JPP Journal of Pharmacy and Pharmacology

JPP 2002, 54: 859–867

Received November 12, 2001
Accepted March 5, 2002
ISSN 0022-3573

Effects of adenosine receptor agonists on guinea-pig isolated working hearts and the role of endothelium and NO

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Abstract

The hypothesis that the coronary vasodilator effects of adenosine receptor agonists are independent of the vascular endothelium or mediators derived therefrom was examined in guinea-pig isolated working hearts. Adenosine receptor agonists, 5′-(*N*-ethylcarboxamido)-adenosine (NECA; two-fold selective for A_2 over A_1 receptors), 2-[*p*-(2-carboxyethyl)phenyl-ethylamino]-5′-*N*-ethylcarboxamidoadenosine (CGS21680; A_{2A} selective), N^6-cyclopentyl-adenosine (CPA; A_1 selective) and N^6-(3-iodobenzyl)adenosine-5′-*N*-methyluronamide (IB-MECA; A_3 selective), were infused (3×10^{-7} M) after endothelium removal by passing oxygen through the coronary circulation. In spontaneously beating hearts, CGS21680 and NECA increased, while CPA decreased, coronary flow. NECA and CPA reduced heart rate, left ventricular pressure and aortic output. The nitric oxide synthase (NOS) inhibitor, N^G-nitro-L-arginine (L-NOARG; 3×10^{-5} M) abolished the vasodilatation by NECA but not CGS21680, indicating that nitric oxide (NO) of a non-endothelial source mediated the NECA response. Coronary vasodilatation by CGS21680 was inhibited by the A_{2A} receptor antagonist, 4-(2-[7-amino-2-(2-furyl)[1,2,4]triazolo [2,3-a][1,3,5]triazin-5-ylamino]ethyl)phenol (ZM241385). Indometacin (10^{-6} M) attenuated the coronary vasodilatation to CGS21680, suggesting a partial role for cyclooxygenase products. IB-MECA had no effect, indicating no A_3 receptor involvement. In paced working hearts, the responses were similar except CPA had no effect on coronary flow or aortic output and CGS21680 increased left ventricular pressure and the maximum rate of ventricular pressure rise. This study has demonstrated functionally effective removal of the endothelium by a novel method of passing oxygen through the coronary vasculature. A coronary vasodilator action of adenosine receptor agonists mediated via A_{2A} receptors is endothelium- and NO-independent, but partially involves cyclooxygenase products.

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Introduction

Adenosine modulates a variety of physiological functions acting via specific cell surface receptors which were initially divided into two subtypes, A_1 and A_2, on the basis of their ability to inhibit or stimulate adenylyl cyclase activity, respectively (Van Calker et al 1979). With the development of selective pharmacological probes and ligands and the use of molecular biology techniques, four different adenosine receptors (A_1, A_{2A}, A_{2B} and A_3) have now been identified (for review see Collis & Hourani 1993; Tucker & Linden 1993). The negative chronotropic and inotropic

actions of adenosine are mediated by A_1 receptors, which have been identified in cardiac tissue by several authors (Bellardinelli et al 1989). In contrast, activation of A_2 receptors, which are believed to be located on vascular smooth muscle (Vials & Burnstock 1993), endothelial (Rubanyi & Vanhoutte 1985) and cardiac cells (Behnke et al 1990; Xu et al 1996), is involved in relaxation of blood vessels. More recently, a novel contractile function of the A_{2A} receptor has been demonstrated in the intact cardiac ventricular cell (Liang & Morley 1996). It has also been suggested that activation of the A_{2A} receptor is capable of opposing the inhibitory effect mediated by the A_1 receptor and serves to maintain the level of cardiac contractility (Xu et al 1996).

The endothelium plays an obligatory role in the relaxation of isolated arteries in response to substances such as acetylcholine, substance P, ATP, ADP and thrombin (De Mey et al 1982; Rubanyi & Vanhoutte 1985). However, the role of endothelium in the relaxation induced by adenosine is controversial. It has been found that vasodilatation produced by adenosine was partially dependent on the presence of an intact endothelium (Headrick & Berne 1990; Kuo & Chancellor 1995), while in other studies of coronary vessels, it is endothelium-independent (Lew & Kao 1999). In the coronary vasculature of guinea-pig isolated hearts, vasodilatation has been shown to be mediated via A_{2A} receptors since it is produced by the selective agonist, CGS21680 (Bellardinelli et al 1998). In small resistance-like coronary arteries in man, the vasodilatation to adenosine is mediated via A_{2B} receptors and is nitric oxide (NO)-independent (Kemp & Cocks 1999).

The effects of adenosine receptor agonists have not been evaluated in isolated working hearts where the functional integrity of the heart remains relatively intact. The aim of this study was therefore to compare the profiles of activity of several selective adenosine receptor agonists on a range of parameters in the guinea-pig working heart. Experiments were performed in spontaneously beating and in paced hearts, the latter so that any effects of changes in heart rate by the adenosine analogues on the other parameters could be eliminated. In addition, the hypothesis to be examined was that the coronary vascular responses to adenosine analogues, including CGS21680, are independent of the endothelium and two likely mediators derived therefrom, cyclooxygenase products and nitric oxide. The removal of the endothelium was by the novel method of passing a blast of oxygen through the coronary vasculature before setting the hearts up and the functional effectiveness of this was evaluated.

