Electrophysiological-anatomic correlates of ATP-triggered vagal reflex in the dog. V. Role of purinergic receptors

Jiang Xu, William Kussmaul, Peter B. Kurnik, Mohamad Al-Ahdav, and Amir Pelleg
Department of Medicine, Drexel University College of Medicine, Philadelphia, Pennsylvania

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EXTRACELLULAR ATP, the ubiquitous adenine nucleotide, acts as a physiological regulator of the cardiovascular system by activating cell surface receptors (23). The latter are called P2 purinergic receptors (P2R), distinct from the P1, the adenosine receptors (P1R) (26). P2R are divided into two families: P2X, ligand-activated cationic channels, and P2Y, G protein-coupled, seven-transmembrane domain receptors. Fifteen P2R have been cloned heretofore. In the heart, ATP exerts negative chronotropic action on sinus node and ventricular pacemaker automaticity, as well as a negative dromotropic action on atrio-ventricular (AV) nodal conduction (17, 18, 22). These actions of ATP are mediated by adenosine, the product of ATP’s rapid degradation by ecto-nucleotidases and by a vagal reflex; when vagal involvement is prevented (e.g., bilateral cervical vagotomy, or atropine), equimolar doses of adenosine and ATP cause similar slowing of heart rate (17, 18, 21).

Our earlier studies attempted to localize the anatomic site at which ATP triggers the vagal reflex, as well as determine the type of receptor that mediates the negative chronotropic action of ATP in the canine heart (10, 12, 13, 20, 22). The following observations in these studies have suggested that the site of ATP’s action is in the left ventricle. 1) Given directly into the sinus nodal artery, ATP is equipotent to adenosine, and its action is devoid of a vagal component. 2) ATP given into either the left main (LM), left circumflex coronary artery (LCA), or left anterior descending coronary artery (LAD) exerted a vagal-dependent negative chronotropic effect on sinus node automaticity (the site-potency rank order of this effect was LM > LCA > LAD). 3) Doses of intracoronal ATP (i.e., 0.1–1.0 μmol/kg) that exerted vagal-dependent negative chronotropic effects were below the threshold dose of intra-right atrial (RA) ATP for triggering a vagal reflex. 4) The negative chronotropic action of intra-RA ATP was not affected by pulmonary denervation. 5) ATP administered into the carotid artery or descending aorta did not trigger a vagal reflex. 6) The site time to peak effect rank order was as follows: RA > aortic root > intracoronary. 7) Bilateral cervical vagotomy abolished the negative chronotropic effect of intracoronary ATP but only attenuated that of intra-RA ATP. 8) The site-potency rank order for a given dose of ATP was intra-left coronary artery > aortic root > intra-RA. Regarding the type of P2R that mediates the stimulatory action of extracellular ATP on vagal afferent nerve terminals, the potency rank order of ATP and its analogs has suggested mediation by a P2X purinergic receptor subtype (P2XR).

The goals of the present study were 1) to confirm that the left ventricle is the sole site in the canine heart at which extracellular ATP activates vagal afferent nerve terminals and 2) to determine the P2X receptor subtype that mediates the action of ATP. For this purpose, the negative chronotropic actions of adenosine and ATP, given at equimolar doses into the right coronary artery (RCA) and LCA, were quantified. Then, the effects of two P2XR antagonists, diinosine pentaphosphate (Ip5I)—a P2X 1 and P2X3R antagonist—and 2’3’-(2,4,6-trinitrophenyl)-ATP (TNP-ATP)—an antagonist of the heteromeric P2X2/3 receptor (P2X23R), as well as the muscarinic cholinergic receptor blocker atropine, on the negative chronotropic action of intra-LCA ATP were determined in a closed-chest canine model.

Address for reprint requests and other correspondence: A. Pelleg, Drexel Univ. College of Medicine, Dept. of Medicine, 245 N 15th St., M.S. #110, Philadelphia, PA 19102-1192 (E-mail: pellega@drexel.edu).

