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Role of A_{2A}-adenosine receptor activation for ATP-mediated coronary vasodilation in guinea-pig isolated heart

[1,4]Knut Ståle Erga, [3]Christoph N. Seubert, [1]Hui-Xiu Liang, [1]Lin Wu, *,[1]John C. Shryock & [1,2,5]Luiz Belardinelli

[1]Department of Medicine, College of Medicine, University of Florida, Gainesville, Florida, U.S.A.; [2]Department of Pharmacology, College of Medicine, University of Florida, Gainesville, Florida, U.S.A. and [3]Department of Anesthesiology, College of Medicine, University of Florida, Gainesville, Florida, U.S.A.

1 Adenosine-5′-triphosphate (ATP) and adenosine are potent coronary vasodilators. ATP is rapidly converted to adenosine by ectonucleotidases. We examined whether coronary vasodilation caused by exogenous ATP is mediated by P_2 receptor activation or by A_{2A}-adenosine receptor activation.

2 Effects of interventions on coronary conductance were determined by measuring coronary perfusion pressure in guinea-pig isolated hearts perfused at a constant flow of 10 ml min^{-1}.

3 ATP and adenosine both caused sustained, concentration-dependent increases of coronary conductance. Maximal responses to both agonists were equivalent. The values of pD_2 ($\pm$s.e.mean) for ATP and adenosine were 6.68 ± 0.04 and 7.06 ± 0.05, respectively. Adenosine was significantly more potent than ATP ($P < 0.0001$, $n = 10$).

4 The values of pIC_{50} for the selective A_{2A}-adenosine receptor antagonist SCH58261 to antagonize equivalent responses to ATP and adenosine were 8.28 ± 0.08 and 8.28 ± 0.06 ($P = 0.99$, $n = 6$), respectively.

5 The non-selective adenosine receptor antagonists xanthine amine congener (XAC) and CGS15943 antagonized similarly the equivalent vasodilations caused by ATP (pIC_{50} values 7.48 ± 0.04 and 7.45 ± 0.06, respectively) and adenosine (pIC_{50} values 7.37 ± 0.13 and 7.56 ± 0.11).

6 In contrast to ATP and adenosine, the two P_2 agonists 2-methylthio-ATP and uridine-5′-triphosphate failed to cause stable increases of coronary conductance, caused desensitization of vasodilator responses, and were not antagonized by SCH 58261, 8-parasulphophenyltheophylline, or XAC.

7 Glibenclamide attenuated coronary vasodilations caused by ATP and adenosine by 88 and 89%, respectively, but failed to attenuate those caused by 2-methylthio-ATP.

8 These results strongly suggest that sustained, submaximal coronary vasodilation caused by exogenous ATP is entirely mediated by adenosine acting upon A_{2A}-adenosine receptors.
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Keywords: Isolated heart; vasodilation; ATP; adenosine; A_{2A}-receptors; P_2-agonists; coronary regulation; ARL67156; AOPCP

Abbreviations: ADA, adenosine deaminase; AdoR, adenosine receptor; AMP-deaminase, 5′-adenylic acid deaminase; AOPCP, α,β-methylene-adenosine diphosphate (5′-nucleotidase inhibitor); ARL67156, 6-N,N-diethyl-β-γ-dibromomethylene-D-adenosine-5′-triphosphate trisodium; ATP, adenosine-5′-triphosphate (agonist for purinergic receptors); ATPγS, adenosine 5′-O-(3-thio-triphosphate) (agonist for purinergic receptors); CGS15493, 9-chloro-2-(2-furyl)[1,2,4]triazolo[1,5-c] quinazolin-5-amine (nonselective adenosine receptor antagonist); DMSO, dimethylsulphoxide; GOPCP, α,β-methylene-guanosine diphosphate (5′-nucleotidase inhibitor); 2-MeSATP, 2-methylthio-ATP (agonist for purinergic receptors, P_{2Y1}-subtype); L-NAME, N^G-nitro-L-arginine methyl ester (inhibitor of nitric oxide synthetase); NOS, nitric oxide synthetase; SCH58261, 5-amino-7-(2-phenylethyl)-2-(2-furyl)-pyrazolo[4,3-ϵ]-1,2,4-triazolo[1,5-c]pyrimidine (A_{2A}-selective adenosine receptor antagonist); 8-SPT, 8-(*p*-sulphophenyl)-theophylline (nonselective adenosine receptor antagonist); $t_{75\%}$, time interval from onset of a response to 75% its maximum; UTP, uridine-5′-triphosphate (agonist for purinergic receptors, P_{2Y2}-subtype); XAC, xanthine amine congener (nonselective adenosine receptor antagonist); ZM241385, 4-(2-[7-amino-2-(2-furyl)[1,2,4]triazolo[2,3-a][1,3,5]triazin-5-yl amino]ethyl)phenol (A_{2A}-selective adenosine receptor antagonist)

Introduction

Adenosine-5′-triphosphate (ATP) causes coronary vasodilation when infused into the coronary circulation (Bunger *et al*., 1975; Giles & Wilcken, 1977). Endogenous ATP is also presumed to cause coronary vasodilation (Burnstock & Kennedy, 1986; Ralevic & Burnstock, 1991). Therefore a role for extracellular ATP in the regulation of coronary tone has been proposed (Ralevic & Burnstock, 1991). ATP can be released from endothelium in response to increases in shear stress (Bodin *et al*., 1991) and from perivascular nerve terminals (Ralevic & Burnstock, 1998; Burnstock & Kennedy, 1986). Inflammatory mediators, ischaemia, and damage to blood vessels also cause ATP release from endothelial cells, neutrophils, cardiomyocytes, erythrocytes and platelets (Borst & Schrader, 1991; Born & Kratzer, 1984; Bodin & Burnstock,

*Author for correspondence.
Current addresses: [4]Department of Physiology, University of Bergen, Årstadveien 19, N-5009 Bergen, Norway; [5]CV Therapeutics Inc., 3172 Porter Drive, Palo Alto, California, U.S.A.

1998; Pearson & Gordon, 1985; Yang *et al*., 1994; Bergfeld & Forrester, 1992; Gordon, 1986; Ralevic & Burnstock, 1991; 1998; Sprague *et al*., 1996; Olsson & Pearson, 1990). ATP is rapidly broken down to adenosine by endothelial ectonucleotidases (Pearson & Gordon, 1985). In the in-situ heart ectoenzymatic conversion of nucleotides to adenosine contributes substantially to the increase of the interstitial adenosine concentration during early regional ischaemia (Kuzmin *et al*., 1998) and during beta adrenergic stimulation (Headrick *et al*., 1996). Likewise exogenous ATP is almost completely metabolized to adenosine during a single passage through the heart (Belardinelli *et al*., 1984; Borst & Schrader, 1991; Fleetwood *et al*., 1989). In humans, intracoronary administration of ATP caused vasodilation accompanied by an increase in coronary sinus adenosine concentration (Nanto *et al*., 1997).

Adenosine causes coronary vasodilation either when administered exogenously or when formed endogenously during metabolic stress (Berne, 1963; Olsson & Pearson, 1990; Duncker *et al*., 1996; Stepp *et al*., 1996). In the heart, adenosine reduces oxygen demand by a negative inotropic and chronotropic as well as an anti-β-adrenergic effect, and increases oxygen supply by its action as a coronary vasodilator. These actions of adenosine result in an increase in the ratio of oxygen supply to oxygen demand in the myocardium (Shryock & Belardinelli, 1997). The presence of A_{2A}-adenosine receptors (A_{2A}-AdoRs) in coronary vessels has been demonstrated by radioligand binding (Belardinelli *et al*., 1996). Activation of these receptors mediates coronary vasodilation by adenosine in guinea-pig isolated hearts (Belardinelli *et al*., 1998). The A_{2A}-AdoR has been classified as a P_1 receptor (Ralevic & Burnstock, 1998).

