P2 purinergic receptor activation enhances cardiac contractility in isolated rat and mouse hearts

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Mei, Qibing, and Bruce T. Liang. P2 purinergic receptor activation enhances cardiac contractility in isolated rat and mouse hearts. Am J Physiol Heart Circ Physiol 281: H334–H341, 2001.—Activation of P2 purinergic receptors exerts a potent positive inotropic effect in the cardiac myocyte. However, it is unknown whether its activation can also cause an increased contractility in intact heart. With the use of isolated rat and mouse hearts, the objective of the present study was to investigate the effect of P2 receptor agonist on the function of the intact heart. In both Langendorff rat hearts and working rat and mouse heart models, the P2X receptor agonist 2-methylthio-ATP (2-meSATP) caused dose-dependent increases in left ventricular developed pressure, rate of contraction, and rate of relaxation. The extent of P2X receptor agonist-stimulated increase in contractility was significantly less than that stimulated by the β-adrenergic agonist isoproterenol. However, the increase in contractility occurred without a significant effect on the basal heart rate, in contrast to that caused by isoproterenol. In isolated rat ventricular myocytes, both ATP and the P2X receptor agonist 2-meSATP stimulated large increases in the myocyte contractile amplitude (107 ± 13% and 99 ± 9%, n = 17 cells from 5 rats and n = 19 cells from 6 rats, respectively). 2-meSATP caused only a slight increase in phospholipase C activity and could stimulate myocyte contractility in the presence of phospholipase C inhibitor U-73122, consistent with the role of a phospholipase C-independent P2X receptor in mediating the positive inotropic effect of 2-meSATP. The data provide evidence for a potentially important physiological role of the cardiac P2X receptor and for the concept that agonist at this receptor may be beneficial for the treatment of cardiac dysfunction.

heart; drugs; ATP; purines; inotropy

P2 PURINERGIC RECEPTOR activation exerts a number of potentially important effects in the cardiovascular system (for reviews, see Refs. 13 and 17). In cardiac myocytes, the endogenous ligand for the P2 receptor, ATP, stimulates a large increase in the cytosolic calcium transient and myocyte contractile amplitude (3, 5, 6, 19). ATP can be released from platelets, endothelial cells, or the ischemic myocardium and may augment contractile state in both healthy and diseased hearts (3, 4, 7, 8, 14, 22). Furthermore, ATP is released as a cotransmitter with norepinephrine from the sympathetic nerve endings and can further enhance the β-adrenergic-stimulated cardiac contractility in an additive or even synergistic manner (24). Although these data clearly demonstrated a pronounced stimulatory effect of ATP and other P2 receptor agonist on the cytosolic calcium level and contractile amplitude of cardiac myocyte, it is unknown whether activation of the P2 receptor can actually cause an increase in the contractility of the intact heart. The effects of P2 receptor agonist on the various important parameters of cardiac function, such as left ventricular developed pressures (LVDP), first derivative of rate of contraction over time (+dP/dt), first derivative of rate of relaxation over time (−dP/dt), and spontaneous heart rate remain to be determined. Therefore, the purpose of the present study was to investigate the effect of P2 receptor agonist on the cardiac function in intact heart preparations. Both the Langendorff and the working heart models were used. To help establish the function of P2 receptor activation in the intact heart, cardiac effects of P2 receptor agonist were determined in both rat and mouse hearts and were directly compared with those of β-adrenergic receptor agonist.

An additional objective was to determine the role of phospholipase C (PLC) in mediating the contractile effect of P2 receptor activation. Isolated rat cardiac ventricular myocytes, which enabled biochemical and cellular studies, were used as a model to investigate the role of PLC, similar to the studies carried out in the chick embryo ventricular myocytes (16).

