British Journal of Pharmacology (1995) 116, 3068-3074 

# The involvement of ATP-sensitive potassium channels and adenosine in the regulation of coronary flow in the isolated perfused rat heart

Michael D. Randall

Department of Physiology and Pharmacology, University of Nottingham Medical School, Queen's Medical Centre, Nottingham NG7 2UH

1 The roles of adenosine 5'-triphosphate (ATP)-sensitive potassium channels ($K_{ATP}$) and endogenous adenosine in the regulation of coronary flow have been assessed in the isolated, buffer-perfused heart of the rat.

2 In the presence of glibenclamide 10 $\mu$M there was a significant ($P<0.001$) reduction in coronary flow from a baseline value of $8.78\pm0.76$ ml $min^{-1}$ $g^{-1}$ to $3.89\pm0.59$ ml $min^{-1}$ $g^{-1}$. This change was accompanied by a significant ($P<0.01$) reduction in cardiac mechanical performance as shown by the decrease in the pressure-rate product from $21,487\pm2,577$ mmHg $min^{-1}$ to $6,950\pm1,104$ mmHg $min^{-1}$.

3 The non-selective adenosine antagonist 8-phenyltheophylline (10 $\mu$M) also caused a significant ($P<0.001$) reduction in coronary flow from a basal value of $10.4\pm0.6$ ml $min^{-1}$ $g^{-1}$ to $6.32\pm0.60$ ml $min^{-1}$ $g^{-1}$. The subsequent addition of glibenclamide, in the presence of 8-phenyltheophylline, brought about a further significant ($P<0.001$) reduction in coronary flow to $3.05\pm0.55$ ml $min^{-1}$ $g^{-1}$ and this value was similar to that in the presence of glibenclamide alone.

4 In hearts perfused under constant flow conditions, exogenous adenosine caused dose-related reductions in coronary perfusion pressure described by a maximum reduction in pressure of $30.7\pm3.9$ mmHg and an $ED_{50}$ of $977\pm813$ pmol. Addition of glibenclamide caused a significant ($P<0.01$) increase in coronary perfusion pressure of $44.7\pm7.2$ mmHg and a significant ($P<0.05$) rightward shift of the dose-response curve for the depressor effects of adenosine ($ED_{50}=13.5\pm3.8$ nmol), with a depression ($P<0.05$) of the maximum ($16.3\pm2.4$ mmHg).

5 In conclusion, both $K_{ATP}$ and endogenous adenosine make major contributions towards coronary vascular tone and the regulation of coronary flow in the rat isolated heart. Furthermore, in the coronary vasculature a significant proportion of the vasodilator action of adenosine is mediated through the activation of $K_{ATP}$.

**Keywords:** ATP-sensitive potassium channels ($K_{ATP}$); glibenclamide; heart; Langendorff preparation; adenosine; coronary flow; coronary vasculature; 8-phenyltheophylline; levcromakalim; sodium nitroprusside

## Introduction

Recent interest has focused on the role played by the vascular smooth muscle adenosine 5'-triphosphate (ATP)-sensitive potassium channel ($K_{ATP}$) in the regulation of vascular tone and blood flow. These channels are regulated by purine derivatives associated with cellular metabolism; intracellular ATP promotes closure, and high ADP favours opening of $K_{ATP}$ (Nichols & Lederer, 1991). Therefore, the activity of these channels may be determined by metabolic status, which has led to the 'ATP hypothesis of blood flow regulation' (Nichols & Lederer, 1991).

In terms of participating in the regulation of basal vascular tone, Jackson (1993) has reported that $K_{ATP}$ plays an important role in determining microvascular tone in the hamster cheek pouch and cremaster muscle under resting conditions. It should be noted, however, that the magnitude of such a role may depend on the vascular bed and also the degree of oxygenation, (Randall, 1994). In the canine coronary vasculature $K_{ATP}$ has been implicated in autoregulation as blockade of these channels abolishes dilator responses associated with arterial occlusion (Komaru *et al.*, 1991; Narishige *et al.*, 1993). In addition these channels also mediate both coronary collateral dilatation (Lamping *et al.*, 1994) and reactive hyperaemic responses (Aversano *et al.*, 1991) in the canine heart. $K_{ATP}$ have also been implicated in hypoxic vasodilatation in the isolated perfused heart of the guinea-pig (von Beckerath *et al.*, 1991). Not only may $K_{ATP}$ participate in coronary vascular regulation under conditions of metabolic stress, but studies in the canine coronary vasculature have demonstrated that glibenclamide induces oscillations in arterial diameters under resting conditions, suggesting a role for $K_{ATP}$ in coronary vasomotion (Nakae *et al.*, 1994). Furthermore, glibenclamide reduces basal coronary flow in the dog *in vivo* (Samaha *et al.*, 1992; Imamura *et al.*, 1992; Billman *et al.*, 1993), identifying a role for these channels in the regulation of basal tone. In addition, electrophysiological data from patch clamp studies on pulmonary arterial vascular smooth muscle cells support the notion that $K_{ATP}$ participates in the regulation of resting membrane potential, with the contribution becoming greater as intracellular ATP levels are reduced (Clapp & Gurney, 1992).