Coronary vasodilation caused by exogenous ATP can theoretically result from either P_1 (adenosine) or P_2 (nucleotide) receptor activation or both. Studies on isolated vessels and isolated hearts support the view that ATP causes coronary vasodilation by P_{2Y} receptor activation (Brown *et al*., 1992; Corr & Burnstock. 1991; Fleetwood & Gordon, 1987; Gordon, 1986; Hopwood & Burnstock, 1987; Matsumoto *et al*., 1997; Olsson & Pearson, 1990; Simonsen *et al*., 1997; Vials & Burnstock, 1994; Godecke *et al*., 1996). However, antagonism of ATP-induced coronary vasodilation by AdoR antagonists implicates activation of A_{2A}-AdoRs in the action of ATP (White & Angus, 1987; Bunger *et al*., 1975; King *et al*., 1990; Kroll & Schrader, 1993). Until now the lack of selective and potent antagonists for either P_{2Y} or A_{2A}-adenosine receptors has been an obstacle to the identification of the type receptor(s) that mediate ATP-induced coronary vasodilation. Recently, a potent and selective antagonist at the A_{2A}-AdoR, 5-amino-7-(2-phenylethyl)-2-(2-furyl)-pyrazolo[4,3-ϵ]-1,2,4-triazolo[1,5-c]pyrimidine (SCH58261) has been characterized (Zocchi *et al*., 1996; Belardinelli *et al*., 1996; 1998). In this study, the A_{2A}-AdoR antagonist SCH58261 was used to test the hypothesis that exogenous ATP causes coronary vasodilation *via* stimulation of A_{2A}-AdoRs in the guinea-pig isolated perfused heart. Responses to the P_2 agonists 2-methylthio-ATP (2-MeSATP) and uridine-5′-triphosphate (UTP) were compared to responses to ATP and adenosine. To confirm that responses to ATP and adenosine were mediated by activation of the same signal transduction pathway, whereas those to 2-MeSATP were mediated by a different transduction pathway, the effects of the nitric oxide synthetase (NOS) inhibitor N^G -nitro-L-arginine methyl ester (L-NAME) and the K_{ATP}-channel blocker glibenclamide on vasodilator responses to ATP, adenosine, and 2-MeSATP were determined.

Methods

Isolated perfused heart preparation

Guinea-pig hearts were isolated and perfused by the Langendorff technique at a constant flow of 10 ml min^{-1} at 36 ± 0.5°C as previously described (Shryock *et al*., 1998). The composition of the perfusate was (in mM): NaCl 118, KCl 4.5, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, pyruvate 2.0, glucose 5.5, Na_2EDTA 0.57, ascorbic acid 0.1 and $NaHCO_3$ 25.0. The perfusate was gassed continuously with 95% O_2 and 5% CO_2 and the pH of the solution was adjusted to 7.4 before use. The protocol for the isolation of hearts was reviewed and approved by the Animal Use Committee of the University of Florida. The mitral valve was cut to prevent accumulation of fluid in the left ventricle. Hearts were paced at a rate of 3.5–3.7 Hz. A surface electrocardiogram was monitored with a unipolar electrode. Electrodes were made from teflon-coated tungsten steel wire of 76-μm diameter (Small Parts Inc., Miami Lakes, FL, U.S.A.). Coronary perfusion pressure was continuously recorded. Coronary conductance (in ml min^{-1} $mmHg^{-1}$) was calculated as coronary flow rate (10 ml min^{-1}) divided by coronary perfusion pressure (mmHg). An equilibration period of 25–40 min preceded experiments. Hearts that failed to develop a stable coronary perfusion pressure of at least 50 mmHg during this initial equilibration period were discarded. Experiments were excluded from analysis if washout of agonists or full antagonism of drug effect failed to return coronary perfusion pressure to a value within 15% of that recorded at baseline. Steady-state responses are reported unless otherwise stated.

Experimental protocols

Coronary vasodilator responses to ATP and adenosine To compare the potencies and maximal effects of adenosine and ATP, cumulative concentration-response relationships for adenosine and ATP to decrease coronary perfusion pressure were determined. Hearts were exposed to increasing concentrations (1–10,000 nM) of either adenosine ($n=10$) or ATP ($n=10$). Perfusion with each concentration of drug was maintained until a stable decrease in coronary perfusion pressure was observed (typically 5 min). The reproducibility of responses was determined in a subset of three hearts for each agonist by exposure of a single heart to two series of increasing concentrations of an agonist, separated by at least 20 min of agonist-free perfusion.

To determine the time courses of ATP- or adenosine-induced vasodilations, two protocols were used that allowed each heart to serve as its own control. First, equieffective and submaximal concentrations of adenosine and ATP were administered as continuous infusions for 5 to 10 min in random sequence to obtain steady-state reductions in coronary perfusion pressure ($n=5$). The time course of each response was analysed to determine the time to reach 75% of maximal coronary vasodilatation ($t_{75\%}$). Second, responses of seven hearts to bolus injections of adenosine and ATP (2 and 4 nmol respectively) in random order were compared. Again, the doses of ATP and adenosine were selected to be submaximal and equieffective. Bolus doses were administered as manual injections of 10–20 μl of 0.2 mM stock solution into the perfusion line. The maximal response and the time interval from the maximal decrease of coronary pressure to 75% recovery towards baseline were measured.

Effects of AdoR antagonists SCH58261, XAC, and CGS15943 on ATP- and adenosine-mediated increases of coronary conductance Three sets of experiments were done to compare the effects of different AdoR antagonists on responses caused by ATP and adenosine. To assess the contribution of A_{2A}-AdoR activation to ATP-induced coronary vasodilation, SCH58261 was used in two complementary protocols. First, the concentration-response relationships for SCH58261 to attenuate coronary vasodilations caused by 100 nM adenosine ($n=6$) and 192 nM ATP ($n=6$) were determined. These concentrations of ATP and adenosine were chosen to cause equivalent decreases in coronary perfusion pressure. Second, cumulative concentration-response relationships for either ATP or adenosine to cause coronary vasodilation were determined in the absence and presence of 60 nM SCH58261 ($n=5$ for each agonist). This concentration of SCH8261 was chosen to achieve selective antagonism of A_{2A}-AdoRs (Zocchi *et al*., 1996). Third, the actions of the nonselective AdoR antagonists XAC and CGS15943 to attenuate agonist-induced increases in coronary conductance were determined. Either adenosine (86 ± 21, $n=4$) or ATP (160 ± 10 nM, $n=5$) was infused to achieve a submaximal decrease in coronary perfusion pressure of ≈20 mmHg. The effect of XAC (10 followed by 100 nM) to attenuate the ATP- and adenosine-induced increases in coronary conductance was measured. In a similar manner hearts were exposed to equieffective concentrations of either ATP (238 ± 38 nM, $n=4$) or adenosine (112 ± 44 nM, $n=3$) in the absence and presence of CGS15943 (10 followed by 100 nM).

Influences of ADA, AMP-deaminase, the ecto-5′-nucleotidase inhibitors AOPCP and GOPCP, and the ecto-ATPase inhibitor ARL67156 on ATP-induced coronary vasodilation To determine whether metabolism of ATP to adenosine was a prerequisite for ATP-mediated coronary vasodilation, vasodilations caused by either ATP or adenosine were evaluated in the absence and presence of ADA. The effects of ADA (6 u ml^{-1}) alone and in the presence of either ATP (121 ± 36 nM, $n=5$) or adenosine (20 and 150 nM, $n=2$), on coronary conductance were determined. In an attempt to distinguish direct ATP-mediated effects from indirect effects due to degradation of ATP to adenosine, additional experiments were carried out with AMP deaminase (0.5 u ml^{-1}), AOPCP (50 μM), GOPCP (50 and 250 μM), and ARL67156 (0.1 mM).