MATERIALS AND METHODS

Methods

Measurement of cardiac functions in intact heart preparations. After intravenous injection of heparin sodium via tail vein (500 U/kg) and intraperitoneal anesthetization with Nembutal (125 mg/kg for rats and 150 mg/kg for mice), the heart, with all major vessels and lungs attached, was excised. The aorta was then cannulated with a 20-gauge catheter and was positioned −2 mm above the coronary ostia. For the Langendorff method, a water-filled latex balloon (no. 3) was inserted into the lumen of the left ventricle via the left atrium according to a previously described method (11). The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
distal end of the balloon-attached catheter was connected to a pressure transducer for measurement of intraventricular distal end of the balloon-attached catheter was connected to a pressure transducer for measurement of intraventricular pressure and $\frac{\text{d}P}{\text{d}t}$. The balloon was inflated to a constantly held diastolic pressure of 5–7 mmHg. The retrograde perfusion via the aorta was carried out by a pressure pump maintaining a column of Krebs-Henseleit solution (KHS) composed of (in mM) 120 NaCl, 4.7 KCl, 2.5 CaCl$_2$, 1.2 MgSO$_4$, 1.2 K$_2$HPO$_4$, 0.5 EDTA, 25 NaHCO$_3$, 2 pyruvate, and 11 glucose; pH 7.4 (following gassing with 95% O$_2$/5% CO$_2$ at 37°C) to provide a constant coronary perfusion pressure of 65 mmHg. We confirmed the coronary perfusion pressure by using a pressure transducer connected via a side port to the aorta perfusion cannula. Drugs were added in the KHS buffer and infused via retrograde perfusion of the coronary artery.

For the working heart model (10, 12), a column of KHS buffer produced a constant hydrostatic pressure of 65 mmHg (for rats) or 55 mmHg (for mice). The opening of the pulmonary vein was connected via a polyethylene (PE)-50 (for mice) or a PE-50 (for mice) catheter to a reservoir of KHS buffer that maintained a “venous return” flow of approximately 12 ml/min (rats) or 5 ml/min (mice) under the resting condition. The venous return was maintained by a constant level of hydrostatic pressure (7–8 mmHg) and yielded a steady rate of venous return. The entering KHS buffer was then switched from retrograde to antegrade perfusion and produced a work-performing heart preparation. The perfusate exited the left ventricle through the aortic cannula, which was connected to the aortic column of KHS buffer with a hydrostatic pressure of 55 mmHg (for mice) or 65 mmHg (for rat). Aortic flow was the amount of perfusate exiting the aortic cannula measured in millimeters per minute. Coronary flow, measured in millimeters per minute, was collected via opening of the pulmonary artery. The sum of aortic flow and coronary flow was the cardiac output. A 23-gauge catheter was inserted into the left ventricle, and its distal end was connected to a pressure transducer to record LV pressures and $\frac{\text{d}P}{\text{d}t}$. The LVDP was the difference between LV systolic and diastolic pressure. A side port of the reservoir allowed direct infusion of $\beta$-adrenergic agonist isoproterenol or P2X receptor agonist 2-methylthio-ATP (2-mSATP) into the KHS buffer that entered the left ventricle via the left atrium, which then entered the coronary circulation after ejection of drug-containing perfusate into the aorta.

The pressure recordings were channeled from amplifiers that had been calibrated by a transducer simulator/calibrator (Kent Scientific; Litchfield, CT). The signals were then digitized via an interface board (model PCM-DAS 168/330, Computer Boards; Mansfield, MA), which provided a high level of performance with analog input channels and digital channels. Data were analyzed by computer software (Work-Bench for Windows+®, Kent Scientific) designed for a personal computer (Dell). The amplified and digitized signals from the transducers were constantly displayed and analyzed. Data acquisition, signal display (LV pressures, $\frac{\text{d}P}{\text{d}t}$, and heart rate), and data analysis programs were run concurrently from the hard drive of the computer. Data points under each basal condition and during infusion of each drug concentration were summarized as means $\pm$ SE. Data obtained with and without drug were analyzed by Student’s $t$-test for possible statistically significant differences. Changes in contractile function were analyzed with a computerized system designed for a personal computer (Dell). The amplified and digitized signals from the transducers were constantly displayed and analyzed. Data acquisition, signal display (LV pressures, $\frac{\text{d}P}{\text{d}t}$, and heart rate), and data analysis programs were run concurrently from the hard drive of the computer. Data points under each basal condition and during infusion of each drug concentration were summarized as means $\pm$ SE. Data obtained with and without drug were analyzed by Student’s $t$-test for possible statistically significant differences. For comparing the effects between groups treated with two different agonists or under different conditions, unpaired $t$-test was used.