$K_{ATP}$ not only responds to changes in intracellular metabolism but may also be activated by a variety of endogenous mediators. These include prostanoids (Bouchard *et al.*, 1994), $\beta$-adrenoceptor agonists (Randall & McCulloch, 1995) and adenosine (Kirsch *et al.*, 1990; Belloni & Hintze, 1991; Merkel *et al.*, 1992; Dart & Standen, 1993). Accordingly, $K_{ATP}$ activity may be highly regulated by a variety of systems.

The present investigation has been carried out to assess the contribution made by $K_{ATP}$ in the regulation of coronary flow in the isolated, buffer-perfused rat heart. To achieve this, the effects of the selective $K_{ATP}$ inhibitor glibenclamide (Sturgess *et al.*, 1985) on coronary flow have been studied under constant pressure perfusion. Given the well-established role for adeno-

sine in blood flow regulation in the heart (Berne, 1963, 1980) the effects of adenosine receptor antagonism on coronary flow have also been determined to assess the contribution made by endogenous adenosine to basal blood flow. Furthermore, the possibility that the effects of adenosine may be mediated by $K_{ATP}$ in the coronary vasculature has been explored.

A preliminary account of part of this work has been presented to the British Pharmacological Society (Randall, 1995).

## Methods

### *Preparation of the isolated Langendorff heart perfused at constant pressure*

Male Wistar rats (220–380 g) were heparinized (1,000 u $kg^{-1}$ i.p.) and anaesthetized with sodium pentobarbitone (60 mg $kg^{-1}$, i.p.: Sagatal, Rhône Mérieux, Harlow, Essex). In each case, following a mid-line thoracotomy, the heart was rapidly excised and placed in ice-cold oxygenated modified Krebs-Henseleit solution (containing (mM): NaCl 118, KCl 4.7, $KH_2PO_4$ 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25, $CaCl_2$ 2, D-glucose 10, Na-pyruvate 2) to arrest cardiac contraction. The aortic stump was then cannulated and the heart perfused retrogradely according to the method of Langendorff (1895) at a constant pressure of 60 mmHg with oxygenated ($Po_2$ = 550–600 mmHg) Krebs-Henseleit buffer. A water-filled latex balloon catheter, coupled to a pressure transducer, was inserted through the pulmonary vein and advanced into the left ventricle in order to measure developed left ventricular pressure (DLVP). In each case left ventricular end diastolic pressure was initially set at 5 mmHg by adjusting the volume of fluid in the balloon. The pressure transducer was coupled to a MacLab 4e recording system (ADInstruments, New South Wales, Australia) and heart rate was derived from the pressure signal. Coronary flow was measured by means of a transit time ultrasonic flow meter (model T106, Transonic Systems Incorporated, Ithaca, New York, U.S.A.) coupled to an extracorporeal flow probe placed in series with the aortic cannula. Under these conditions drugs were added to the perfusion fluid to achieve the desired concentration.

### *Preparation of the isolated Langendorff heart perfused under constant flow conditions*

The heart was prepared in exactly the same way as described above for constant pressure perfusion except that perfusion was performed by means of a peristaltic pump (Watson-Marlow, 504S), at a flow rate to achieve a coronary perfusion pressure of approximately 90 mmHg. In these experiments drugs were injected as bolus doses close to the aortic inflow cannula in volumes of 30–100 $\mu$l and the effects on both coronary perfusion pressure and mechanical performance were recorded.

### *Experimental protocol*

To assess the effects of glibenclamide (10 $\mu$M) on coronary flow under constant pressure conditions preparations were equilibrated for 15 min, after which time coronary flow and cardiac mechanical performance were recorded over the next 15 min and the time-averaged values obtained. Glibenclamide or vehicle was then added to the perfusion fluid and following a 15 min equilibration period the effects on coronary flow and mechanical performance were recorded for another 15 min. The vasorelaxant viability of the preparation was then assessed by the addition of 100 $\mu$M sodium nitroprusside.

In order to assess the effects of 8-phenyltheophylline 10 $\mu$M (8-PT) on coronary flow preparations were equilibrated for 15 min, after which coronary flow and cardiac mechanical performance were recorded for the next 15 min. 8-PT was then added to the perfusion fluid and following a 15 min period the effects on coronary flow and mechanical performance were recorded for 15 min. Following this any additional effect of glibenclamide was determined by addition of glibenclamide (10 $\mu$M) in the presence of 8-PT and the effects determined after a further 15 min.