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The present data support the hypothesis that extracellular ATP triggers a cardio-cardiac vagal depressor reflex by activating P2X2/3R located on vagal sensory nerve terminals localized in the left ventricle of the canine heart.

A preliminary report of the present data has been previously published in abstract form (24).

MATERIALS AND METHODS

The Institutional Animal Use and Care Committee of Drexel University College of Medicine approved the experimental protocol.

Experimental model. Mongrel dogs of either sex (n = 13; 24 ± 2 kg) were sedated with acepromazine (0.2 mg/kg im), anesthetized with pentobarbital sodium (30 mg/kg + 3 mg·kg⁻¹·h⁻¹ iv), intubated with an endotracheal tube, and ventilated with room air supplemented as necessary with O₂ using a Harvard respirator. A thermal mattress was used to maintain body temperature at a physiological range; body temperature was monitored with a rectal thermometer. Heparin (50 U/kg iv) was given to prevent thrombus formation. A peripheral vein was cannulated for the administration of the anesthetic, atropine, and the physiological saline solution. An electronic pressure transducer-tipped catheter (Millar) was inserted via the left femoral artery and positioned in the descending aorta. Several catheters, introduced via a Deseret sheath positioned in the right femoral artery and positioned in the LCA, Judkins left coronary diagnostic catheter (JL3, 6 F; Cordis, Miami FL) and Judkins left coronary guiding catheter (JL3.5, 6 F; Cordis); RCA, Judkins right coronary guiding catheter (JR 3.5, 6 F, Cordis) and internal mammary catheter (6 F, Cordis). ISOVUE-370 contrast media and Philips BV29 fluoroscopy system were used for coronary angiography and positioning of catheters.

Protocol. After a stabilization period of 30 min, adenosine, ATP (0.1 μmol/kg), and control solution (physiological saline solution) were administered at random initially into the right coronary artery and, subsequently, into the LCA. When administered into the LCA, two additional doses of adenosine and ATP were used: 0.01 and 1.0 μmol/kg. ATP was administered into the LCA also before and after intra-LCA Ip5I (5 mg) or TNP-ATP (5 mg), and intravenous atropine (0.2 mg/kg). Our previous studies have shown that adenosine and ATP at a dose of 0.1 μmol/kg are devoid of any effect on either sinus node automaticity or AV nodal conduction when given systemically (i.e., intravenously). Ample time was given for the recovery of baseline conditions between consecutive injections whenever necessary. Only one antagonist (i.e., TNP-ATP, Ip5I, or atropine) was used in a given animal.

Data acquisition and analysis. The following parameters were monitored and recorded using Gould Data Acquisition System (no. 5900), TEAC tape recorder (XR-5000), Dell Computer (Precision 410), data acquisition software (Datagaq), Astromed (no. 9500) chart recorder, and Ciba-Corning blood gas systems (no. 200): standard lead I and II ECGs, systemic arterial blood pressure, and blood pH, PaO₂, and PaCO₂. The negative chronotropic effects of adenosine and ATP were calculated as a percentage of the largest prolongation of sinus cycle length (%ΔSCL), measured as P-P interval, according to the following formula: %ΔSCL = [(SCLmax - SCLb)/SCLb] × 100, where SCLmax is the longest SCL induced by the test compound, and SCLb is the baseline SCL. One-way ANOVA and Scheffé’s post hoc comparison were used to determine significant differences in %ΔSCL at the level of P < 0.05. Data are presented as means ± SE.

RESULTS

Baseline data. Following the stabilization period, SCL was 445 ± 23 ms and systolic blood pressure was 149 ± 6 mmHg (n = 13).

Intra-RCA adenosine and ATP. Because of the relatively small size of the RCA in the dog, successful uncomplicated positioning of the intracoronary infusion catheter was achieved in a limited number of animals. Given at a low dose (0.1 μmol/kg) as a bolus injection into the right coronary artery, ATP and adenosine had a negligible effect on sinus node automaticity, that is, %ΔSCL of 31 ± 20 and 23 ± 16 (n = 5) (P = NS), respectively; at the higher dose of 1.0 μmol/kg, the effect was 38 and 130 (n = 2), respectively. Irrespective of the dose, the time to peak effect after the administration of ATP and adenosine was always 7–10 s (compared with <2 s for intra-LCA administration; see below). Control (physiological saline solution; same volume as that of the test compound solutions) injections were without effect.