Preparation of homogeneous populations of cardiac ventricular myocytes isolated from adult Sprague-Dawley rats. Cardiac ventricular myocytes were prepared according to a modification of previously described procedure (23). In brief, hearts from 250-g adult Sprague-Dawley rats were perfused in a retrograde manner through the aorta with calcium-free HEPES/KHS buffer containing (in mM) 34.8 HEPES, 118 NaCl, 4.0 KCl, 15 glucose, 1.2 KH$_2$PO$_4$, pH 7.4, as well as collagenase for 30 min at 37°C. All solutions were bubbled with 100% oxygen. Hearts were then minced into small pieces with scissors and subjected to a 10-min extraction in the HEPES/KHS buffer (containing collagenase) in a shaking water bath at 37°C. Dislodged and isolated cells were neutralized with a medium containing 10% bovine serum albumin (BSA), 20% Dulbecco’s modified Eagle’s medium (DMEM), and HEPES/KHS buffer (70%, by volume) with a calcium concentration of 0.36 mM. The supernatant was removed by decantation with a pipette, and the combined pellets were resuspended in fresh washing media. After the cells were allowed to settle for 10 min, the washing medium was removed. The cells were resuspended in fresh washing media and settled carefully over a BSA bed (6% BSA in DMEM), which helped eliminate round, contracted myocytes as well as endothelial and smooth muscle cells. Rod-shaped ventricular cells were allowed to settle for 10–15 min, at which time the BSA solution was removed completely. The final pellet was resuspended in media containing 6% fetal bovine serum in DMEM with a calcium concentration of 1.8 mM, and the cells were plated in 35-mm culture plates for contractility studies. Typical preparations yielded $\approx 80–90$% rod-shaped viable ventricular myocytes.

Coating of plates with laminin. Contractility studies were performed using 12-mm glass coverslips coated with laminin (1 $\mu$g/ml) in 35-mm culture plates. Plates containing laminin-inDMEM solution were stored for 24 h at 4°C. The solution was then removed, and cells were plated as described above.

Measurement of myocyte contractile amplitude. Measurement of myocyte contractile amplitude was performed by using an optical video system as previously described (1, 23). A 12-mm glass coverslip was placed on the stage of an inverted phase contrast microscope in a perfusion chamber warmed up to 37°C and superfused at a rate of 1 ml/min with HEPES-buffered solution containing (in mM) 5 HEPES, 0.9 CaCl$_2$, 4 KCl, 140 NaCl, 0.5 MgCl$_2$, and 11 glucose (pH 7.4). Myocytes were field stimulated at a rate of 2 Hz with platinum electrodes connected to a voltage stimulator. Light-dark contrast at the edge of the myocyte provided a marker for measurement of the amplitude of motion. The amplitude of myocyte motion remained unchanged for at least 10 min, indicating the stability of the preparation. Myocytes were then perfused for 3 min with the same HEPES-buffered solution containing the P2 receptor or $\beta$-adrenergic receptor agonist. Changes in contractile amplitude were monitored, and recordings were made at 1-min intervals. The contractility measurement was made on only one cell per coverslip and each plate contained three coverslips.

Measurement of phosphoinositide response. Inositol phosphates were determined according to the basic method of Berridge et al. (2) and further modified as described by Podrasky et al. (16). Cells were preincubated with 10 $\mu$Ci/ml of myo-$[^{3}H]$inositol for 4 h and washed with inositol-free DMEM containing 15 mM LiCl and incubated in this LiCl buffer for 10 min at 37°C before being exposed to ATP or other nucleotide analogs. After extraction with 1 ml of chloroform-methanol-HCl (1:2:0.5 vol/vol/vol), the various inositol phosphates were separated on a 1.0 ml anion exchange column (AG × 8 resin, formate form), and d-myoinositol 1-phosphate [Ins(1)P], d-myoinositol 1,4-bisphosphate [Ins(1,4)P$_2$], and d-myoinositol 1,4,5-trisphosphate [Ins(1,4,5)P$_3$] were eluted sequentially with 100 mM formic acid/200 mM ammonium formate, 100 mM formic acid/600 mM ammo-
nium formate, and 100 mM formic acid/1 M ammonium formate, respectively. Columns were calibrated with each inositol phosphate standard to confirm the complete separation of Ins(1)P, Ins(1,4)P2, and Ins(1,4,5)P3. Recovery of each inositol phosphate was >95%. In other experiments, myocytes were preincubated with 5 μCi/ml of myo-[3H]inositol for 18 h, and the effects of P2 receptor agonists were then determined.