Coronary vasodilator responses to the P_2-agonists 2-MeSATP and UTP in the absence and presence of AdoR antagonists To determine the time courses and reproducibility of coronary responses to P_{2Y} agonists, coronary vasodilator responses caused by 2-MeSATP and UTP administered for three separate 5-min periods were assessed (three hearts for each agonist). These experiments indicated that the coronary vasodilator responses to both 2-MeSATP and UTP decreased upon repeated administration of the agonist. Near-maximal vasodilation was achieved within 45 s, but was not sustained for the duration of agonist administration. Therefore, experiments to determine the attenuation by AdoR antagonists of P_{2Y} receptor-mediated responses were designed using exposures to either 2-MeSATP or UTP that were of sufficient duration to cause maximal vasodilation, but brief enough to avoid significant desensitization of the response. Coronary vasodilator responses to 45 or 60 s infusions of 2-MeSATP (10 nM) or UTP (5 μM), respectively, were measured in the absence and presence of 100 nM SCH58261 ($n=4$ for 2MeSATP and $n=3$ for UTP). Each heart was exposed to the same agonist three times, separated by at least 15 min of drug-free perfusion. Infusion of SCH58261 was started 15 min prior to and maintained for the duration of the second exposure to an agonist. Using a similar protocol, the attenuation by 60 nM SCH58261 of coronary vasodilations caused by 10 min infusion of 100 nM ATPγS was measured. ATPγS has been described as a stable analogue of ATP (Cusack *et al*., 1983). Attenuations by the nonselective AdoR antagonist XAC (5 μM) of increases in coronary conductance caused by equieffective concentrations of ATP, adenosine, 2-MeSATP, and UTP were determined in another series of experiments. XAC (5 μM) is expected to attenuate responses mediated by all types of AdoRs (Muller & Stein, 1996). Hearts were exposed twice in random order to either 200 nM ATP and 5 μM UTP ($n=3$) or 100 nM adenosine and 10 nM 2-MeSATP ($n=4$). The durations of exposure to ATP, UTP, adenosine and 2-MeSATP were 5 min, 2 min, 5 min, and 45 s, respectively. Agonist administrations were separated by 15 min of drug-free perfusion. Infusion of XAC was started 10 min prior to and maintained for the duration of the second exposure to the agonists. The effect of a high concentration of the nonselective AdoR antagonist 8-SPT (30 μM) on coronary vasodilations caused by ATP (200 nM for 5 min, $n=3$), adenosine (100 nM for 5 min, $n=3$), and 2-MeSATP (20 nM for 45 s, $n=4$) was assessed in a third group of hearts.

Effects of the NOS inhibitor L-NAME and the K_{ATP}-channel blocker glibenclamide on coronary vasodilator responses to ATP and adenosine To determine whether coronary vasodilator responses to ATP, adenosine, and 2-MeSATP involve different signal transduction mechanisms, the NOS inhibitor L-NAME and the K_{ATP}-channel blocker glibenclamide were used. Hearts ($n=4-6$ for each group) were exposed to equieffective concentrations of either ATP (200 nM), adenosine (100 nM) or 2-MeSATP (20 nM) first in the absence and then in the presence of either L-NAME (50 μM) or glibenclamide (0.5 μM). After washout of glibenclamide a third vasodilator response to the agonist was elicited. After the agonist response in the presence of L-NAME was recorded, hearts were exposed to a near-maximal concentration of sodium nitroprusside (1 μM) to establish that the coronary vasculature was still responsive to a vasodilator agent.

Chemicals

Adenosine, ATP, UTP, 2-MeSATP, adenosine 5′-O-(3-thiotriphosphate) (ATPγS), α,β-methylene-adenosine diphosphate (AOPCP), α,β-methylene-guanosine diphosphate (GOPCP), dimethylsulphoxide (DMSO), ADA (type VII), and 5′-adenylic acid deaminase (AMP deaminase) were purchased from Sigma Chemical (St. Louis, MO, U.S.A.). 8-[4[[[[(2-Aminoethyl)amino]carbonyl]methyl]oxy]phenyl]-1,3-dipropylxanthine, xanthine amine congener (XAC), 9-chloro-2-(2-furyl)[1.2.4]triazolo[1,5-c]quinazolin-5-amine (CGS15943), 8-(*p*-sulphophenyl)-theophylline (8-SPT), L-NAME, ARL-67156, and glibenclamide were from Research Biochemicals International (Natick, MA, U.S.A.). SCH58261 was a gift from E. Ongini (Schering-Plough Research Institute, Milan, Italy). Stock solutions (10 mM) of SCH58261, XAC, glibenclamide, and CGS15943 were prepared in DMSO and stored at -80°C until use. Stock solutions were further diluted with saline prior to each experiment. The highest concentration of DMSO in the perfusate was 0.03%. This concentration of DMSO had no significant effect on coronary perfusion pressure (Shryock *et al*., 1998). All other compounds were dissolved in either water or saline. AOPCP

was incubated with ADA (0.02 U per μmol AOPCP) for 30 min to degrade adenosine that is present as a contaminant in the AOPCP preparation (Borst & Schrader, 1991). AMP deaminase was filtered (3 and 0.2 μm pore size, Millipore) to remove particulate material.

Data analysis and statistics

All values are presented as mean $\pm$ s.e.mean. The two tailed Student *t*-test as appropriate for paired or unpaired measurements was used to determine the significance of differences between two treatments. Either ANOVA or repeated measures ANOVA (followed by appropriate post-hoc testing) was used for analysis of the significance of differences among three or more treatments in an experiment. Differences were indicated as significant for P values $\leqslant 0.05$.

Concentration-response relationships were analysed by fitting the data with a nonlinear regression algorithm (Marquardt-Levenberg) to a multiparameter logistic equation by using Table Curve (v2.02, Jandel Scientific, San Raphael, CA, U.S.A.). The concentration of agonist that caused 50% of the maximum response (EC_{50}) was estimated for each heart from the curve yielding the best fit. Mean EC_{50} and apparent K_b values were calculated as the negative antilogarithms of the mean values of pEC_{50} (pD_2) and pK_b, as the latter are normally distributed (Kenakin, 1997).

Antagonist equilibrium dissociation constants (apparent K_b values) for SCH58261 were determined by Gaddum analysis. This method involves the estimation of equieffective agonist concentrations in the presence and absence of a fixed antagonist concentration (Lazareno & Birdsall, 1993) according to the following equation:

$$K_b = \frac{[\text{Antagonist}]}{\left(\frac{EC_{50,\text{ with Agonist}}}{EC_{50,\text{ Agonist alone}}}\right) - 1}$$

Results

Comparison of coronary vasodilator responses to ATP and adenosine

The time courses of coronary vasodilator responses to exogenously administered adenosine and ATP were remarkably similar (Figure 1). Responses to steady-state infusions of equieffective concentrations of adenosine (75 nM) and ATP (150 nM) were sustained for the duration of agonist administration (Figure 1a). The time intervals for responses to adenosine and ATP to reach 75% of maximal were 98 ± 8 s ($n=12$) and 86 ± 6 s ($n=13$), respectively. Likewise, bolus administrations of equieffective doses of adenosine (2 nmol) and ATP (4 nmol) caused coronary vasodilator responses with similar time courses (Figure 1b). The increases in coronary conductance caused by 2 nmol adenosine and 4 nmol ATP were 0.101 ± 0.007 and 0.105 ± 0.008 ml min^{-1} mmHg^{-1}, respectively. The time intervals from the maximal increases in coronary conductance to 75% recovery of the vasodilator responses towards baseline coronary conductance were 4.4 ± 0.2 min for adenosine and 4.2 ± 0.2 min for ATP (Figure 1b).