Materials and Methods

Heart perfusion

Male Dunkin–Hartley guinea-pigs (300–380 g) were maintained in accordance with NIH guidelines (publication 85-23) for animal care and were anaesthetized by intraperitoneal injection of sodium thiopental (50 mg kg^{-1}). The heart was quickly excised and immersed into heparinized ice-cold saline solution. The aorta was dissected free and in the hearts where the endothelium was denuded, a short blast of oxygen was applied via the aorta. This was achieved by cannulating the aorta with a tube attached to a small oxygen canister and delivering dry oxygen at 2–5 psi for 15 s at room temperature. The aorta was then cannulated for retrograde aortic perfusion in the Langendorff mode with a filtered modified Krebs–Henseleit buffer containing (mM): NaCl 118; KCl 4.7; $NaHCO_3$ 25; $MgCl_2$ 1.2; KH_2PO_4 1.2; glucose 11; pyruvate 0.5 and $CaCl_2$ 1.25. The perfusion solution was continuously gassed with an O_2–CO_2 (95/5%) mixture (pH 7.4) and maintained at 37°C throughout the experiment.

Retrograde perfusion of the heart started immediately at a perfusion pressure of 80 cm H_2O for 10 min and then switched to perfusion according to the working-heart technique (Neely et al 1967). Preload was held at 10 cm H_2O and afterload was maintained at 80 cm H_2O. The perfusate was not re-circulated and the hearts were allowed to equilibrate for 15 min before beginning different interventions.

Global mechanical function was continuously recorded by a catheter-tipped manometer (Millar Instruments, Houston, TX) placed into the left ventricle by inserting the needle through the ventricular wall. Left ventricular pressure, the maximum rate of ventricular pressure rise (dP/dt max), the maximum rate of ventricular pressure decline (dP/dt min) and heart rate were acquired directly on a recorder (Gould Electronics, Cleveland, OH). Aortic output and coronary flow were determined volumetrically by collecting, respectively, the outflow from the cannulated aorta and the overflow from the heart originating from the pulmonary artery, the volumes in 1 min being recorded in a measuring vessel.

In some experiments, hearts were paced at constant rate. The hearts were set up in working mode and the sinoatrial nodal region and part of the right atrium were excised (Clemo et al 1987). After 5 min of the 15-min equilibrium period, a pair of platinum electrodes was placed on the wall of the right ventricle and the heart paced at 4 Hz with square-wave pulses of 5 ms duration and supramaximal voltage (approximately 20 V).

Experimental protocol

During the 15-min stabilization period, baseline values for the haemodynamic parameters were determined. All the drugs were infused via the aortic perfusion at a flow rate of 10% of the basal coronary flow using a low-flow infusion pump (Harvard Apparatus, Type 11 Digital Infusor, Edenbridge, Kent, UK). To confirm that the blast of oxygen was sufficient to remove the vascular endothelium, the vasodilator response to an infusion of acetylcholine (10^{-6} M) was examined in endothelium-intact and -denuded hearts. Adenosine agonists were infused as single concentrations (3×10^{-7} M) which had been found in previous studies to produce 50–100% of the maximal responses at A_1 receptors for N^6-cyclopentyl-adenosine (CPA; Gardner & Broadley 1999), at A_2 receptors for 5′-(*N*-ethylcarboxamido)-adenosine (NECA; Losinski & Alexander 1995), at A_{2A} receptors for 2-[*p*-(2-carboxyethyl)phenylethylamino]-5′-*N*-ethylcarboxamidoadenosine (CGS21680; Bellardinelli et al 1998) and at A_3 receptors for N^6-(3-iodobenzyl)adenosine-5′-*N*-methyluronamide (IB-MECA; Reeves et al 1997). Infusions of acetylcholine or the adenosine agonists were maintained for 40 min. To investigate further the mechanism involved in the effect of NECA and CGS21680, they were also examined in the presence of N^G-nitro-L-arginine (L-NOARG, 3×10^{-5} M), indometacin (10^{-6} M) or the selective A_{2A} antagonist, ZM241385 (4-(2-[7-amino-2-(2-furyl)[1,2,4]triazolo[2,3-a][1,3,5]-triazin-5-ylamino]ethyl)phenol, 10^{-7} M). L-NOARG, indometacin or ZM241385 were infused 10 min before and throughout the agonist infusion.

Drugs

CGS21680, CPA, indometacin and NECA were obtained from Sigma (St Louis, MO). L-NOARG was obtained from Research Biochemicals International (RBI, Natick, MA). IB-MECA was synthesised in house in the chemistry Department at SmithKline Beecham, France. ZM241385 was a gift from Zeneca Pharmaceuticals. CGS21680, CPA, NECA, ZM241385 and IB-MECA were made up as stock solutions (10^{-3} M) in 50:50 polyethylene glycol–distilled water, followed by serial dilution in Krebs solution. Indometacin was made up as a stock solution (10^{-3} M) in 1% ethanol and diluted with Krebs solution. Acetylcholine (10^{-2} M) and L-NOARG (10^{-3} M) were made up as stock solutions in distilled water and diluted with Krebs solution. L-NOARG (10^{-3} M) was made up as a stock solution in distilled water and diluted with Krebs solution. An infusion of final dilution of 50:50 polyethylene glycol–distilled water in Krebs solution at 1 mL min^{-1} did not change the haemodynamic parameters other than a small transient injection artefact.

Statistical analysis

Each parameter was measured at 5-min intervals after the start of the drug infusion and expressed as a percentage of the pre-drug resting level. The mean ± s.e.m. for n experiments was then calculated. Statistical analysis was performed using analysis of variance followed by Student–Newman–Keuls test. A P value of less than 0.05 was considered statistically significant.

Results

Effects of removal of endothelium and inhibition of NOS

To confirm that the blast of oxygen removed the endothelium, the effect of acetylcholine (10^{-6} M) was compared in intact hearts and in hearts receiving the oxygen blast. In endothelium-intact preparations, acetylcholine increased coronary flow, an effect which was abolished in the presence of the nitric oxide synthesis inhibitor, L-NOARG (Figure 1). In contrast, after applying the blast of oxygen, the response to acetylcholine was significantly attenuated. A small vasodilator response remained, but this did not differ significantly from the control. In the endothelium-denuded preparation, a slow onset vasoconstriction response to acetylcholine was observed in the presence of L-NOARG (Figure 1).