Materials

Myo-[3H]inositol was obtained from DuPont-New England Nuclear (Boston, MA). ATP, ADP, AMP, α,β-methylene ATP, β,γ-methylene ATP, 2-meSATP, and isoproterenol were obtained from Sigma (St. Louis, MO). Collagenase (type 2) was from Worthington Biochemicals (Lakewood, NJ). Three-month-old Sprague-Dawley rats and CD-1 mice were obtained from Charles River (Cambridge, MA).

RESULTS

Effect of 2-meSATP on Cardiac Function in Langendorff and Working Rat Heart Preparations

Although activation of the P2 purinergic receptor can cause a significant stimulation of the contractile amplitude in isolated cardiac myocytes, the question arises regarding whether the receptor can also mediate an increase in the contractility of the intact heart. To study this question, the contractile effect of 2-meSATP in isolated Langendorff-perfused heart preparation was determined. The P2X receptor agonist caused a significant increase in +dP/dt and −dP/dt in a dose-dependent manner (Fig. 1A). The maximal stimulation occurred at 100 nM of 2-meSATP and showed increases of 22 ± 3.5% and 17.3 ± 2.8% for +dP/dt and −dP/dt, respectively (n = 5, P < 0.05, paired t-test). LVDP also increased significantly in the presence of 100 nM 2-meSATP (% increase was 14.5 ± 1.6%, means ± SE, n = 5, P < 0.01, paired t-test). There was no significant change in the heart rate or coronary flow at any of the 2-meSATP concentrations (P > 0.1). These data indicate that activation of the P2X receptor can enhance cardiac performance without an attendant increase in the heart rate in the Langendorff model. In comparison, the maximal β-adrenergic stimulated increases in LVDP, +dP/dt, and −dP/dt were 142 ± 31, 370 ± 28, and 282 ± 59%, respectively (means ± SE, n = 5, P < 0.001, and paired t-test) and were significantly larger than the corresponding maximal increases in these parameters by 2-meSATP (P < 0.05, t-test). Whereas the P2X receptor agonist was able to stimulate cardiac contractility without affecting the heart rate, isoproterenol stimulated both the basal contractility (Fig. 1B) and heart rate. The percent increase in heart rate stimulated by 10 nM isoproterenol was 35 ± 4% (n = 5, P < 0.05, paired t-test).

The effect of P2X receptor agonist was then tested in the working heart model. 2-meSATP also caused a significant increase in +dP/dt and −dP/dt in a concentration-dependent manner with the maximal effect occurring at 100 nM (Fig. 1A) (% stimulation was 30 ± 4 and 24.5 ± 3, n = 5, P < 0.05, paired t-test). There was no significant stimulatory or inhibitory effect on the basal heart rate at any of the P2X agonist concentrations.

P2X receptor agonist can stimulate contractility and enhance performance in working mouse heart. A working mouse heart model was also used to further confirm the cardiac effects of P2X receptor activation in the intact heart. Similar to the data obtained in Langendorff and working rat heart models, 2-meSATP also caused dose-dependent increases in +dP/dt and −dP/dt (maximal percent stimulation of 24.5 ± 5.5% and 19.5 ± 2%, respectively, n = 5, P < 0.05, paired t-test) without significant effect on the heart rate (Fig. 2). Similar to the result obtained in the Langendorff model, there was no observed arrhythmia at any of the P2X agonist concentrations in the working heart preparation. The P2 receptor agonist also caused a significant increase in the cardiac output (Table 1).