Concentration-response relationships for adenosine and ATP to increase coronary conductance are shown in Figure 2. Maximal increases in coronary conductance caused by adenosine and ATP were not significantly different; the corresponding increases were 0.149 ± 0.015 ($n=10$) and 0.152 ± 0.012 ($n=10$) ml min^{-1} mmHg^{-1}, respectively. Adenosine was about twice as potent as ATP. The values of EC_{50} ($pD_2 \pm$ s.e.mean) for adenosine and ATP were 86 nM (7.06 ± 0.05, $n=10$) and 211 nM (6.68 ± 0.04, $n=10$), respectively. To confirm that hearts did not desensitize to the presence of either adenosine or ATP during the course of an experiment, two consecutive concentration-response relationships for adenosine and ATP were obtained in each of three hearts. Mean values of pEC_{50} for adenosine to increase coronary conductance during the first and second series of agonist exposures were 7.04 ± 0.01 and 6.93 ± 0.05, respectively ($P=0.18$, NS). Likewise, mean values of pEC_{50} for ATP to increase coronary conductance were 6.52 ± 0.06 during the first and 6.53 ± 0.05 during the second series of agonist exposures ($P=0.89$, NS). The maximal increases in coronary conductance during the first and second exposures to adenosine were 0.163 ± 0.017 and 0.164 ± 0.016 ml min^{-1} mmHg^{-1}. Those during the first and second exposures to ATP were 0.127 ± 0.006 and 0.129 ± 0.005 ml min^{-1} mmHg^{-1}. These maximal responses during the first and second exposures to either adenosine or ATP were not significantly different. Thus, neither time-dependent deterioration nor agonist-induced desensitization by ATP or adenosine was observed.

Effect of AdoR antagonists on ATP- and adenosine-mediated increases in coronary conductance

The potent and highly selective A_{2A}AdoR antagonist SCH58261 (Zocchi *et al*., 1996; Belardinelli *et al*., 1998) caused

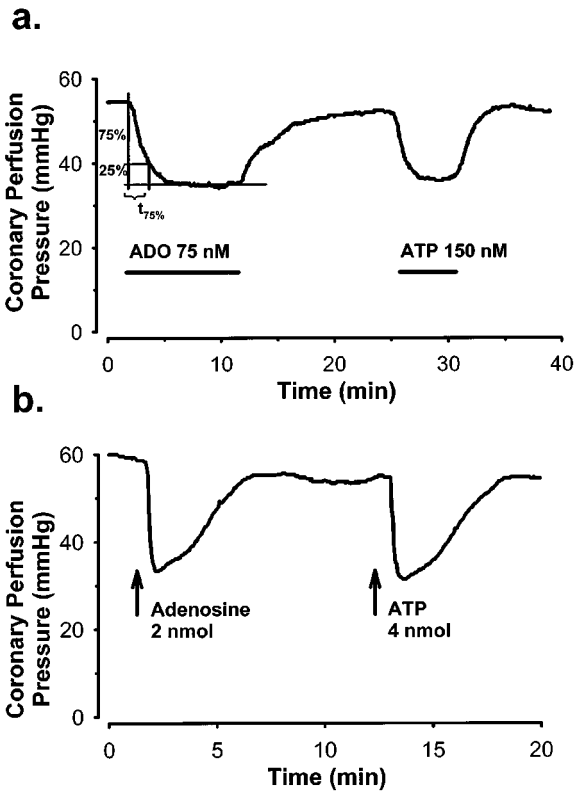

Figure 1 Chart records of coronary perfusion pressure comparing responses to a continuous infusion (a) and a bolus administration (b) of adenosine and ATP in guinea-pig isolated hearts. (a) Time course of responses to adenosine (75 nM for 10 min) and ATP (150 nM for 5 min). Solid bars indicate the time of drug infusion. Measurement of the time interval ($t_{75\%}$) from onset of the vasodilator response to 75% of the maximal vasodilation is illustrated for the adenosine response. (b) Responses to bolus administration of 2 nmol adenosine and 4 nmol ATP in the same heart. Bolus doses were administered by manually injecting 10–20 μl of 0.2 mM stock solution into the perfusion line. Vertical arrows indicate the time of bolus administration.

equivalent attenuations of ATP- and adenosine-mediated coronary vasodilations. Concentration-response relationships for reversal by SCH58261 of comparable ATP- and adenosine-induced increases in coronary conductance are shown in Figure 3. Values of IC_{50} ($pIC_{50} \pm$s.e.mean) for SCH58261 to attenuate vasodilator responses caused by adenosine and ATP were 5.26 nM (8.28 ± 0.08) and 5.25 nM (8.28 ± 0.06), respectively. In a complementary series of experiments (Figure 4) concentration-response relationships for coronary vasodilatation caused by adenosine and ATP were obtained in the absence and presence of 60 nM SCH58261. SCH58261 did not decrease coronary conductance by itself but caused similar parallel rightward shifts of the concentration-response relationship for each agonist (Figure 4). The rightward shift of the concentration-response relationship by SCH58261 increased the EC_{50} ($pEC_{50} \pm$s.e.mean) values from 82 nM (7.09 ± 0.09) to 831 nM (6.08 ± 0.17) for adenosine ($P = 0.0015$, $n = 5$) and from 170 nM (6.77 ± 0.04) to 1346 nM (5.87 ± 0.09) for ATP ($P = 0.0001$, $n = 5$). The apparent K_b ($pK_b \pm$s.e.mean) values for SCH58261 calculated by Gaddum analysis (see Methods) were 6.7 nM (8.17 ± 0.14) with adenosine as the agonist and 8.8 nM (8.06 ± 0.07) with ATP as the agonist. These values of pK_b were not significantly different.

To determine if the apparent similarity in the attenuation by SCH58261 of vasodilations caused by ATP and adenosine is an experimental artifact caused by previously unrecognized properties of SCH58261, the effects of the two nonselective AdoR antagonists XAC and CGS15943 on comparable ATP- and adenosine-mediated increases of coronary conductance (0.092 ± 0.009 and 0.101 ± 0.005 ml min^{-1} $mmHg^{-1}$, respectively, P = NS) were tested. XAC (10 and 100 nM) reversed 29 ± 4 and 82 ± 2% of the ATP and 24 ± 8 and 78 ± 4% of the adenosine-mediated increases in coronary conductance (Figure 5a). The estimated pIC_{50} values for XAC to antagonize increases of coronary conductance caused by ATP and adenosine were 7.48 ± 0.04 and 7.37 ± 0.13 ($P = 0.40$), respectively. Similarly, CGS15943 reversed comparable adenosine- and ATP-mediated increases in coronary conductance of 0.104 ± 0.014 and 0.074 ± 0.005 ml min^{-1} $mmHg^{-1}$ with estimated pIC_{50} values of 7.56 ± 0.11 and 7.45 ± 0.06 ($P = 0.40$), respectively (Figure 5b). Hence, both antagonists reversed equivalent increases in coronary conductance caused by adenosine and ATP with similar potencies and in a concentration-dependent manner.

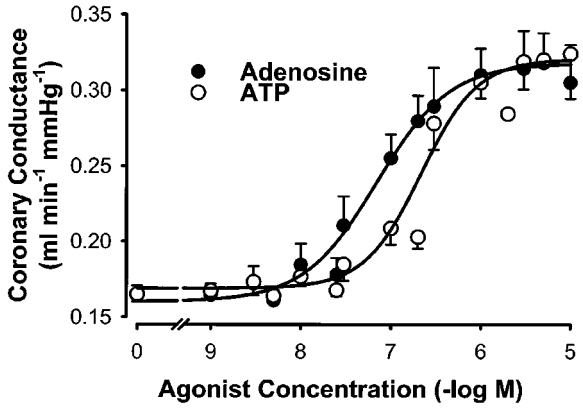

Figure 2 Concentration-response relationships for adenosine and ATP to increase coronary conductance in guinea-pig isolated heart. Two groups of 10 hearts were exposed to a series of increasing concentrations of either ATP or adenosine. Symbols represent the mean of three to 10 observations. Data from each experiment were individually analysed and fit.