Intra-LCA adenosine and ATP. Intra-LCA adenosine did not significantly affect sinus node automaticity. Specifically, at a dose of 0.01, 0.1, and 1.0 μmol/kg, %ΔSCL was 1.0 (n = 2), 6 ± 4 (n = 8), and 14 ± 14 (n = 3), respectively (P = NS). In contrast, ATP given at a molar dose equivalent to that of adenosine exerted a pronounced dose-dependent negative chronotropic effect on sinus node automaticity, manifested in marked abrupt prolongation of SCL. A typical example of the effects of intra-RCA and intra-LCA adenosine and ATP (0.1 μmol/kg) is shown in Fig. 1. As can be seen, neither adenosine nor ATP affected sinus rate when given into the RCA; similarly, intra-LCA adenosine had no effect on sinus rate. In contrast, intra-LCA ATP had a pronounced transient negative chronotropic effect, which peaked in <2 s after its administration. After 0.01, 0.1, and 1.0 μmol/kg ATP, %ΔSCL was 116 ± 34 (n = 3), 162 ± 28 (n = 12), and 203 ± 35 (n = 6), respectively (each, P < 0.05; Fig. 2). This effect of ATP was rapid in onset (that is, ≤2 s after its administration) and short lasting. The pattern of an abrupt prolongation of the P-P interval was suggestive of sinus exit block induced by ATP. Repetitive administrations of ATP exerted reproducible effects, indicating lack of desensitization of the mediating receptor. Control (physiological saline solution; same volume as that of ATP solution) injections were without effect.

Blood pressure. Transient drop in blood pressure was observed subsequent to the injections of either ATP or adenosine in either the RCA or the LCA (Table 1). The time to peak effect was 11–12 s, significantly longer (P < 0.05) than the time to peak effect of ATP on heart rate (<2 s), indicating that these effects were mediated by the direct dilatory actions of ATP and adenosine on the peripheral resistive vasculature.

Effects of TNP-ATP, Ip5I, and atropine. Intra-LCA TNP-ATP completely abolished the negative chronotropic effect of the low dose of ATP (0.01 μmol/kg; n = 2) and markedly attenuated that of 0.1 μmol/kg; that is, %ΔSCL was reduced from 179 ± 30 to 27 ± 12 (n = 5, P < 0.05). A typical example of the effect of TNP-ATP on the chronotropic action of ATP (0.01 μmol/kg) is shown in Fig. 3. As can be seen, baseline SCL of 640 ms (Fig. 3, top trace) was maximally prolonged to 1,260 ms (Fig. 3, middle trace); when ATP was given again 20 s after TNP-ATP (5 mg; intra-LCA bolus), it...
DISCUSSION

New information. The present results suggest that the heteromeric P2X2/3R located on left ventricular vagal nerve terminals mediates the central reflex triggered by intra-LCA ATP. Because these nerve endings are considered to play a critical mechanistic role in neurally mediated syncope, it is tempting to speculate that extracellular ATP is involved in this syndrome. In addition, it can be proposed that ATP, released into the extracellular space from ischemic myocytes, is an endogenous mediator of atropine-sensitive bradyarrhythmias associated with infero-posterior wall myocardial infarction.

Background and previous findings. Extracellular ATP is a physiological regulator in the cardiovascular system; in the heart, ATP exerts pronounced negative chronotropic and dromotropic actions on cardiac pacemakers and AV nodal conduction, respectively (23). Two decades ago, we showed that these effects of ATP are mediated by adenosine, which is the product of ATP’s rapid degradation by ecto-nucleotidases, and by a central vagal reflex; when vagal involvement was excluded (e.g., bilateral cervical vagotomy, atropine pretreatment), ATP and adenosine were equipotent (17, 18). Since then, we have been studying the vagal component of ATP’s action in the canine heart with the following objectives: 1) to determine the anatomic site at which ATP triggers the afferent neural traffic to the medulla and 2) to identify the type of receptor that mediates this action of ATP (10, 13, 14, 20–22). Data obtained in these studies have suggested that ATP stimulates vagal sensory nerve terminals localized in the left ventricle of the canine heart and that this action is mediated by P2X receptors. The present data give further support to this interpretation and suggest that P2X2/3R is the receptor subtype activated by ATP.