To examine the ability of the potassium channel activator levcromakalim to increase baseline coronary flow, preparations were perfused under constant pressure conditions. A dose-response curve was constructed for levcromakalim by close arterial injections of the potassium channel activator and the effects on both coronary flow and mechanical performance were determined.

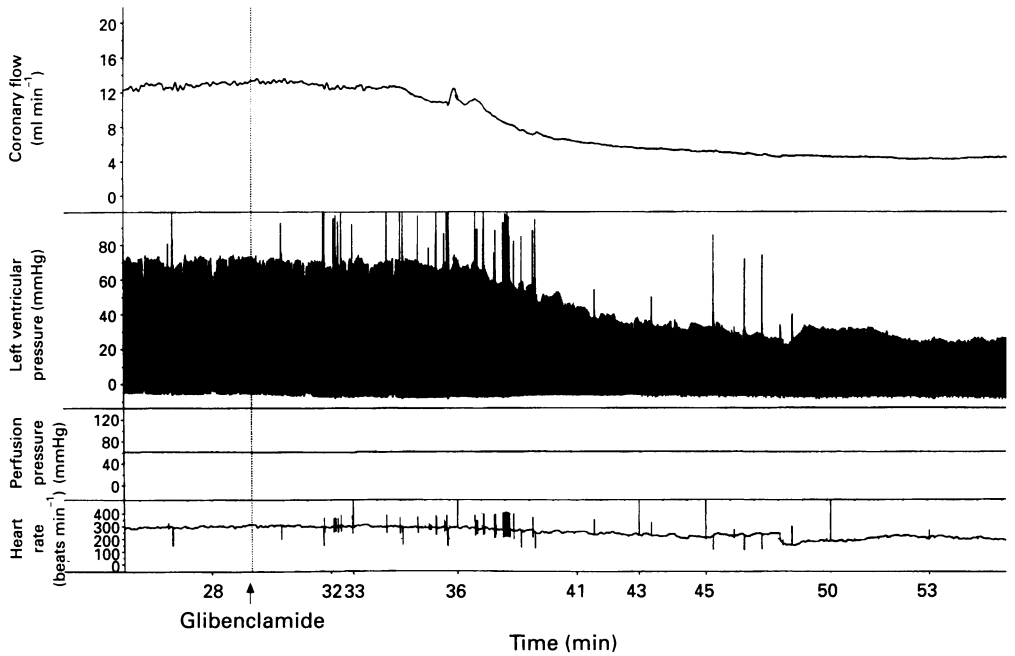


**Figure 1** A representative trace showing the effects of glibenclamide on the cardiac variables (coronary flow, left ventricular pressure, coronary perfusion pressure and heart rate). The initial portion of the trace shows the baseline values and is followed by the addition of glibenclamide (10 $\mu$M) to the perfusion fluid at 30 min with the subsequent equilibration.

In order to determine whether adenosine activates $K_{ATP}$ in the coronary vasculature dose-response curves were constructed for exogenous adenosine under constant flow conditions. Glibenclamide (10 $\mu$M) was then added to the perfusion fluid and dose-response curves were again constructed for adenosine following a 15 min wash-in period. Administration of glibenclamide resulted in a consistent increase (ca. 45 mmHg) in coronary perfusion pressure. In order to control for the possible effects of this increase in tone on vasorelaxant responses another dose-response curve was constructed for adenosine in the presence of glibenclamide, but at a level of tone equivalent to the initial baseline (ca. 90 mmHg). This was achieved by reducing the flow rate to yield a perfusion pressure close to the initial level.

### *Quantitation and statistical analysis*

All data are given as the mean ± s.e.mean and were compared by either analysis of variance or Student's *t* tests (paired or unpaired) as appropriate. Cardiac mechanical performance was quantified as the pressure-rate product (PRP), which is the mathematical product of DLVP and heart rate. $ED_{50}$ values for vasodilator responses were obtained from individual dose-response curves as the dose at which the half-maximal relaxant response occurred. These variables were determined by fitting the data to the logistic equation:

$$R = \frac{R_{max}\ A^{n_H}}{ED_{50}{}^{n_H} + A^{n_H}}$$

where R is the response, A the dose of vasorelaxant, $R_{max}$ the maximum response, $n_H$ the slope function and $ED_{50}$ the dose of the vasorelaxant giving half the maximal response. The curve fitting was carried out using KaleidaGraph software (Synergy, Reading, PA, U.S.A.) running on a Macintosh LC III computer. The $ED_{50}$ values were converted to the logarithmic values for statistical analysis.