Removal of the endothelium did not significantly affect the resting cardiac and coronary vascular parameters of spontaneously beating working hearts. The baseline values in endothelium-intact and -denuded hearts, respectively, for aortic output (40.5±5.9 and 41.0±6.9 mL min^{-1}), coronary flow (14.5±1.9 and 12.5±0.9 mL min^{-1}), left ventricular pressure (68.0±6.5 and 76.5±5.3 mmHg), dP/dt max 1300±91 and 1550±176 mmHg s^{-1}), dP/dt min (1525±125 and 1400±100 mmHg s^{-1}) and heart rate (225±10 and 250±13 beats min^{-1}) were not significantly different.

Adenosine analogues in spontaneously beating hearts

Coronary flow measured in spontaneously beating guinea-pig isolated hearts in which the endothelium had been removed remained stable (12.5±0.8 mL min^{-1}) for

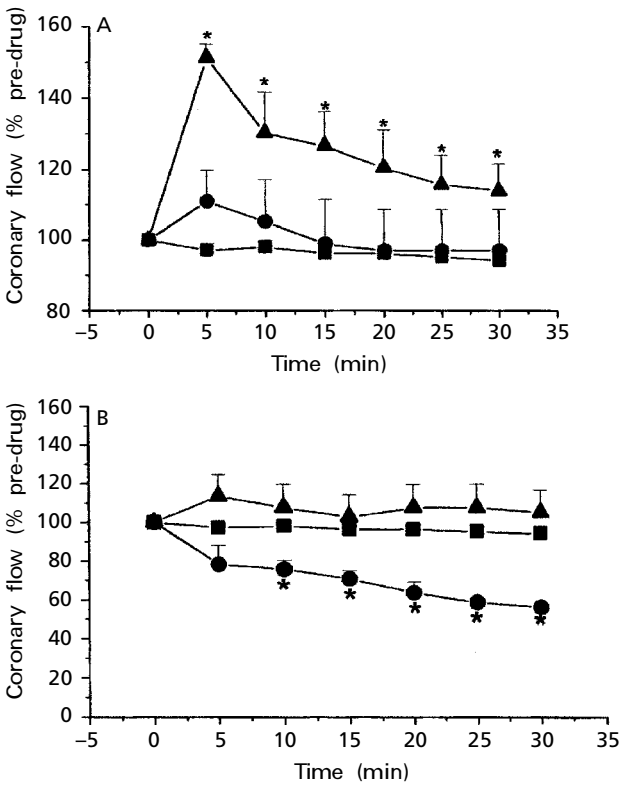

Figure 1 Effect of acetylcholine (10^{-6} M), infused over 30 min, on the coronary flow (expressed as percentage of pre-drug value) of guinea-pig working hearts in the absence (A) and presence (B) of L-NOARG (3×10^{-5} M). Preparations were either endothelium-intact (▲) or -denuded (●). A control, without acetylcholine (■), was performed. Each point represents the mean (n = 4) change in coronary flow expressed as a percentage of the pre-drug level (±s.e.m.). $^{*}P < 0.05$, compared with the control at each time point.

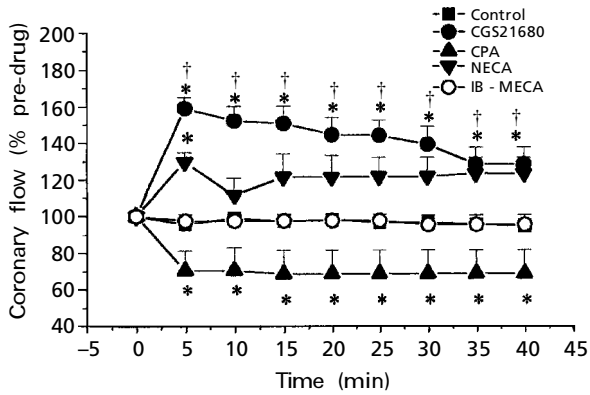

Figure 2 The effects of CGS21680 (3×10^{-7} M), NECA (3×10^{-7} M), CPA (3×10^{-7} M) or IB-MECA (3×10^{-7} M) on coronary flow (expressed as percentage of pre-drug value) of guinea-pig spontaneously beating working hearts denuded of endothelium. A control, in the absence of agonist (■), was performed. Each point represents the mean±s.e.m., n = 4 experiments. $^{*}P < 0.05$, CGS21680-, NECA- or CPA-treated groups vs control (in the absence of agonist); $^{\dagger}P < 0.05$, CGS21680-treated group vs CPA group. Analysis of variance followed by Newman–Keuls test was used for comparison.

at least 40 min. Both of the adenosine A_2 receptor agonists, CGS21680 and NECA, increased coronary flow (159±6% and 129±5% of pre-drug level, respectively) compared with the control (97.1±1.9%). In contrast, the A_1 receptor agonist, CPA, rapidly reduced coronary flow to 70.4±10.8% of the basal value (Figure 2). Both NECA and CPA markedly reduced heart rate (33.3±1.0% and 28.5±1.4%, respectively) and dP/dt max (46.3±6.5% and 17.2±5.2%) compared with controls (98.3±1.7% for heart rate and 107±2% for dP/dt max); dP/dt min and left ventricular pressure were also reduced (Table 1). Consequently, both compounds virtually abolished aortic output. During perfusion with CGS21680, these parameters of cardiac function remained close to control values (Table 1). IB-MECA (3×10^{-7} M) produced no change in coronary flow compared with the control (97.4±3.6% and 97.1±1.9%, respectively, at 5 min) (Figure 2). During perfusion with IB-MECA, the other parameters also remained close to control values (Table 1).