Similar to the data obtained in the rat heart, the maximal isoproterenol stimulated increases in the +dP/dt and −dP/dt were 90 ± 7% and 47 ± 4%, respectively (n = 5, P < 0.05, paired t-test, Fig. 3A). The isoproterenol-stimulated increases in +dP/dt and −dP/dt were significantly larger than the corresponding increases stimulated by the maximally effective concentration of 2-meSATP (P < 0.05, t-test). The increase in cardiac output produced by isoproterenol was also significantly higher than that elicited by 2-meSATP (Table 1, P < 0.05, t-test). Similar to the rat heart, isoproterenol caused a marked increase in the basal heart rate (Fig. 3C) (P < 0.05, paired t-test).

Activation of P2 purinergic receptor increased the contractile amplitude of individual rat cardiac myocytes. To confirm that the same P2 receptor agonists can stimulate the contractility of individual isolated cardiac myocytes, effects of the agonists on the contractile amplitude were determined in cardiac myocytes isolated from Sprague-Dawley rats that were age and weight matched with those used in the intact heart studies. ATP was able to stimulate a marked increase in the myocyte contractile amplitude, with a half-maximal effective concentration (EC50) of 0.13 ± 0.01 μM and a maximal increase of 107 ± 13% (n = 17 cells from 5 rats with 3–4 myocytes per animal, means ± SE, P < 0.001, paired t-test) (Fig. 4). ADP and AMP had little stimulatory effect on the myocyte contractility with maximal increases of only 32 ± 8 (15 cells from 5 rats with 3 myocytes per rat, means ± SE, P < 0.05, paired t-test) and 18 ± 5% (n = 13 cells from 4 rats with 3 or 4 myocytes per animal, P < 0.05, paired t-test), respectively. Among the various ATP analogs, 2-meSATP was the most potent and efficacious agonist in stimulating the myocyte contractility with an EC50 of 0.08 ± 0.01 μM and a maximal increase of 99 ± 9% (n = 19 cells from 6 rats with 3 or 4 myocytes per animal, means ± SE, P < 0.001, paired t-test) (Fig. 4). The P2 receptor agonist α,β-methylene ATP was ineffective at stimulating myocyte contractility with a maximal increase of 19.4 ± 4.1% at 10 μM (17 cells from 5 rats with 3 or 4 cells per animal, P < 0.05, paired t-test). β,γ-Methylene-ATP was less effective at stimulating myocyte contractility with a maximal increase of <10%
Fig. 1. A: effects of 2-methylthio-ATP (2-meSATP) and isoproterenol on cardiac functions in the Langendorff and working rat heart preparations. Retrograde and antegrade perfusions of the aorta and coronary artery in the isolated rat heart were carried out using the Langendorff and working heart models, respectively (see MATERIALS AND METHODS). P2 receptor agonist 2-meSATP was added in the Krebs-Henseleit solution (KHS) buffer directly at the indicated concentrations to perfuse the heart via the coronary artery in a cumulative manner. Squares represent data obtained in the Langendorff model, whereas the circles represent data obtained in the working heart model. Typical data are shown and are representative of 5 rats. 

B: effects of isoproterenol on cardiac functions in the Langendorff rat heart preparation. Retrograde perfusion of the aorta and coronary artery in the isolated rat heart was carried out using the work-performing model (see MATERIALS AND METHODS). β-adrenergic receptor agonist isoproterenol was added in the KHS buffer directly at the indicated concentrations to perfuse the heart via the coronary artery in a cumulative manner. Typical data are shown and are typical of 4 other rats. $\ddot{d}P/dt$ and $-dP/dt$, first derivative of contraction and relaxation rate over time, respectively.
(n = 12 cells from 4 rats with 3 cells per animal). These data are consistent with the hypothesis that a 2-meSATP-sensitive P2 receptor is involved in mediating the positive inotropic response to ATP.