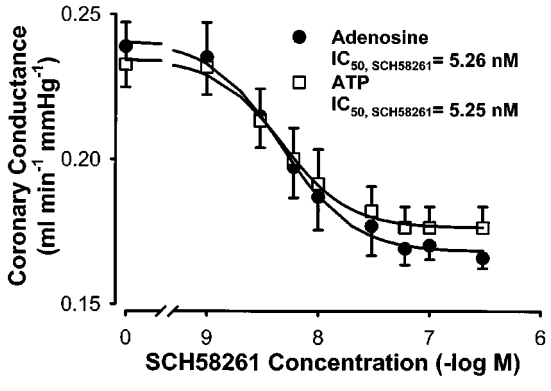

Figure 3 Concentration-response relationships for SCH 58261 to attenuate increases in coronary conductance caused by either adenosine (100 nM) or ATP (average concentration 193 nM) in guinea-pig isolated hearts. In separate experiments it was found that in the absence of SCH58261 the responses to 100 nM adenosine or 200 nM ATP were sustained for more than 40 min (the time typically needed to complete SCH58261 infusion). Mean values of coronary conductance in the absence of agonist were 0.146 ± 0.003 ($n = 6$) and 0.158 ± 0.006 ml min^{-1} $mmHg^{-1}$ ($n = 6$) for hearts treated with adenosine and ATP, respectively. Symbols represent mean values; error bars represent s.e.mean.

Effects of ADA, AMP-deaminase, and the ectonucleotidase inhibitors AOPCP, GOPCP, and ARL67156 on ATP-induced coronary vasodilation

ADA (6 u ml^{-1}) attenuated the increases in coronary conductance caused by both adenosine and ATP. ADA by itself did not affect coronary conductance. Consistent with previous results (Belardinelli *et al*., 1998). ADA reversed by 100 and 93% the steady-state coronary vasodilations induced by 20 and 150 nM adenosine ($n = 2$), respectively. ADA (6 u ml^{-1}) attenuated by 46 ± 4% the increase in coronary conductance caused by 121 ± 36 nM ATP ($n = 5$). However, in two hearts the reversal of the ATP response was not sustained for the duration of ADA administration. In an effort to reduce

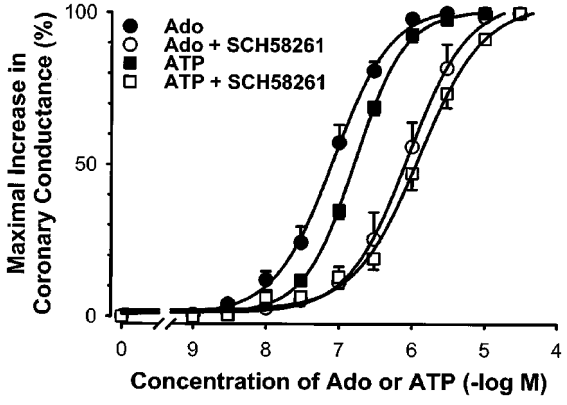

Figure 4 Normalized concentration-response relationships for adenosine (Ado) and ATP to increase coronary conductance alone and in the presence of 60 nM SCH 58261. Coronary conductances in the absence of agonist (0.167 ± 0.010 ml min^{-1} $mmHg^{-1}$ for adenosine-treated hearts and 0.167 ± 0.008 ml min^{-1} $mmHg^{-1}$ for ATP-treated hearts) did not differ significantly. The maximal increases in coronary conductance in the absence and presence of SCH 58261 were 0.29 ± 0.025 and 0.29 ± 0.022 ml min^{-1} $mmHg^{-1}$ for adenosine and 0.33 ± 0.030 and 0.31 ± 0.036 ml min^{-1} $mmHg^{-1}$ for ATP, respectively. Symbols represent mean values; error bars represent s.e.mean ($n = 5$ experiments for each agonist). The untransformed data were used for statistical analysis.

the degradation of ATP to adenosine and thereby distinguish the direct and indirect effects of ATP, AMP deaminase and the ecto-5′-nucleotidase inhibitors AOPCP and GOPCP were used. Unfortunately, AMP deaminase (0.5 u ml^{-1}) alone caused a progressive decrease in coronary conductance, and AOPCP (50 μM) alone increased coronary conductance by 0.141 ± 0.003 ml min^{-1} $mmHg^{-1}$ ($n=3$). GOPCP by itself had no effect on coronary conductance but delayed the onset and decreased the maximal vasodilator responses to both ATP and adenosine in a similar manner. Therefore neither AMP deaminase nor AOPCP or GOPCP were suitable for a quantitative study of vasodilator responses to ATP in our preparation.

The ecto-ATPase inhibitor ARL67156 did not change coronary conductance in either the absence or presence of 200 nM ATP. Values of coronary conductance in the absence of drug (control) and in the presence of 0.1 mM ARL67156 were 0.172 ± 0.007 and 0.173 ± 0.006 ml min^{-1} $mmHg^{-1}$ ($n=6$), respectively. The increases of coronary conductance during consecutive exposures of hearts to 200 nM ATP alone then 200 nM ATP in the presence of 0.1 mM ARL67156, followed by 200 nM ATP again after washout of ARL67156 were 0.115 ± 0.013, 0.089 ± 0.013, and 0.88 ± 0.008 ml min^{-1} $mmHg^{-1}$, respectively ($P=$NS for ATP+ARL67156 *vs* ATP alone). Analysis of the ATP concentration in samples of the coronary effluents collected during periods of perfusion of hearts with 200 nM ATP was done to determine if ARL67156 prevented the metabolism of ATP by the guinea-pig heart. Results of HPLC analysis indicated that the concentration of ATP in coronary effluents was <10 nM when hearts ($n=5$) were perfused with 200 nM ATP either in the absence or presence of 0.1 mM ARL67156. Thus, ARL67156 appeared neither to prevent the metabolism of ATP by the guinea-pig isolated heart nor to reduce the vasodilator action of ATP.

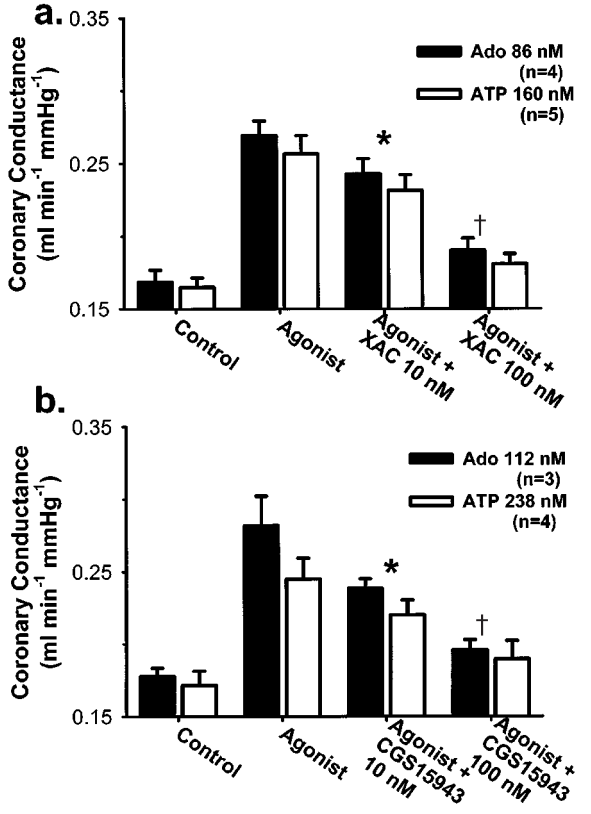

Figure 5 Reversals of equivalent adenosine- (Ado) and ATP-mediated coronary vasodilations by two nonselective and structurally different adenosine receptor antagonists, XAC (a) and CGS 15943 (b), in guinea-pig isolated hearts. Bars represent mean values and error bars represent s.e.mean of data from three to five experiments. $^{*}P<0.05$ compared to agonist alone; $^{\dagger}P<0.05$ compared to 10 nM XAC or CGS 15943. Responses to Ado and ATP alone were significantly different from control ($P<0.001$). Responses to Ado and ATP were not significantly different. Significance of differences was determined by one-way repeated-measures ANOVA followed by Tukey's test.