P2 purinergic receptors. Several lines of evidence suggest that P2X2/3R is the P2R subtype that mediates the action of ATP on left ventricular vagal sensory nerve terminals in the canine heart. The P2XR receptors, seven of which have been cloned heretofore, are ligand-binding cationic channels that are commonly found on excitable cells. Their signal transduction pathways are membrane delimited, which enables rapid response to agonist activation. This rapid response to agonist activation provides support for P2XR receptors’ role in neurotransmission, in general, and rapid activation of central and local reflex loops, in particular (4, 16). Homomorphic P2X2R and heteromeric P2X2/3R are found on peripheral terminals of small-diameter sensory neurons (6, 15, 33), and a phenotype characterized by coexpression of P2X2R and P2X2/3R is found in nodose ganglion cells (29). Thus it is not surprising that localized application of ATP at the canine epicardial sites triggers afferent neural traffic in the nodose ganglion (2).

We have previously shown that in the canine heart, intra-left coronary α,β-methylene-ATP (α,β-mATP) is a potent stimulator of left ventricular vagal afferent nerve terminals (13). Of the seven P2XRs, only the homomeric P2X1R, P2X3R, and P2X7R, as well as the heteromeric P2X2/3R, are sensitive to α,β-mATP (32). P2X1 and P2X3 are rapidly desensitized after stimulation by agonists; however, in the present model, repeated administrations of ATP and its similarly active analogs were not associated with desensitization, thus arguing against the involvement of P2X1R and P2X3R in this action of ATP. In addition, Isla, a P2X3R antagonist, did not alter the action of ATP. Taken together, these observations suggest that P2X2/3R mediates the action of ATP on vagal sensory nerve terminals in the left ventricle of the canine heart.
Indeed, P2X2/3R does not exhibit rapid desensitization; it is sensitive to α,β-mATP, and it is potently blocked by TNP-ATP (16). That TNP-ATP completely abolished the vagal reflex triggered by intracoronary ATP in the present study is congruent with the aforementioned conclusion.

**Type of afferent fibers.** It is interesting to note that ATP can also generate afferent neural activity in capsaicin-sensitive vagal C fibers by stimulating bimodal (i.e., chemosensitive and mechanosensitive) nerve terminals in the canine lungs by activating P2XR (19). The ability of P2XR agonists to induce C fiber-dependent nociception has already been demonstrated (9). Whether this action of ATP on pulmonary C fiber nerve terminals is responsible for neurogenic (i.e., vagal dependent) bronchoconstriction induced by ATP (14) remains to be determined because ATP can also stimulate the pulmonary rapidly adapting receptors of afferent Aδ fibers (A. Pelleg and C. Hurt, unpublished observations). The ability of ATP to stimulate both capsaicin-sensitive (i.e., C fibers) and capsaicin-insensitive (Aδ) sensory nerve terminals is in agreement with previous observations of ATP-induced inward current in capsaicin-sensitive and capsaicin-insensitive small-sized and medium-sized dorsal root ganglion neurons, respectively (31). Although the type of fibers carrying the ATP-induced afferent neural activity from the heart has not been studied heretofore, several lines of evidence suggest that ATP stimulates the capsaicin-insensitive (Aδ) sensory fibers rather than the C fibers. Specifically, heteromeric P2X2/3R mediates the activation by ATP of capsaicin-insensitive primary afferent neurons (30). In addition, cell bodies of sensory Aδ and C fibers are located in the nodose and jugular ganglion, respectively (35), and accordingly, VR1 capsaicin receptor transcripts were not detected in nodose ganglia (5). Finally, ATP stimulates epicardial mechanosensitive and chemosensitive afferent nerve terminals whose cell bodies are in the nodose ganglion (2). Taken together, these data indicate that extracellular ATP stimulates sensory vagal afferent Aδ terminals in the left ventricle by activating P2X2/3R.