Because ATP is coupled to a pronounced stimulation of the phosphatidyl-4,5-bisphosphate-specific PLC (PIP2-PLC) activity (P < 0.001 vs. basal, paired t-test, n = 5) (Fig. 5A), it is possible that this PLC is involved in mediating the positive inotropic effect of P2 receptor activation. However, 2-meSATP had very little, if any, stimulatory effect on the PIP2-PLC activity (P > 0.1, paired t-test, Fig. 5A), arguing against a role of PLC in mediating the positive inotropic response to 2-meSATP. The aminosteroid U-73122, a known inhibitor of the receptor-mediated activation of PLC (19, 20), was used to further study whether PLC plays a role in the 2-meSATP-stimulated increase in myocyte contractility. The inhibitory effect of U-73312 in the rat cardiac myocyte was shown by the total abolition of the PLC activity stimulated by ATP (data not shown), consistent with the findings of Podrasky et al. (16) and others (20, 21). 2-meSATP was able to stimulate an increase in myocyte contractility in the presence of 10 μM of U-73122 (P < 0.05 vs. the contractile amplitude obtained in the presence of U-73122 alone, paired t-test, n = 16 myocytes from 6 rats with 2–4 cells per animal) (Fig. 5B). The increase in contractile amplitude stimulated by 2-meSATP in the presence of U-73122 (93 ± 8% increase, n = 16 cells) was similar to the increase caused by the same 2-meSATP concentra-

tion in the absence of U-73122 (99 ± 9%, n = 19 cells) (P > 0.1, t-test).

In comparison with the β-adrenergic-stimulated increase in myocyte contractility, the positive inotropic effect of 2-meSATP was significantly less than that induced by isoproterenol (100 nM), which typically caused a 255 ± 37% increase in the contractile amplitude relative to unstimulated cells (n = 13 cells from 5 rats with 2–3 myocytes per animal, P < 0.01, t-test). The maximal extent of stimulation by 2-meSATP was ~30% of that caused by isoproterenol.

**DISCUSSION**

Previous studies (3, 5, 6, 13, 16, 19, 24) have demonstrated that ATP and ATP analogs are capable of stimulating a pronounced positive inotropic effect in isolated cardiac ventricular myocytes and in intact papillary muscle. In addition to a marked increase in the cardiac myocyte contractile amplitude, there was also a significant stimulation of the cytosolic calcium transient. Whereas these data showed a clear increase in the contractile amplitude of isolated cardiac myocyte in the presence of P2 receptor agonist, it is unknown whether the P2 receptor agonist can stimulate contractility in the intact heart.

In the present study, we investigated whether the P2 receptor agonist-stimulated increase in the contractility of isolated cardiac myocyte can be translated into an increase in the contractile performance of the intact heart by using both the Langendorff and the work-performing heart models. In the Langendorff preparation, the ability of the heart to respond to receptor agonist can be determined by adding agonist to the buffer perfusing the heart via coronary artery directly (10, 12). The work-performing heart model offers several advantages that were not available in the Langendorff model. First, the perfusing buffer was infused into the left ventricle via the left atrium, allowing the heart to function as a pump in a more physiological manner. Second, the coronary perfusion was performed

<table>
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<tr>
<th>Parameters</th>
<th>Basal</th>
<th>2-meSATP</th>
<th>Basal</th>
<th>Iso</th>
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<tbody>
<tr>
<td>+dP/dt</td>
<td>3,002 ± 153</td>
<td>3,503 ± 242</td>
<td>2,863 ± 197</td>
<td>5,053 ± 476</td>
</tr>
<tr>
<td>-dP/dt</td>
<td>2,526 ± 86</td>
<td>3,163 ± 219</td>
<td>2,611 ± 211</td>
<td>3,376 ± 286</td>
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<tr>
<td>CO</td>
<td>5.9 ± 0.3</td>
<td>6.3 ± 0.3</td>
<td>6.56 ± 0.33</td>
<td>8.74 ± 0.32</td>
</tr>
<tr>
<td>HR</td>
<td>428 ± 14</td>
<td>440 ± 15.4</td>
<td>443 ± 18.5</td>
<td>624 ± 26</td>
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Values are means ± SE for 10 mice in 2-methylthio-ATP (2-meSATP) group and 9 mice in isoproterenol (Iso) group. CO, cardiac output; HR, heart rate; dP/dt, first derivative of contraction and relaxation over time, respectively. Antegrade perfusion in the isolated mouse heart was carried out using the work-performing model as described in MATERIALS AND METHODS. 2-meSATP and Iso were present at 100 nM. Basal, baseline values obtained before infusion of the agonists. Increases in +dP/dt, -dP/dt, and CO by both agonists were statistically significant (P < 0.05). Whereas 2-meSATP did not change HR, Iso caused a significant stimulation of HR (P < 0.05, paired t-test).
via a systolic-diastolic gradient more closely resembling the perfusion in vivo. Third, the working heart was continuously loaded with constant levels of baseline preload and afterload and allowed measurement of cardiac output. This enabled determination of the effect of β-adrenergic or P2 receptor agonist under the same basal loading condition.