Role of NO, endothelium and prostanoids in the vasodilator response to CGS21680 and NECA

To investigate the involvement of NO in the vasodilator effects of CGS21680 and NECA, their actions were compared in the absence or presence of the NO synthase inhibitor (L-NOARG) in endothelium-denuded spontaneously beating working hearts. The vasodilatation induced by CGS21680 was not significantly altered by L-NOARG (161±14% vs 159±6%, Figure 3A). In contrast, L-NOARG abolished the vasodilator response induced by NECA (to 96.3±10.0% of pre-drug level) (Figure 3A). When the endothelium remained intact, a vasodilator response was observed with CGS21680 infusion, although it was significantly less than that in endothelium-denuded preparations; the values at 5 min after commencing the infusion were 130±6% and 159±6%, respectively (Figure 3B).

To confirm that the increase in coronary flow produced by CGS21680 was mediated via A_{2A} receptors, the selective A_{2A} antagonist, ZM241385, was found to abolish the vasodilator response to CGS21680 (to 94.4±7.9%; Figure 3B) in endothelium-denuded hearts.

In endothelium-denuded hearts, the cyclooxygenase inhibitor, indometacin (10^{-6} M), significantly attenuated

Table 1 Effects of infusions of adenosine analogues on various parameters of guinea-pig spontaneously beating working hearts.

	Aortic output	Coronary flow	Left ventricular pressure	dP/dt max	dP/dt min	Heart rate
Control	98.2±1.2	97.1±1.9	99.3±2.6	107±2	98.7±1.2	98.3±1.7
CGS21680	86.6±2.1	159±6*	105±3	107±4	97.2±1.6	90.0±0.0
NECA	5.2±3.7*	130±5*	80.2±5.3*	46.3±6.5*	40.0±7.8*	33.3±1.0*
CPA	0±0*	70.4±10.8*	49.7±12.0*	17.2±5.2*	17.7±3.7*	28.5±1.4*
IB-MECA	99.3±2.9	97.4±3.6	97.8±3.5	97.8±4.2	102±4	102±2
CGS21680+L-NOARG	87.5±12.5	161±14*	107±6	97.2±4.2	101±8	102±2
NECA+L-NOARG	0±0*	96.3±10.0	65.0±13.2*	31.4±5.7*	38.0±2.9*	41.8±5.4*
CGS21680 (intact)	101±4	130±6*	102±3	115±13	83.2±5.9	99.0±13.4
CGS21680+ZM241385	100±3	94.4±7.9	97.3±4.6	98.1±8.3	83.2±10.2	95.8±4.7

The effects of CGS21680 (3×10^{7} M), NECA (3×10^{-7} M), CPA (3×10^{-7} M) or IB-MECA (3×10^{-7} M) were examined in endothelium-denuded hearts. The effects of CGS21680 (3×10^{-7} M) and NECA (3×10^{-7} M) in the presence of L-NOARG (3×10^{-5} M), and that of CGS21680 (3×10^{-7} M) in the presence of ZM241385 (10^{-7} M) were also examined in endothelium-denuded hearts. CGS21680 was also examined in endothelium-intact hearts. Responses are measured at 5 min after commencing the infusion and are presented as the mean (n = 4) change expressed as a percentage of pre-drug level (±s.e.m.). Statistical significance was determined by analysis of variance followed by Newman–Keuls test. *$P < 0.05$ vs control. Absolute baseline levels for the control group were: aortic output, 41.6±2.8 mL min^{-1}; coronary flow, 13.2±0.73 mL min^{-1}; left ventricular pressure, 66.4±11.0 mmHg; dP/dt max (maximum rate of ventricular pressure rise), 1500±266.5 mmHg s^{-1}; dP/dt min (maximum rate of ventricular pressure decline), 1400±236.6 mmHg s^{-1}; heart rate, 260.0±14.1 beats min^{-1}.

the increase in coronary flow by CGS21680 from 159±6% to 123±7%, although the vasodilator response was still significantly greater than the control (97.1±1.9%) (Figure 4). Again, there was no significant change in the other parameters.

Adenosine analogues in paced working hearts

In endothelium-denuded hearts paced at a constant frequency, CGS21680 and NECA increased coronary flow (129±3% and 119±1% at the 5-min peak effect, respectively) compared with the control (95.2±1.6%). In contrast, CPA produced no significant effect on coronary flow (Table 2). There was no significant effect on aortic output by infusions of CGS21680 or CPA (98±9.6% and 92.2±4.9% at 5 min) compared with controls (102±3%), whereas NECA caused a significant reduction of aortic output (78.8±6.2%). During infusions of CGS21680, there were significant improvements of left ventricular pressure and dP/dt max compared with controls, whereas CPA reduced these parameters and they were unaffected by NECA infusion (Table 2).

Discussion

Studies were performed in guinea-pig isolated working heart preparations to examine the effects of endothelium on the responses to adenosine receptor agonists. The effectiveness of the method of endothelium removal was first assessed, which was to apply a short blast of oxygen via the aorta before perfusion of the heart. This procedure substantially attenuated the coronary vasodilator response to acetylcholine, the remaining response being not significantly different from the control in the absence of acetylcholine. This indicates that the coronary vascular endothelium had been effectively removed by the blast of oxygen. An alternative method of endothelium removal is to perfuse hearts with Triton X-100. This has also been shown to remove endothelium as evidenced by abolition of the vasodilator response to acetylcholine without pathological changes on morphological examination. However, there were marked changes in contractile function of papillary muscles removed from these hearts suggesting that there were detrimental effects of this method of endothelium removal (Li et al 1993), In this study, one might have expected to see a vasoconstriction to acetylcholine after endothelial removal, as occurs in isolated arterial preparations (Furchgott & Zawadzki 1980). A vasoconstrictor response was, however, observed in denuded hearts in the presence of L-NOARG which suggests that the endothelium was not completely removed or that acetylcholine releases NO from a non-endothelial source (e.g. vascular smooth muscle). In hearts with intact endothelium in the presence of L-NOARG to inhibit NO generation, acetylcholine had neither vasodilator nor vasoconstrictor actions. This can be explained by