Coronary vasodilations caused by P_2 receptor agonists and lack of response to adenosine receptor antagonists

Because ATP is thought to cause coronary vasodilation at least in part by activating P_2-purinergic receptors, vasodilator responses to ATP and adenosine were compared with those of prototypical P_2-receptor agonists 2-MeSATP and UTP. 2-MeSATP (10 nM) and UTP (5 μM) both caused coronary vasodilation (Figure 6). As illustrated in Figure 6, the time courses of vasodilator responses caused by 2-MeSATP and UTP differed markedly from those caused by either ATP or adenosine (Figure 1a). The times to achieve 75% of the maximal response ($t_{75\%}$) were 18 ± 1 s ($n=10$) and 20 ± 1 s ($n=10$) for 2-MeSATP and UTP, respectively. Thus the $t_{75\%}$ for responses caused by 2-MeSATP and UTP was more than 4 fold shorter than the $t_{75\%}$ for responses caused by ATP and adenosine (see above). Coronary vasodilations caused by 2-MeSATP and UTP were not sustained for the duration of drug administration (Figure 6). Repeated 5-min exposures to either 2-MeSATP or UTP caused desensitization of the vasodilator response (data not shown). Despite the desensitization, reproducible responses to three 45-s administrations of 2-MeSATP (10 nM) and to three 60-s administrations of UTP

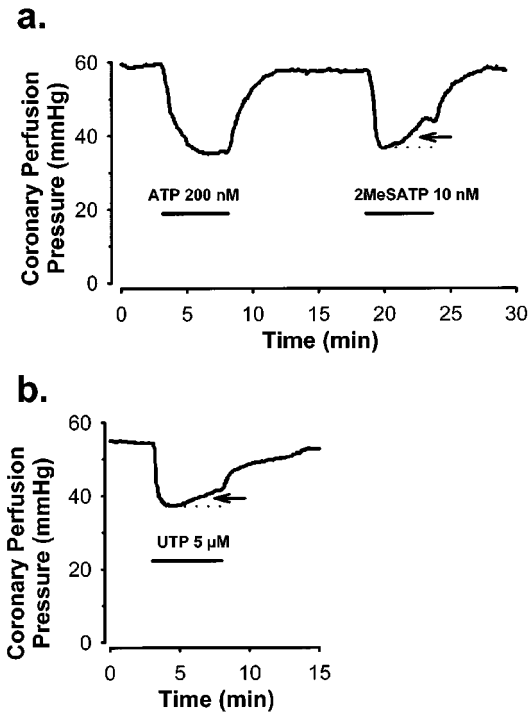

Figure 6 Chart recordings comparing typical decreases of coronary perfusion pressure caused by ATP (a) and the P_{2Y} agonists 2-MeSATP (a) and UTP (b) in guinea-pig isolated heart. Solid horizontal bars indicate the time of drug administration. This experiment was repeated three times for each P_{2Y} agonist with similar results. Note: the vasodilations caused by 2-MeSATP and UTP are not sustained (arrows), whereas those caused by ATP (a) and adenosine are sustained (Figure 1a).

(5 μM) could be obtained (data not shown). Neither the vasodilator responses to 2-MeSATP nor those to UTP were affected by the presence of either 60 or 100 nM SCH58261, respectively (Figure 7). Similarly, whereas 5 μM XAC antagonized the increases in coronary conductance caused by adenosine and ATP by 99 ± 1 and 92 ± 3%, respectively, the responses to 2-MeSATP and UTP were unaffected by XAC (Figure 8a). 8-PST, another nonselective AdoR antagonist, reversibly antagonized the increases in coronary conductance caused by ATP and adenosine, but failed to diminish those caused by 2-MeSATP (Figure 8b). In contrast to 2-MeSATP and UTP, ATPγS (100 nM) caused a sustained increase of coronary conductance from 0.183 ± 0.012 to 0.247 ± 0.012 ml min^{-1} mmHg^{-1} ($n=3$) with a time course similar to the time courses of increases of coronary conductance caused by ATP and adenosine. SCH58261 (60 nM) reversed the coronary vasodilation caused by ATPγS by 80 ± 5%.

Effects of glibenclamide and L-NAME on vasodilations caused by ATP, adenosine, and 2-MeSATP

Block of K_{ATP} channels with 0.5 mM glibenclamide nearly abolished vasodilations caused by ATP and adenosine, but failed to attenuate significantly those caused by 2-MeSATP (Figure 9a). The increases in coronary conductance caused by 200 nM ATP and 100 nM adenosine were 0.079 ± 0.004 and 0.098 ± 0.005 ml min^{-1} mmHg^{-1} in the absence and 0.009 ± 0.002 and 0.011 ± 0.004 ml min^{-1} mmHg^{-1} in the presence of 0.5 mM glibenclamide, respectively ($P<0.001$, $n=4$ each). Glibenclamide attenuated ATP-induced vasodilations by 88.4% and adenosine-induced vasodilations by 88.9% ($P=0.40$). In contrast to vasodilations caused by ATP and adenosine, those caused by 2-MeSATP were not significantly attenuated by glibenclamide ($P=0.21$). Glibenclamide (0.5 mM) alone caused a small decrease of coronary conductance of 0.014 ± 0.002 ml min^{-1} mmHg^{-1} (mean$\pm$s.e.mean, $n=16$).

Inhibition of NOS nearly abolished vasodilations caused by equieffective concentrations of ATP, adenosine and 2-MeSATP (Figure 9b). In the absence of L-NAME the agonist-induced increases in coronary conductance caused by 200 nM ATP, 100 nM adenosine and 20 nM 2-MeSATP were 0.102 ± 0.007, 0.110 ± 0.007, and 0.088 ± 0.010 ml min^{-1} mmHg^{-1}. L-NAME attenuated these vasodilator responses by 92.4 ± 2.5, 97.4 ± 1.2, and 79.8 ± 5.6% for ATP, adenosine, and 2-MeSATP, respectively. Attenuation by L-NAME was similar for all agonists studied ($P=0.38$. NS). In keeping with a role for NO in determining basal coronary tone (Kelm & Schrader, 1990), L-NAME by itself caused a small but significant decrease in coronary conductance of 0.025 ± 0.009 ml min^{-1} mmHg^{-1}.

Discussion

The receptor(s) mediating coronary vasodilatation during infusion of ATP in the coronary circulation is controversial. The results of this study indicate that the sustained coronary vasodilator response of the guinea-pig isolated heart to a submaximal concentration of exogenous ATP is caused entirely by adenosine acting upon A_{2A}-AdoRs. Experiments done under equilibrium conditions with the selective A_{2A}-AdoR antagonist SCH58261 (Figures 3 and 4) demonstrated that both ATP and adenosine caused coronary vasodilation *via* stimulation of A_{2A}-AdoRs. Four additional and complimen-

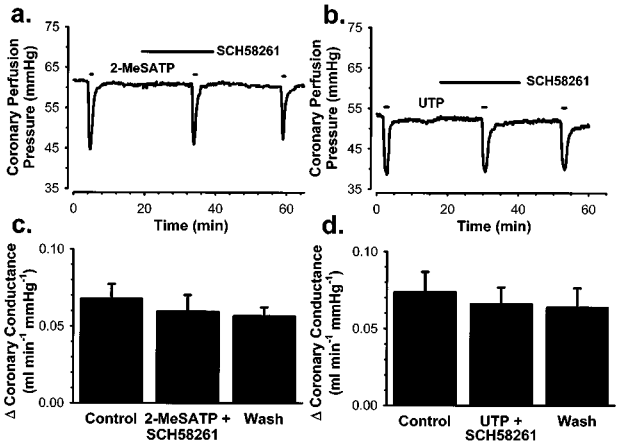

Figure 7 Effects of SCH58261 on the increases of coronary conductance caused by the P_{2Y} agonists 2-MeSATP and UTP in guinea-pig isolated hearts. (a,b) Analogue records of the decreases in coronary perfusion pressure caused by three consecutive exposures to either 10 nM 2-MeSATP for 45 s (a) or 5 μM UTP for 1 min (b). The second exposures to 2-MeSATP and UTP were done in the presence of SCH58261 at concentrations of 60 and 100 nM, respectively. Solid horizontal bars indicate the time of drug administration. (c,d) Summary of data demonstrating the lack of antagonism by 60 and 100 nM SCH58261 of increases in coronary conductance caused by 2-MeSATP (c) and UTP (d), respectively. Values of mean and s.e.mean of coronary conductance in the absence of drug (control) were 0.182 ± 0.006 ml min^{-1} mmHg^{-1} ($n=7$). Bars represent mean values and error bars represent s.e.mean of data from three to four experiments.