Several lines of evidence support the concept that the P2X receptor agonist can enhance the performance of the intact heart. First, in both the Langendorff and the work-performing rat heart models, the P2X receptor agonist 2-meSATP stimulated significant increases in $+\frac{dP}{dt}$ and $-\frac{dP}{dt}$. Second, the stimulatory effect on cardiac function by the P2X receptor agonist is present in more than one species. Similar to the data obtained in work-performing rat heart, 2-meSATP also stimulated significant increases in $+\frac{dP}{dt}$ and $-\frac{dP}{dt}$ in the working mouse heart. Third, the dose-dependent nature of the stimulatory effect and the similarity in

Fig. 3. Effects of isoproterenol on cardiac functions in the working mouse heart preparation. Antegrade perfusion of the aorta and coronary artery in the isolated mouse heart was carried out using the work-performing model (see MATERIALS AND METHODS). Isoproterenol was added in the KHS buffer directly at the indicated concentrations to perfuse the heart via the coronary artery in a cumulative manner. Data are means ± SE for values obtained in 5 mice. Percent increase (above basal) in $+\frac{dP}{dt}$ and $-\frac{dP}{dt}$ (A) and in heart rate (C) was significant at all agonist concentrations. Increase in cardiac output (B) was significant at 10 and 100 nM of the β-adrenergic agonist. *$P < 0.05$, paired t-test.

Fig. 4. Effect of P2X receptor agonist 2-meSATP on cardiac myocyte contractile amplitude. Cardiac ventricular myocytes were isolated from adult Sprague-Dawley rats (see MATERIALS AND METHODS). Myocytes were paced at 2 Hz by field stimulation, and after a 5-min equilibration period, myocytes were superfused with HEPES-buffered medium containing the indicated concentrations of ATP or 2-meSATP. Representative tracings for ATP and 2-meSATP-stimulated increase in contractile amplitude are shown.
were also observed in myocytes prelabeled with 5 mM 

The mechanism by which activation of the cardiac P2X receptor exerts a positive inotropic effect is unclear. The P2X receptor is a ligand-gated cation channel capable of permeating sodium and calcium when it is activated. It is possible that an influx of sodium and calcium into the cardiac myocyte leads to an increase in the sarcoplasmic reticulum content of calcium and hence a greater level of calcium transients and myocyte contractility. Further investigation is required to test this hypothesis. A potential limitation of the data obtained in the intact heart study is that 2-meSATP may be degraded by the ectonucleotidases. Although 2-thioether derivatives of adenine nucleotide are less susceptible to degradation by nucleotidases than are the unmodified adenine nucleotides (25), the extent of resistance of 2-meSATP to ectonucleotidases relative to that of ATP is modest (15). However, ATP itself was also able to stimulate a marked increase in the +dP/dt in the Langendorff rat heart preparation (a maximal increase of 28 ± 4% at 100 nM, n = 6 rats, means ± SE). Thus, even with possible degradation of ATP and 2-meSATP, the continuous perfusion of the heart with agonist-containing media likely resulted in sufficient concentration of the active agonist at or near the myocyte P2X receptor.

The maximal P2 agonist-induced increases in the +dP/dt and −dP/dt ranged from 10 to 25% of those

the maximal extent of stimulation in both Langendorff and working heart models and in the two species argue against a nonspecific contractile effect of the P2 agonist.

