptors with epicardial PPADS (8 μmol). PPADS itself did not change the activity of these afferents. In addition, after blockade with PPADS, these afferents still consistently responded to application of BK (0.59 ± 0.16 to 3.48 ± 0.58 vs. 0.68 ± 0.17 to 3.54 ± 0.64 imp/s, before vs. after PPADS). In the second group, six additional C fiber afferents (CV = 0.64 ± 0.13 m/s) responded consistently to ATP (2 μmol) following administration of the vehicle (PBS; Fig. 8B). The locations of the 12 afferent nerve endings are provided in Fig. 1.

DISCUSSION

We made four major novel findings in the present study. First, epicardial application of ATP increased discharge activity of ischemically sensitive cardiac afferents in a dose-dependent manner. Second, similar to ATP, epicardial application of α,β-meATP and ADP also excited ischemia-sensitive cardiac afferents. However, ADP, a selective P2Y agonist, only weakly stimulated six of the eight afferents compared with ATP, a P2 receptor agonist. Third, blockade of P2 receptors with PPADS attenuated the responses of cardiac afferents to myocardial ischemia and abolished the action of ATP on the ischemically sensitive afferents. Last, epicardial ATP did not stimulate the...
ischemia-insensitive cardiac spinal afferents, although the afferents did respond to epicardial application of BK. Thus these data strongly suggest that endogenously produced ATP contributes to activation of cardiac spinal afferents during ischemia through stimulation of P2 receptors.

The role of extracellular ATP in stimulation of somatic sensory nerve fibers leading to nociceptive pain and the associated reflex responses have been studied extensively (5, 10, 28, 29). In contrast, the effect of extracellular ATP on cardiac spinal sensory nerve function has not been evaluated. Application of ATP to the base of a blister in humans induces pain (5). Iontophoresis of ATP to the forearm skin of healthy humans produces a modest sensation of burning that lasts for several minutes (29). In the skin-nerve preparation of rats, ATP directly excites cutaneous Aδ and C fiber nociceptors (28). Neurophysiological data indicate that between 40 and 96% of cultured DRG sensory neurons respond to ATP by increasing cellular free Ca2+ concentrations or depolarization (3, 7, 46) through a mechanism involving ATP-gated ion channels (8, 55). In addition, ATP evokes pulmonary-cardiac depressor reflexes by stimulating pulmonary and cardiac vagal C fiber sensory nerve endings (33, 45). These results suggest that extracellular ATP has the potential to activate cardiac spinal afferents, although, until the current study, there has been no direct evidence to support this speculation.

Extracellular ATP produces its physiological and pharmacological effects through activation of P2 receptors. The subtypes of P2 receptors include ionotropic P2X receptors and metabotropic P2Y receptors (35, 56). P2X receptors activate ligand-gated ion channels, whereas P2Y receptors exert their actions through a G protein-coupled mechanism. In the nervous system, there is little evidence suggesting that P2Y receptors play an important role in activation of sensory nerves (see detail below). In contrast, there is abundant evidence indicating that P2X receptors are important for excitation of sensory nerve fibers and neurons in peripheral sensory ganglia (10, 28, 35). Seven subtypes of P2X receptors, including P2X1–7, have been cloned and characterized since the early 1990s (1, 10). Immunohistochemical studies have shown that all other P2X receptor subtype mRNAs and proteins, except P2X7, are expressed in sensory neurons located in the DRG and nodose and trigeminal ganglia, although P2X3 has the highest level of expression (11, 15, 60). Early in vivo electrophysiological studies indicate that activation of P2X receptors by the selective P2X receptor agonist α,β-meATP leads to excitation of cutaneous afferents (52). Application of α,β-meATP in normal and carrageen-inflamed skin of rats excites peripheral nerve endings of Aδ and C fibers in the skin (28). In studies of isolated neurons, investigators observed that ATP and α,β-meATP evoke rapid transient inward currents in IB4-labeled small-diameter neurons of rat DRG through acti-
vation of P2X receptors (9, 14, 46). Furthermore, two recent studies of mice lacking the P2X receptor gene reveal that the ionotropic P2X receptor channels are responsible for the response of sensory neurons to ATP (12, 49). We show in the present investigation that thinly myelinated and unmyelinated ischemia-sensitive cardiac fibers respond robustly to α,β-meATP (Fig. 3), indicating that P2X receptors are involved in the ATP-mediated activation of cardiac spinal afferents.