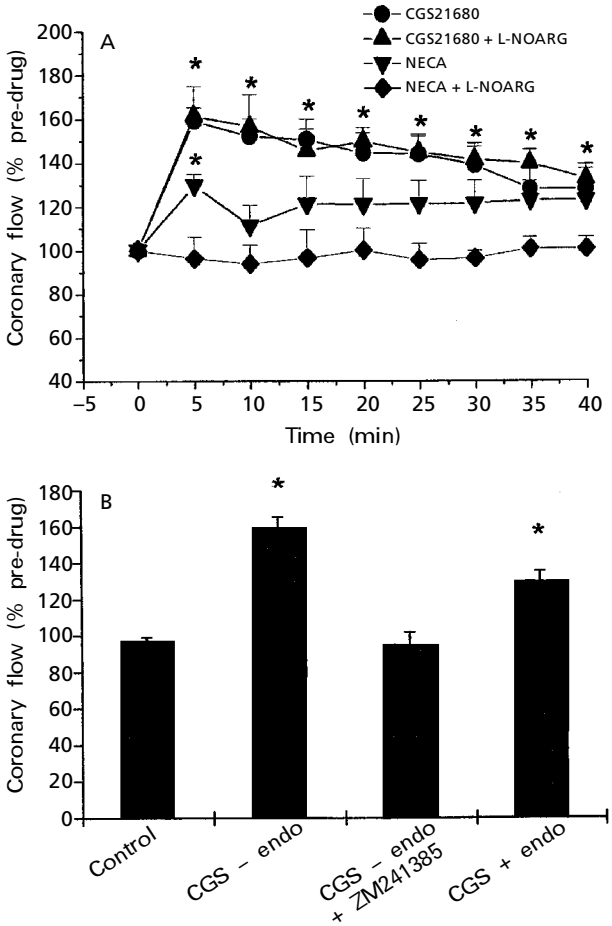

Figure 3 Effects of NECA (3×10^{-7} M) and CGS21680 (3×10^{-7} M) on the coronary flow (expressed as percentage of pre-drug value) of guinea-pig spontaneously beating working hearts. A. Responses of endothelium-denuded hearts to NECA and CGS21680 in the absence and presence of L-NOARG (3×10^{-5} M). B. Effects on the coronary flow of CGS21680, measured at 5 min after commencing the infusion, in the absence (CGS − endo) or presence (CGS + endo) of endothelium and of endothelium-denuded hearts in the presence of ZM241385 (10^{-7} M). Each point represents the mean ± s.e.m., n = 4 experiments. *$P < 0.05$, CGS21680-treated groups vs control group. Analysis of variance followed by Newman–Keuls test was used for comparison.

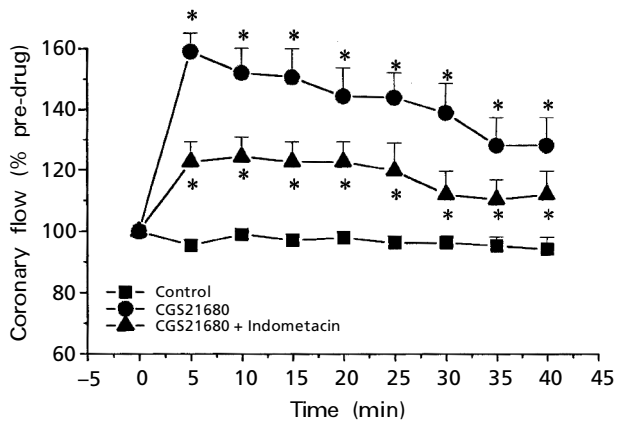

Figure 4 Effects of CGS21680 (3×10^{-7} M) on the coronary flow (expressed as percentage of pre-drug value) of guinea-pig endothelium-denuded spontaneously beating working hearts in the absence (●) and presence (▲) of indometacin (10^{-6} M). A control, in the absence of CGS21680 (■), was performed. Each point represents the mean ± s.e.m., n = 4 experiments. *$P < 0.05$, CGS21680-treated groups vs control group. Analysis of variance followed by Newman–Keuls test was used for comparison.

acetylcholine releasing a vasodilator substance from the endothelium other than NO which opposes the direct vasoconstrictor actions of acetylcholine. Acetylcholine releases vasodilator prostacyclin from cultured endothelial cells of rabbit heart (Kan et al 1997) and the release of prostacyclin from rat mesenteric vasculature by acetylcholine has been shown to be entirely endothelium-dependent (Peredo et al 1997). Notwithstanding these issues, the fact that in the presence of L-NOARG, there is a vasoconstriction to acetylcholine after endothelium removal suggests that this method of endothelium removal is effective. The effects of the adenosine analogues in preparations subjected to this procedure can therefore be considered to be essentially endothelium-independent.

The effects of four adenosine analogues, NECA (2-fold selective for A_2 over A_1 receptors), CGS21680 (A_{2A} selective), CPA (A_1 selective) and IB-MECA (A_3 selective) (Fredholm et al 2001), were investigated on various haemodynamic parameters in guinea-pig spontaneously beating and paced working heart preparations. Previous work involving the use of adenosine analogues has shown that there is a population of A_2 purinoceptors present in the guinea-pig coronary vasculature (Vials & Burnstock 1993). The vasodilator activity of the selective A_{2A} receptor agonist CGS21680 observed here in both spontaneously beating and paced working hearts confirms observations in non-working Langendorff hearts (Bellardinelli et al 1998), that these are of the A_{2A} subtype. The coronary vasodilator responses to CGS21680 and NECA were observed in endothelium-denuded spontaneous and paced heart preparations suggesting that A_2 receptor-mediated coronary vasodilatation in the guinea-pig working heart preparation is not necessarily mediated through endothelial-dependent pathways. An endothelium-independent vasodilatation confirms studies in pig isolated coronary arteries (Lew & Kao 1999). The vasodilator response to CGS21680 was, in fact, less when the coronary endothelium was intact than when it was removed. This suggests that an

Table 2 Effects of infusions of adenosine analogues on various parameters of guinea-pig paced working hearts.