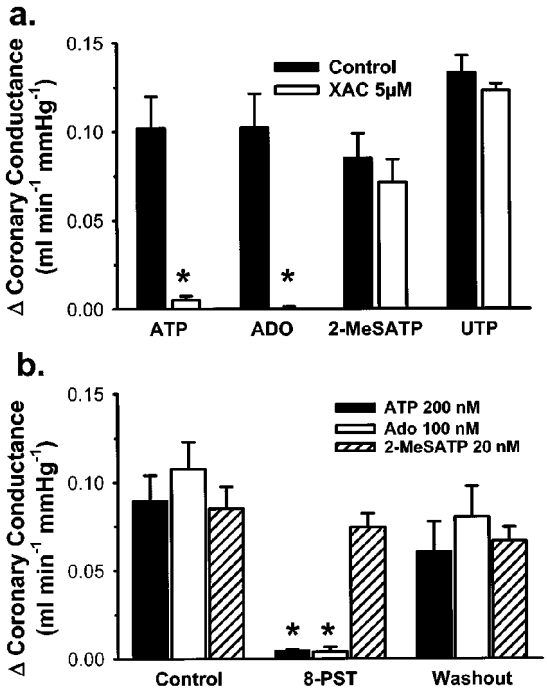

Figure 8 (a) Effect of 5 μM XAC on the increases in coronary conductance caused by 200 nM ATP, 100 nM adenosine, 20 nM 2-MeSATP, and 5 μM UTP. (b) Effect of 30 μM 8-PST on increases in coronary conductance caused by 200 nM ATP, 100 nM adenosine, and 20 nM 2-MeSATP. The durations of exposure to ATP, adenosine, 2-MeSATP, and UTP were 5 min 5 min, 45 s, and 2 min, respectively. In the absence of agonist, neither XAC nor 8-PST decreased coronary conductance significantly. The mean values of coronary conductance before exposure of hearts to drugs were 0.161 ± 0.004 (a) and 0.165 ± 0.008 (b) ml min^{-1} mmHg^{-1}. Bars represent mean$\pm$s.e.mean of determinations in three to four hearts. *$P<0.05$ compared to agonist alone. Significance of differences was determined by repeated-measures ANOVA followed by Tukey's test.

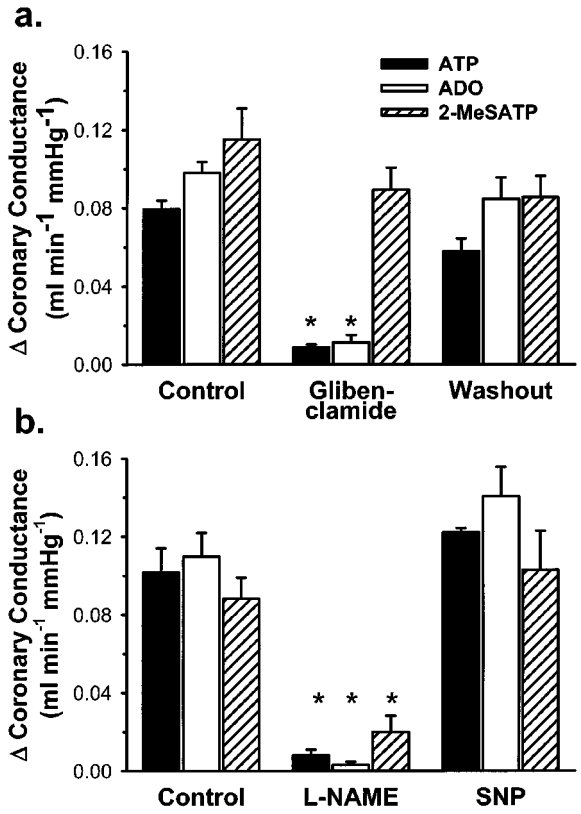

Figure 9 Effects of K_{ATP} channel block (a) and inhibition of NOS (b) on vasodilations caused by ATP, adenosine, or 2-MeSATP. (a) Attenuation by 0.5 μM glibenclamide of increases in coronary conductance caused by ATP and adenosine, but not those caused by 2-MeSATP. (b) Attenuation by 50 μM L-NAME of increases in coronary conductance caused by ATP, adenosine, and 2-MeSATP. ATP, adenosine and 2-MeSATP were administered at concentrations of 200, 100, and 20 nM for 5 min, 5 min, and 45 s, respectively. After the agonist response in the presence of L-NAME was recorded, hearts were exposed to a near-maximal concentration of sodium nitroprusside (1 μM, SNP) to elicit an agonist-independent vasodilation. Bars represent mean $\pm$ s.e.mean of determinations in four to six hearts. The mean and s.e.mean of values of coronary conductance for all hearts ($n=27$) in the absence of drug were 0.161 ± 0.003 ml min^{-1} $mmHg^{-1}$. *$P<0.05$ compared to agonist alone. Significance of differences was determined by repeated-measures ANOVA followed by Tukey's test.

tary lines of evidence support the conclusion that coronary vasodilation caused by exogenous ATP was entirely mediated by adenosine acting upon A_{2A}-AdoRs. First, coronary vasodilations caused by adenosine and ATP were similar in time course and maximal effect (Figure 1). Second, two structurally different non-selective AdoR antagonists (XAC and CGS15943) attenuated with similar potencies equivalent increases in coronary conductance caused by ATP and adenosine (Figures 5 and 8). Third, L-NAME, glibenclamide and ADA attenuated the responses to both ATP and adenosine. Fourth, coronary vasodilations caused by 2-MeSATP and UTP, presumably acting on P_{2Y} receptors, differed markedly from coronary vasodilations caused by adenosine and ATP in time course (Figure 6) and were not antagonized by SCH58261, XAC and 8-PST (Figures 7 and 8).

Role of adenosine receptor activation in coronary vasodilations caused by ATP

Previous reports both confirm and contradict our findings. Consistent with our results are reports that theophylline and CGS15943 significantly attenuated ATP-mediated coronary vasodilation in isolated hearts (Bunger *et al*., 1975; Kroll & Schrader, 1993; Hopwood & Burnstock, 1987). In contrast are reports that AdoR antagonists caused no significant attenuation of ATP-mediated coronary vasodilation (Brown *et al*., 1992; Vials & Burnstock, 1994; Giles & Wilcken, 1977). This discrepancy may be explained in part by differences in preparation, species and experimental design. For example, ATP is reported to be more potent than adenosine as a coronary vasodilator in the dog (Giles & Wilcken, 1977) and rat (Fleetwood & Gordon, 1987), whereas ATP and adenosine appear to be approximately equipotent as coronary vasodilators in the guinea-pig (Bunger *et al*., 1975; this study). This suggests that either the densities or subtypes of receptors that mediate responses to ATP are different in different species, or that different rates of metabolism of intravascularly-administered ATP in different species may lead to different concentrations of ATP at ATP receptors. ATP is rapidly metabolized by endothelial cells in the coronary circulation (Cusack *et al*., 1983; Pearson & Gordon, 1985; Gordon *et al*., 1986; Meghji *et al*., 1995). Therefore during exogenous administration of ATP, the concentration of ATP in the receptor compartment is likely to be much lower than the concentration of ATP in the coronary perfusate.