Compared with ionotropic P2X receptors, the possible role for metabotropic P2Y receptors in activation of the sensory nervous system has received limited attention. Eight mammalian subtypes of P2Y receptors, including P2Y<sub>1,2,4,6,11,12,13,14</sub>, have been cloned to date (10, 58). Among the P2Y receptors, P2Y<sub>1,2,4,6</sub> are G<sub>q</sub> protein coupled, whereas P2Y<sub>12,13,14</sub> are G<sub>i</sub> coupled (39). In situ hybridization studies have documented that P2Y<sub>1,2,4,6</sub> receptor mRNAs are present in rat DRG neurons (48, 61). P2Y<sub>1</sub>-like immunoreactivity also has been observed in cell bodies of rat nodose ganglia (16). However, the data obtained in studies of P2Y receptor function are controversial. For example, UTP, a selective agonist for P2Y<sub>2</sub> and P2Y<sub>4</sub> receptors, does not affect the release of either cGRP or substance P from isolated DRG sensory neurons in rats (32). UTP also does not evoke a muscle chemoreflex during activation of muscle afferent fibers (31). Excitation of P2Y<sub>1,12,13,14</sub> receptors by selective agonists, including ADP, adenosine 5′-O-(2-thiodiphosphate), or 2-methyladenosine 5′-triphosphate, inhibits activation of N-type Ca<sup>2+</sup> channels of DRG neurons in rats that, in turn, leads to antinociceptive effects (26, 27). In contrast, studies of ATP-mediated activation of cardiac spinal afferent function are supported by our observation that blockade of P2Y<sub>1</sub> and P2Y<sub>2</sub> receptors increase the excitability of sensory neurons. For instance, ATP potentiates Ca<sup>2+</sup> extrusion by the plasma membrane Ca<sup>2+</sup> pump of small-diameter rat DRG neurons, causing a decrease in amplitude and duration of the action potential after hyperpolarization that leads to an increase in excitability of these neurons. This effect is prevented by blockade of P2Y<sub>1</sub> receptors with the selective antagonist A3P5P (26, 54). In rat DRG neurons and HEK-293 cells expressing recombinant TRPV<sub>1</sub> receptors, through activation of P2Y<sub>1</sub> receptors, ADP and ATP potentiates capsaicin-induced ion currents and lower the threshold for activation of heat-sensitive currents from 42 to 35°C (51). Furthermore, activation of P2Y<sub>1</sub> receptors with ADP evokes the sensation of pain in human subjects during stimulation of cutaneous sensory nerve fibers (4, 5). Activation of P2Y<sub>2</sub> receptors with either UTP or ATP leads to a prolonged burst of action potentials in rat DRG sensory neurons (41). The later observations are supported by our current data showing that ADP stimulates ischemically sensitive cardiac spinal afferents through activation of P2Y receptors.

We also observed that application of ATP to the surface of the heart stimulates ischemically sensitive cardiac afferent nerve endings, whereas administration of ATP in the left atrium using the same dose or even higher doses does not activate these afferents, suggesting that it is necessary to stimulate P2 receptors on afferent nerve endings to cause their depolarization. In support of this contention, previous studies have documented that ATP breakdown occurs very rapidly, since its half-life is about 0.2 s when perfused in the circulatory system (42, 47) because there are numerous ectonucleotidases that hydrolyze this nucleoside very quickly. Additionally, our laboratory and others have shown that cardiac spinal afferent nerve endings are distributed near the epicardial surface where we found it was necessary to apply exogenous ATP to excite afferent fibers (2). Finally, previous studies have demonstrated that the P2X and P2Y receptors are present in the DRG (11, 15, 60, 61), and these DRG P2 receptors transported to the axonal nerve ending in the heart similar to the transport of DRG vanilloid receptors (62). As such, ATP most likely stimulates cardiac afferents through activation of the P2 receptors located on afferent endings.