**Physiological and clinical implications.** Cardiopulmonary bimodal (mechanosensitive and chemosensitive) vagal sensory nerve terminals in the left ventricle have been implicated in the mechanism of vasovagal syncope (1). Specifically, it is believed that the initial autonomic response in the syncope cascade of events of increased sympathetic input to the heart, caused by the drop of systemic blood pressure, is followed by a paradoxical increase in vagal input to the heart and sympathetic withdrawal mediated by the cardiopulmonary receptors, including left ventricular mechanosensitive vagal nerve terminals. Thus, instead of initiating a reflex response that would accommodate the hemodynamic stress, the ensuing vagal afferent activity ultimately triggers inappropriate vasodilation and bradycardia that lead to syncope (3). Because extracellular ATP can also activate these receptors, it is tempting to speculate that ATP could be mechanistically involved in vasovagal syncope as one of the endogenous mediators postulated to enhance the sensitivity of the cardiopulmonary receptors responsible for the paradoxical increase in vagal input to the heart. Indeed, in 7 of 30 patients with unexplained syncope and a negative baseline head-up-tilt-table test (HUT), the administration of ATP (intravenous bolus) before a second HUT resulted in a positive test (25).

ATP and adenosine are released into the extracellular space under pathophysiological conditions associated with acute myocardial ischemia (23). Adenosine has been implicated with anginal pain through the activation of sympathetic chemosensitive nerve endings (27, 28). Atropine-responsive bradycardias associated with acute myocardial infarction have been hypothesized to be mediated by a vagal reflex triggered within the heart (7, 34). Thus, on the basis of previous and present data, it can be hypothesized that ATP is a mediator locally released during acute myocardial infarction that plays a

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**Table 1. Effects of intracoronary ATP and Ado on blood pressure**

<table>
<thead>
<tr>
<th>Dose, μmol/kg</th>
<th>BPmin, mmHg*</th>
<th>Time to Peak Effect, s</th>
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<tbody>
<tr>
<td>ATP</td>
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<tr>
<td>0.1</td>
<td>120 ± 9</td>
<td>12 ± 0</td>
<td>10</td>
</tr>
<tr>
<td>1.0</td>
<td>92 ± 13</td>
<td>11 ± 0</td>
<td>11</td>
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<tr>
<td>Ado</td>
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<tr>
<td>0.1</td>
<td>97 ± 8</td>
<td>11 ± 1</td>
<td>5</td>
</tr>
<tr>
<td>1.0</td>
<td>74 ± 6</td>
<td>12 ± 1</td>
<td>8</td>
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Data are the minimal arterial diastolic pressure (BPmin), as well as the time to peak effect. The effects of ATP and adenosine (Ado) were transient, and baseline BP was restored in <60 s following the administration of either ATP or Ado. *P < 0.05 each vs. baseline.

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**Fig. 3.** Typical example of the effect of intracoronary 2',3'-O-(2,4,6-trinitrophenyl)-ATP (TNP-ATP) on the chronotropic effect of intracoronary ATP. **Top:** baseline conditions; baseline sinus cycle length (SCLb) is 640 ms. **Middle:** control injection. ATP (0.01 μmol/kg) given as a rapid bolus into the LCA maximally prolonged SCL from 640 ms to 1,260 ms with a pattern of transient sinus exit block. **Bottom:** Same dose of ATP given in the same mode 20 s following intra-LCA TNP-ATP (5 mg, rapid bolus) is devoid of any effect. Arrowheads mark time of administration of ATP. SCLmax, longest SCL induced by test compound. P, QRS, and T designate atrial and ventricular depolarization and ventricular repolarization, respectively.
mechanistic role in the associated bradyarrhythmias by activating the P2X2/3R receptors on vagal sensory nerve terminals.

REFERENCES