Experiments designed to determine P_{2Y} receptor-mediated responses frequently involve administration of agonists as a bolus to minimize potential desensitization of P_{2Y} receptor-mediated responses. The transient high concentration of ATP may cause activation of P_2 receptors that have both high and low affinities for ATP. Activation of these receptors may lead to either a faster onset of coronary vasodilation caused by ATP or an initial P_{2X} receptor-mediated coronary vasoconstriction that is followed by vasodilation (Godecke *et al*., 1996; Gordon, 1986; Hopwood & Burnstock, 1987; Olsson & Pearson, 1990). Thus it is possible that the bolus administration of high concentrations of ATP may allow the investigator to observe activation by ATP of P_2 receptors that cause transient changes in coronary conductance that were not seen in this study. However, such non-equilibrium conditions limit the usefulness of receptor antagonists for characterization of the specific receptors that mediate an agonist-induced response (Kenakin, 1997). Therefore in our experiments steady-state responses to submaximal concentrations of ATP and adenosine were investigated. Under these conditions, we found that the response to ATP was mediated by activation of A_{2A}-AdoRs.

Mechanism of A_{2A}-AdoR activation by ATP

Hypothetically, ATP by itself may activate A_{2A}-AdoRs, or ATP may be broken down to adenosine which activates A_{2A}-AdoRs. Pirotton & Boeynaems (1993) demonstrated that ATP and its thio-analogues are not ligands at the A_{2A}-AdoR. These investigators reported that ADA and AOPCP nearly abolished the displacement by ATP and by its thio-analogues of specific binding of the highly selective A_{2A}-AdoR agonist [^{3}H]-CGS21680 to bovine striatal membranes. Consistent with these results ADA attenuated coronary vasodilations caused by adenosine and ATP in our experiments. However, as noted in previous studies (Ragazzi *et al*., 1991), the attenuation by ADA of ATP-mediated responses was incomplete. The inability of ADA to fully attenuate ATP-mediated coronary vasodilations may indicate that dephosphorylation of ATP occurs in close proximity to the A_{2A}-AdoR. If ecto-nucleotidases were located near the A_{2A}-AdoR, then adenosine formed from exogenous ATP may activate the receptor before

it is either transported into cells or degraded by ADA (Meghji *et al*., 1995; Bruns, 1980; 1990). In support of this idea are reports that ecto-5′-nucleotidase, G protein-coupled receptors, and adenylyl cyclase are localized in caveolae (Gordon, 1986; Shaul & Anderson, 1998).

It is possible that ATP was degraded very quickly to adenosine in the guinea-pig coronary vasculature, and that none of the administered ATP was able to reach the vascular P_2 receptors in our experiments. Our previous findings (Belardinelli *et al*., 1984) indicated that only 2.5% of ATP added to the coronary perfusate was recovered in the effluent of the guinea-pig isolated heart. The inhibition of ectonucleotidases should reduce vasodilation caused by ATP if dephosphorylation of ATP to adenosine is necessary for ATP-mediated coronary vasodilation. Guibert *et al*. (1998) reported that inhibition of ectonucleotidase activity reduced the A_{2A}-AdoR-mediated dilation of endothelium-denuded portal vein strips caused by ATP. Similarly, inactivation of ectonucleotidase by monoclonal antibodies reduced the A_{2B}-AdoR-mediated decrease of endothelial permeability caused by adenine nucleotides (Lennon *et al*., 1998). Furthermore, administration of the ecto-5′-nucleotidase inhibitor AOPCP reduced the formation of adenosine from exogenously administered ATP by the isolated perfused guinea-pig heart (Borst & Schrader, 1991). In our preparation, the inhibitor of 5′-nucleotidase activity AOPCP (50 μM) caused coronary vasodilation by itself, whereas the inhibitor of ectoATPase ARL67156 failed either to prevent the metabolism of ATP or to reduce the vasodilator action of ATP. Therefore, neither AOPCP nor ARL67156 were suitable and selective inhibitors of the degradation of ATP to adenosine in our preparation. Because enzyme inhibitors failed to prevent degradation of ATP in the guinea-pig heart, and antagonists that are selective and specific for P_2 receptors are currently not available, we cannot assess directly if ATP activates P_2 receptors prior to its degradation to adenosine. Although we cannot conclude that activation of P_2 receptors by ATP is without effect on coronary conductance, our results suggest that P_2 receptor activation by ATP does not contribute to vasodilator responses at steady state.

Role of P_{2Y} receptor activation for coronary vasodilation caused by ATP

Coronary vasodilations caused by 2-MeSATP and UTP differed markedly from coronary vasodilations caused by adenosine and ATP in time course and sensitivity to AdoR antagonists. Although not highly selective, 2-MeSATP and UTP are the prototypical agonists for P_{2Y1} and P_{2Y2} receptors, respectively (Ralevic & Burnstock, 1998; Fredholm *et al*., 1994). The P_{2Y1} (formerly P_{2Y}) and P_{2Y2} (formerly P_{2U}) receptors have been implicated in coronary vasodilation (Fredholm *et al*., 1994; Ralevic & Burnstock, 1998; Godecke *et al*., 1996). The coronary vasodilations caused by 2-MeSATP and UTP (Figures 6 and 7) indicate that P_{2Y1} and P_{2Y2} receptor subtypes are present in the guinea-pig coronary vasculature. Whereas 2-MeSATP is a more potent agonist than ATP at the P_{2Y1} receptor, UTP is as potent as ATP at the P_{2Y2} receptor (Fredholm *et al*., 1994). However, ATP was 20 fold more potent than UTP to cause vasodilation in our preparation. This result suggests that P_{2Y2} receptor activation does not contribute significantly to ATP-mediated coronary vasodilation. Furthermore, coronary vasodilations caused by 2-MeSATP and UTP were transient and more rapid in onset than those caused by ATP. In contrast to coronary vasodilations caused by ATP and adenosine, coronary vasodilations caused by UTP and 2-MeSATP were not antagonized by SCH58261 (Figures 3 and 7) and XAC (Figure 8). These findings indirectly support the interpretation that ATP-induced vasodilation of the guinea-pig coronary vasculature results from activation of A_{2A}-AdoRs rather than activation of P_{2Y} receptors.

The vasodilator responses caused by ATPγS, described as a P_{2Y2} agonist (Fredholm *et al*., 1994), were similar in time course to those caused by adenosine and ATP. Furthermore, they were nearly completely reversed by SCH58261. Because ATPγS by itself does not bind to A_{2A}-AdoRs (Pirotton & Boeynaems. 1993), these findings suggest that ATPγS was metabolized to adenosine in our preparation. Degradation by ATP-pyrophosphohydrolases of ATP analogues to AMP, and degradation of AMP to adenosine have been documented in guinea-pig and rat hearts (Imai *et al*., 1989; Fleetwood *et al*., 1989).

Attenuations by glibenclamide and L-NAME of vasodilator responses caused by ATP, adenosine, and 2-MeSATP

Both glibenclamide and L-NAME markedly reduced the vasodilator responses caused by ATP and adenosine (Figure 9). L-NAME also attenuated vasodilations caused by 2-MeSATP (Figure 9b). This finding suggests that nitric oxide plays a role in the receptor-effector signal transduction of both A_{2A}-adenosine and P_{2Y1} receptors (Vials & Burnstock, 1993; Kelm & Schrader, 1990). In contrast, glibenclamide did not attenuate the vasodilations caused by 2-MeSATP (Figure 9a), indicating that activation of K_{ATP} channels is not required for P_{2Y1} receptor-mediated vasodilation. This result clearly distinguishes the actions of ATP and adenosine from those of 2-MeSATP and is consistent with activation of a single receptor by ATP and adenosine.

In summary, our results show that sustained, submaximal coronary vasodilation caused by exogenous ATP is entirely mediated by adenosine acting upon A_{2A}-AdoRs. The lack of a significant contribution of P_{2Y} receptor activation to the observed coronary vasodilation caused by ATP indicates the high capacity of the guinea-pig coronary vasculature to dephosphorylate ATP to adenosine (Belardinelli *et al*., 1984) and the efficient coupling of A_{2A}-AdoR activation to coronary vasodilation (Shryock *et al*., 1998). Prolonged intravascular release of nucleotides occurs in the setting of myocardial ischaemia and as a result of vascular injury and platelet activation (e.g. myocardial revascularization) (Born & Kratzer, 1984). Our results indicate that intravascular release of nucleotides will lead to A_{2A}-AdoR-mediated coronary vasodilation.

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