Several lines of evidence suggest the possibility that endogenous ATP contributes to activation of ischemically sensitive cardiac afferents. First, as our current data show, exogenous ATP is capable of exciting cardiac spinal afferents through activation of P2X as well as P2Y receptors. Second, brief global myocardial ischemia enhances the release of ATP in the coronary venous effluent (6). Myocardial hypoxia likewise leads to a rapid increase of ATP concentration in the coronary effluent of isolated perfused hearts (57). Last, various cells, including myocytes, platelets, and nerve terminals, release large quantities of ATP in the interstitial space by exocytosis or leakage following cell lysis under the pathophysiological circumstances, including ischemia, hypoxia, and tissue acidosis (50, 59). Our hypothesis that ATP, released during myocardial ischemia, stimulates cardiac spinal afferent nerve endings is further supported by our observation that blockade of P2 receptors with PPADS significantly attenuates the ischemia-mediated increase in cardiac spinal afferent activity. PPADS blocks most of the subtypes of P2X ion channels and subtypes of P2Y<sub>G<sub>q</sub>-coupled</sub> receptors, but not P1 receptors (38, 58).

Cardiac spinal afferents can be classified as ischemia-sensitive and ischemia-insensitive in function (17, 18, 43). The ischemia-sensitive cardiac afferents likely serve as cardiac nociceptors that respond to noxious stimuli like myocardial ischemia and play an important role in evoking cardiac sympathetic reflexes and angina pectoris (17, 19, 24). The function of ischemia-insensitive cardiac afferents is less clear. Our data suggest that extracellular ATP likely acts specifically as a nociceptive stimulus because epicardial application of ATP stimulates ischemia-sensitive, but not ischemia-insensitive, cardiac sympathetic afferents. In support of this observation, Hamilton and his colleagues (28) have found that ATP stimulates cutaneous Aδ and C fiber nociceptors but not nociceptive fibers (28). Thus extracellular ATP likely acts as a specific noxious stimulus for excitation of cardiac nociceptors during ischemia.

In conclusion, with the use of combined neurophysiological and pharmacological approaches, the present study provides novel evidence demonstrating that endogenously produced ATP stimulates ischemia-sensitive, but not ischemia-insensitive, cardiac spinal afferents during myocardial ischemia. The action of extracellular ATP on these cardiac afferents is mediated through activation of P2 receptors likely located on the cardiac afferent endings. These new data broaden our understanding of the function of the endogenously released ATP in response to myocardial ischemia. Future studies are needed to elucidate the contributions of each of the P2X and P2Y subtype receptors in stimulation of ischemically sensitive cardiac afferents by using more selective P2X or P2Y subtype receptor agonists and/or antagonists.

Different ischemic mediators may affect cardiac afferent activity during specific time frames in an interactive and/or
multifactorial manner during myocardial ischemia. Previous studies from our laboratory have demonstrated that histamine affects afferent activity predominately during the later stages of ischemia (18), whereas endothelin appears to affect both early and later stages of ischemia (24). The present study suggests that endogenous ATP affects predominately the early afferent response to ischemia.

These differences in timing of stimulation indicate the need for further exploration of interactions between ATP and other ischemic mediators on cardiac afferent activity.

ACKNOWLEDGMENTS

We gratefully acknowledge the technical assistance of Jesse Ho and Alvin Nguyen. We also thank undergraduate students Steven Wu and Sherwin Barvarz for help with experimental procedures.

GRANTS

J. Longhurst holds the Larry K. Dodge and Susan Samuei Endowed Chairs. This study was supported by National Heart, Lung, and Blood Institute Grant HL-66217.

DISCLOSURES

No conflicts of interest are declared by the authors.

REFERENCES