	Aortic output	Coronary flow	Left ventricular pressure	dP/dt max	dP/dt min
Control	103±3	95.2±1.6	93.7±5.1	90.9±3.7	85.8±4.4
CGS21680	98.4±9.6	129±4*	107±2*	111±4*	106±7*
NECA	78.8±6.2*	119±1*	95.8±1.2	99.5±7.7	90.0±3.6
CPA	92.2±4.9	91.0±6.3	84.6±2.3*	74.5±1.9*	69.7±2.0*

The effects of CGS21680 (3×10^{-7} M), NECA (3×10^{-7} Ml) or CPA (3×10^{-7} M) were examined in guinea-pig endothelium-denuded paced hearts. They are presented as the mean (n = 4) change at 5 min after commencing drug infusion, expressed as a percentage of the pre-drug level (±s.e.m.). Statistical significance was determined by analysis of variance followed by Newman–Keuls test. *$P < 0.05$ vs control. Absolute baseline levels for the control group were: aortic output, 27.8±1.8 mL min^{-1}; coronary flow, 14.8±1.03 mL min^{-1}; left ventricular pressure, 69.0±9.7 mmHg; dP/dt max (maximum rate of ventricular pressure rise), 2175±361.4 mmHg s^{-1}; dP/dt min (maximum rate of ventricular pressure decline), 1775±444.2 mmHg s^{-1}.

opposing vasoconstrictor substance is released by CGS21680 from the endothelium, such as endothelin.

Major differences were observed between the NECA and CGS21680 vasodilator responses in the spontaneously beating hearts, which was not entirely unexpected as they activate different sets of receptors; A_2/A_1 receptors and A_{2A} receptors, respectively. This suggests that the mechanisms underlying their vascular responses may be different. In the endothelium-denuded preparations, the NO inhibitor L-NOARG (Rees et al 1990) failed to block the vasodilator response to CGS21680, suggesting that it was NO-independent. The vasodilator response to NECA, however, was abolished, indicating that it is NO-dependent, the source of the NO being primarily non-endothelial, since the endothelium had been removed.

The vasodilator response to NECA in the absence of endothelium in our study could be due to direct stimulation of the A_2 receptors in smooth muscle, possibly of the A_{2B} subtype, which in turn causes release of NO. The existence of L-arginine-NO in smooth muscle has previously been suggested (Schini & Vanhoutte 1991). Thus, vascular smooth muscle may produce a relaxing factor (vascular smooth muscle-derived relaxing factor (MDRF)) with pharmacological and chemical properties similar to those of NO (Wood et al 1990). As previously noted, the vasodilatation to CGS21680 was not inhibited by L-NOARG and was endothelium independent. The possibility that this NO-independent vasodilator response to CGS21680 was due to the release of prostanoids was examined in the presence of the cyclooxygenase inhibitor, indometacin. The response was partially inhibited by indometacin in the endothelium-denuded preparations, suggesting the involvement of vasodilator prostanoids. Felsch et al (1994) showed that there was an increase in the release of prostacyclin in the effluent from guinea-pig hearts after stimulation of adenosine A_{2A} receptors with CGS21680 which was endothelium independent. The site of release was suggested to be the vascular smooth muscle. In rabbit isolated hearts, the same receptors that elicit vasodilatation also released prostacyclin (Karwatowska-Prokopczuk et al 1988). In confirmation of our results, Vials & Burnstock (1993) also concluded that prostanoids only play a minor role in the vasodilator response to the A_{2A} receptor agonist, CGS216890. The selective A_{2A} adenosine receptor antagonist ZM241385 (Poucher et al 1995) abolished the coronary vasodilator response to CGS21680 in the guinea-pig working heart preparation, confirming the direct involvement of the A_{2A} receptor in this response. Whether endogenously released adenosine might influence the coronary vascular responses to exogenously added agonists could be assessed from the experiments involving ZM241385. It was of interest that the basal coronary flow in the presence of ZM241385 (10.0±0.8 mL min^{-1}) was significantly less than that before CGS21680 infusion (15.0±1.5 mL min^{-1}). While this could have been due to a concomitant fall in aortic output, it could also reflect a small blockade of vasodilator activity of endogenous adenosine. The effect was small and is therefore unlikely to influence the results of this study markedly.

CGS21680 had no effect on the other parameters in the spontaneously beating working heart, suggesting no involvement of A_{2A} receptors in the control of cardiac rate and force of contraction. There were, however, increases in left ventricular pressure and dP/dt max of the paced hearts, which could have arisen from the more substantial increases in coronary flow with CGS2680 than with NECA. Both NECA and CPA reduced rate of

contraction in the spontaneously beating hearts, presumably due to stimulation of A_1 receptors. NECA and CPA also reduced the measures of cardiac contractility (left ventricular pressure, dP/dt max and aortic output) in the spontaneously beating hearts. This could be attributed to the changes in heart rate. No direct inhibitory changes in ventricular contractility would be expected since there are no negative inotropic responses attributable to A_1 receptor stimulation in ventricular muscle (Wilson & Broadley 1989). Indeed, in paced hearts, NECA failed to cause changes in left ventricular pressure and dP/dt max although aortic output was reduced. CPA, however, did reduce left ventricular pressure and dP/dt max, which is not easily explained.