Because only low concentration of ATP was required to induce an increase in the calcium level and myocyte contractile amplitude and because of an apparent dose-response relationship of the ATP effect, a cardiac myocyte P2 receptor is likely involved in mediating this effect. Although the identity of this P2 receptor is unknown, recent study shows that a PLC-independent mechanism appears to mediate the positive inotropic effect of P2 receptor agonist in a cultured chick embryo cardiac myocyte model (16). Several lines of evidence support the conclusion that a PLC-independent pathway is also involved in mediating the positive inotropic response to 2-meSATP in the rat cardiac myocyte. First, 2-meSATP could induce a marked stimulation of the myocyte contractility with very little if any effect on the PLC activity. Second, the PLC-specific inhibitor U-73122 did not affect the ability of 2-meSATP to stimulate myocyte contractility. The extent of 2-meSATP-stimulated increase in contractility in the presence of U-73122 was similar to that obtained in the absence of U-73122. Finally, although 2-meSATP can activate multiple subtypes of the P2X receptor, it appears to be selective at the P2X family than at the P2Y subfamily (9, 18). In contrast to P2Y receptors, which are coupled to PLC or adenylyl cyclase, the P2X receptors are ligand-gated ion channels and are not coupled to PLC. Thus the inotropic effect of 2-meSATP is likely mediated via activation of a P2X receptor on the cardiac myocyte. Taken together, the data obtained using the isolated rat cardiac myocyte are similar to those obtained in the chick embryo cardiac myocyte and suggest that a P2X receptor is also likely involved in mediating the positive inotropic response to 2-meSATP in the rat heart. The positive inotropic effect of 2-meSATP was less than that produced by the β-adrenergic agonist isoproterenol. In general, the magnitude of P2X receptor agonist-stimulated increase in contractile amplitude was ~35–40% of that stimulated by the β-adrenergic agonist.

The mechanism by which activation of the cardiac P2X receptor exerts a positive inotropic effect is unclear. The P2X receptor is a ligand-gated cation channel capable of permeating sodium and calcium when it is activated. It is possible that an influx of sodium and calcium into the cardiac myocyte leads to an increase in the sarcoplasmic reticulum content of calcium and hence a greater level of calcium transients and myocyte contractility. Further investigation is required to test this hypothesis. A potential limitation of the data obtained in the intact heart study is that 2-meSATP may be degraded by the ectonucleotidases. Although 2-thioether derivatives of adenine nucleotide are less susceptible to degradation by nucleotidases than are the unmodified adenine nucleotides (25), the extent of resistance of 2-meSATP to ectonucleotidases relative to that of ATP is modest (15). However, ATP itself was also able to stimulate a marked increase in the +dP/dt in the Langendorff rat heart preparation (a maximal increase of 28 ± 4% at 100 nM, n = 6 rats, means ± SE). Thus, even with possible degradation of ATP and 2-meSATP, the continuous perfusion of the heart with agonist-containing media likely resulted in sufficient concentration of the active agonist at or near the myocyte P2X receptor.

The maximal P2 agonist-induced increases in the +dP/dt and −dP/dt ranged from 10 to 25% of those
caused by the β-adrenergic agonist. Thus activation of the P2 receptor exerts a modest positive inotropic effect. However, the positive inotropic effect of P2 receptor agonist was not accompanied by any significant chronotropic effect. This is in marked contrast to the significant positive chronotropic effect that accompanied the β-adrenergic agonist-stimulated positive inotropic effect. Thus it is possible that the P2 receptor agonist-stimulated increase in contractility would occur without the expense of a rate-related increase in oxygen consumption. This property may make such agonist a beneficial agent in the treatment of left ventricular dysfunction and heart failure. Furthermore, an enhanced rate of relaxation by the P2X agonist suggests that such agonist may exert a beneficial effect by improving cardiac performance with enhanced contractility and relaxation. Whether the P2 receptor-mediated positive inotropic effect also exhibits rapid agonist-induced desensitization, like desensitization of β-adrenergic receptor by its agonist, is unclear and requires further investigation. Future studies are needed to determine whether agonist at this P2 receptor represents a novel therapeutic target. Overall, the data provide evidence for a potentially important physiological role of the cardiac P2X receptor. The release of the endogenous ligand ATP may further improve cardiac performance in healthy and/or diseased hearts.

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