The A_1 selective agonist CPA reduced coronary flow rate in the guinea-pig spontaneously beating hearts, indicative of coronary vasoconstriction. Since this did not occur in paced hearts, it is unlikely to be a direct effect on the coronary vasculature but the result of an A_1 effect to depress heart rate with a consequent reduction of left ventricular developed pressure as proposed by Webb et al (1990). However, studies by others (Wiklund et al 1987, 1989; Stoggall & Shaw 1990; Neely & Matot 1996) together with our previous observations in the aorta and pulmonary artery (Broadley & Maddock 1996), suggest that a direct vasoconstriction is possible. Since NECA caused a similar reduction in heart rate to CPA but with a concurrent coronary vasodilatation, we consider that the reduced coronary flow by CPA in spontaneously beating hearts is more likely to be due to a direct vasoconstrictor action.

The A_3 receptor agonist, IB-MECA, at 3×10^{-7} M, produced no effect on the spontaneously beating working heart. This shows that, at this concentration, IB-MECA could not elicit any responses attributable to A_1 (negative chronotropy) or A_{2A} receptor (coronary vasodilatation) stimulation. In non-working rabbit and rat hearts there were also no changes in ventricular function or heart rate although in rat, but not rabbit, hearts there was a small coronary vasodilatation by IB-MECA (50 nM) that was attributed to A_{2A} receptor activation (Lasley et al 1999). The concentration used in this study should have been sufficient to stimulate A_3 receptors, since others have shown significant responses at this concentration, including enhanced antigen-induced release of serotonin (5-hydroxytryptamine) from rat mast cells (Reeves et al 1997) and neutrophil degranulation (Bouma et al 1997).

In conclusion, this study has demonstrated functionally effective removal of the endothelium from guinea-pig hearts by a novel method of passing oxygen through the coronary vasculature. A coronary vasodilator action of adenosine receptor agonists mediated via A_{2A} receptors is endothelium- and NO-independent, but partially involves cyclooxygenase products. A separate endothelium-independent A_2 receptor-mediated coronary vasodilator action that is mediated via NO was also identified.

References

Behnke, N., Muller, W., Neumann, J., Schmitz, W., Scholz, H., Stein, B. (1990) Differential antagonism by 1,3-dipropylxanthine-8-cyclopentylxanthine and 9-chloro-2-(2-furanyl)-5,6-dihydro-1,2,4-triazolo(1,5-c)quinazolin-5-imine of the effects of adenosine derivatives in the presence of isoprenaline on contractile response and cyclic AMP content in myocytes. Evidence for the coexistence of A_1- and A_2-adenosine receptors on cardiomyocytes. *J. Pharmacol. Exp. Ther.* **254**: 1017–1023

Bellardinelli, L., Linden, J., Berne, R. M. (1989) The cardiac effects of adenosine. *Prog. Cardiovasc. Dis.* **32**: 73–97

Bellardinelli, L., Shyrock, J. C., Snowdy, S., Zhang, Y., Monopoli, A., Lozza, G., Ongini, E., Olsson, R. A., Dennis, D. M. (1998) The A_{2a} adenosine receptor mediates coronary vasodilatation. *J. Pharmacol. Exp. Ther.* **284**: 1066–1073

Bouma, M. G., Jeunhomme, T. M. M. A., Boyle, D. L., Dentener, M. A., Voitenok, N. N., van den Wildenberg, F. A. J. M., Buurman, W. A. (1997) Adenosine inhibits neutrophil degranulation in activated human whole blood. *J. Immunol.* **158**: 5400–5408

Broadley, K. J., Maddock, H. L. (1996) P_1-purinoceptor-mediated vasodilatation and vasoconstriction in hypoxia. *J. Auton. Pharmacol.* **16**: 363–366

Clemo, H. F., Bourassa, A., Linden, J., Bellardinelli, L. (1987) Antagonism of the effects of adenosine and hypoxia on atrioventricular conduction time by two novel alkylxanthines: correlation with binding to adenosine A_1 receptors. *J. Pharmacol. Exp. Ther.* **242**: 478–484

Collis, M. G., Hourani, S. M. O. (1993) Adenosine receptor subtypes. *Trends Pharmacol. Sci.* **14**: 360–366

De Mey, J. G., Claeys, M., Vanhoutte, P. M. (1982) Endothelium-dependent effects of acetylcholine, adenosine triphoshate, thrombin and arachidonic acid in the canine femoral artery. *J. Pharmacol. Exp. Ther.* **222**: 166–173

Felsch, A., Stocker, K., Borchard, U. (1994) Adenosine A_1 and A_2 receptor agonists alter cardiac functions and prostacyclin release in the isolated guinea-pig heart. *Eur. J. Pharmacol.* **263**: 261–268

Fredholm, B. B., Ijzerman, A. P., Jacobson, K. A., Klotz, K.-A., Linden, J. (2001) International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors. *Pharmacol. Rev.* **53**: 527–552

Furchgott, R. F., Zawadzki, J. V. (1980) The obligatory role of endothelial cells in the regulation of arterial smooth muscle by acetylcholine. *Nature* **288**: 373–376

Gardner, N. M., Broadley, K. J. (1999) Analysis of the atypical characteristics of adenosine receptors mediating negative inotropic and chronotropic responses of guinea-pig isolated atria and papillary muscles. *Br. J. Pharmacol.* **127**: 1619–1626

Headrick, J. P., Berne, R. M. (1990) Endothelium-dependent and -independent relaxations to adenosine in guinea pig aorta. *Am. J. Physiol.* **28**: H62–H67

Kan, H., Ruan, Y., Malik, K. U. (1997) Signal transduction mechanism(s) involved in prostacyclin production elicited by acetylcholine in coronary endothelial cells of rabbit heart. *J. Pharmacol. Exp. Ther.* **282**: 113–122

Karwatowska-Prokopczuk, E., Ciabattoni, G., Wennmalm, A. (1988) Effect of adenosine on the formation of prostacyclin in the rabbit isolated heart. *Br. J. Pharmacol.* **94**: 721–728

Kemp, B. K., Cocks, T. M. (1999) Adenosine mediates relaxation of human small resistance-like coronary arteries *via* A_{2B} receptors. *Br. J. Pharmacol.* **126**: 1796–1800

Kuo, L., Chancellor, J. D. (1995) Adenosine potentiates flow-induced dilation of coronary arterioles by activating K_{ATP} channels in the endothelium. *Am. J. Physiol.* **38**: H541–H549

Lasley, R. D., Narayan, P., Jahania, M. S., Partin, E. L., Kraft, K. R., Mentzer, R. M., (1999) Species-dependent hemodynamic effects of adenosine A_3-receptor agonists IB-MECA and Cl-IB-MECA. *Am. J. Physiol.* **276**: H2076–H2084

Lew, M. J., Kao, S. W. (1999) Examination of adenosine receptor-mediated relaxation of the pig coronary arteries. *Clin. Exp. Pharmacol. Physiol.* **26**: 438–443

Li, K., Rouleau, L. K., Andries, L. J., Brutsaert, D. L. (1993) Effect of dysfunctional vascular endothelium on myocardial performance in isolated papillary muscles. *Circ. Res.* **72**: 768–777

Liang, B. T., Morley, J. F. (1996) A new cyclic AMP-independent, Gs-mediated stimulatory mechanism via the adenosine A_{2a} receptor in the intact cardiac cell. *J. Biol. Chem.* **271**: 18678–18685

Losinski, A., Alexander, S. P. H. (1995) Adenosine receptor-mediated relaxation of guinea-pig precontracted, isolated trachea. *Br. J. Pharmacol.* **116**: 2425–2428

Neely, C. F., Matot, I. (1996) Pharmacological probes for A_1 and A_2 adenosine receptors in vivo in feline pulmonary vascular bed. *Am. J. Physiol.* **39**: 610–619

Neely, J. R., Liebermeister, H., Battersby, B. J., Morgan, H. E. (1967) Effect of pressure development on oxygen consumption by isolated rat heart. *Am. J. Physiol.* **212**: 804–814

Peredo, H. A., Feleder, E. C., Adler-Graschinsky, E. (1997) Differential effects of acetylcholine and bradykinin on prostanoid release from rat mesenteric bed: role of endothelium and nitric oxide. *Prostaglandins Leukot. Essent. Fatty Acids* **56**: 253–258

Poucher, S. M., Keddie, J. R., Singh, P., Stoggall, S. M., Caulkett, P. W. R., Jones, G., Collis, M. G. (1995) The *in vitro* pharmacology of ZM 241385, a potent, non-xanthine, A_{2a} selective adenosine receptor antagonist. *Br. J. Pharmacol.* **115**: 1096–1102

Rees, D. D., Palmer, R. M. J., Schulz, R., Hodson, H. F., Moncada, S. (1990) Characterisation of three inhibitors of endothelial nitric oxide synthase in vitro and in vivo. *Br. J. Pharmacol.* **101**: 746–752

Reeves, J. J., Jones, C. A., Sheehan, M. J., Vardey, C. J., Wheelan, C. J. (1997) Adenosine A_3 receptors promote degranulation of rat mast cells both in vitro and in vivo. *Inflammation Res.* **46**: 180–184

Rubanyi, G. N., Vanhoutte, P. M. (1985) Hypoxia releases vasoconstricting substance from the canine vascular endothelium. *J. Physiol.* **364**: 45–56

Schini, V. B., Vanhoutte, P. M. (1991) L-arginine evokes both endothelium-dependent and independent relaxation in L-arginine depleted aortas of the rat. *Circ. Res.* **68**: 209–216

Stoggall, S. M., Shaw, J. S. (1990) The coexistence of adenosine A_1 and A_2 receptors in guinea-pig aorta. *Eur. J. Pharmacol.* **190**: 329–335

Tucker, A. L., Linden, J. (1993) Cloned receptors and cardiovascular responses to adenosine. *Cardiovasc. Res.* **27**: 62–67

Van Calker, D., Muller, M., Hamprecht, B. (1979) Adenosine regulates via two different types of receptors, the accumulation of cyclic AMP in cultured cells. *J. Neurochem.* **33**: 999–1005

Vials, A., Burnstock, G. (1993) A_2-purinoceptor-mediated relaxation in the guinea-pig coronary vasculature: a role for nitric oxide. *Br. J. Pharmacol.* **109**: 424–429

Webb, R. I., McNeal, R. B., Barclay, B. W., Yasay, G. D. (1990) Hemodynamic effects of adenosine agonists in conscious spontaneously hypertensive rat. *J. Pharmacol. Exp. Ther.* **254**: 1090–1099

Wiklund, N. P., Cederquist, B., Matsuda, H., Gustafsson, L. E. (1987) Adenosine can excite pulmonary artery. *Acta Physiol. Scand.* **131**: 477–478

Wiklund, N. P., Cederquist, B., Gustafsson, L. E. (1989) Adenosine enhancement of adrenergic neuroeffector transmission in guinea-pig pulmonary artery. *Br. J. Pharmacol.* **96**: 425–433

Wilson, A. N. A., Broadley, K. J. (1989) Analysis of the direct and indirect effects of adenosine on atrial and ventricular cardiac muscle. *Can. J. Physiol. Pharmacol.* **67**: 294–303

Wood, K. S., Buga, G. L., Byrns, R. E., Ignarro, L. J. (1990) Vascular smooth muscle-derived relaxing factor (MDRF) and its close similarity to nitric oxide. *Biochem. Biophys. Res. Commun.* **170**: 80–88

Xu, H., Stein, B., Liang, B. (1996) Characterisation of a stimulatory adenosine A_{2a} receptor in adult rat ventricular myocyte. *Am. J. Physiol.* **270**: H1655–H1661