### *Drugs*

All solutions were prepared on the day of the experiment. Glibenclamide (Research Biochemicals Incorporated, Natick,

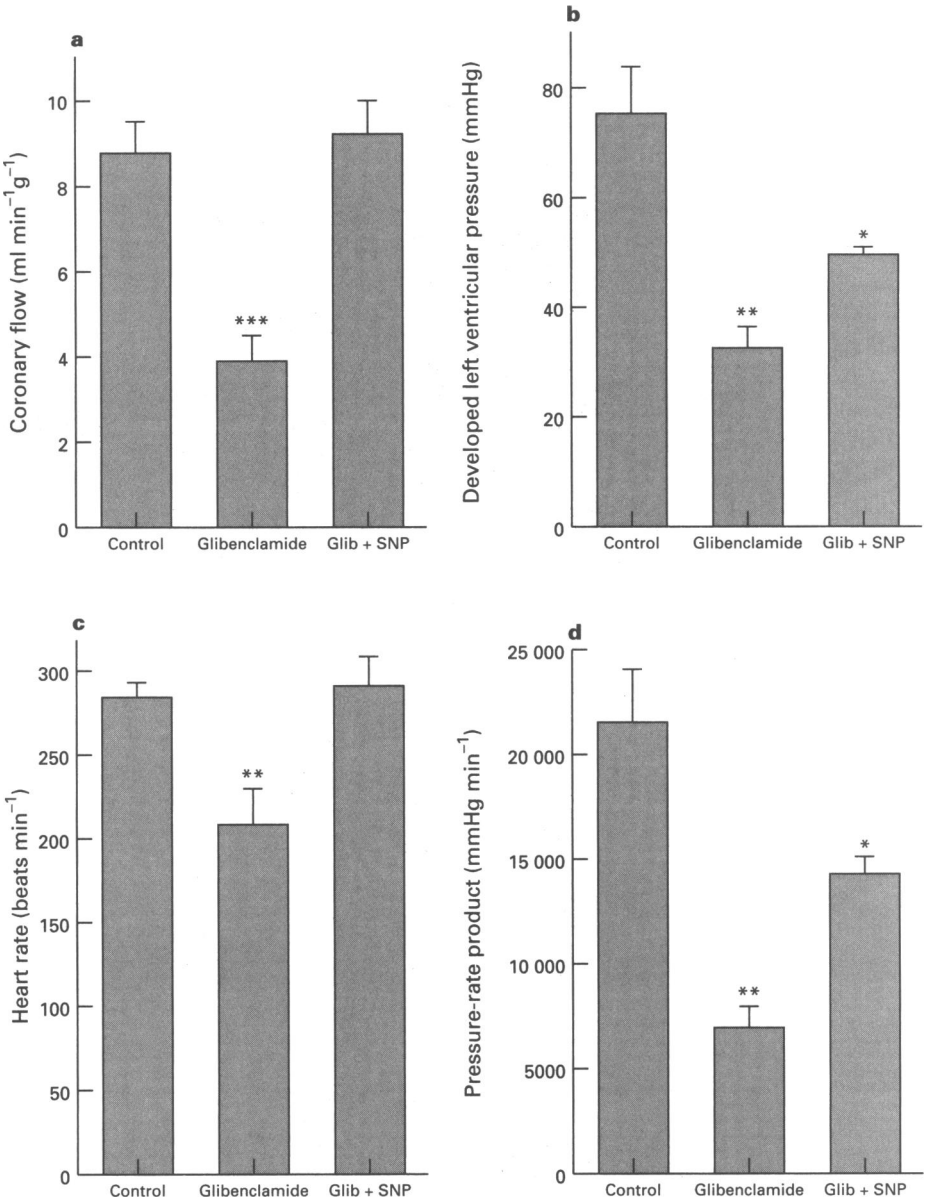


**Figure 2** Cardiac variables in the isolated buffer perfused heart of the rat under control conditions, in the presence of glibenclamide (10 $\mu$M) and in the presence both glibenclamide 10 $\mu$M (Glib) and sodium nitroprusside 100 $\mu$M (SNP). (a) Shows the effects on coronary flow, (b) developed left ventricular pressure, (c) heart rate and (d) mechanical performance assessed by the pressure-rate product. *$P<0.05$, **$P<0.01$ and the ***$P<0.001$ indicate statistically significant differences from the controls. The data were obtained from 6 preparations and are given as mean ± s.e.mean (indicated by the vertical bars).

MA, U.S.A.) was dissolved in dimethylsulphoxide (DMSO) as a $10^{-2}$M stock solution and the final concentration of DMSO in the perfusion fluid was 0.001% (v/v). 8-Phenyltheophylline (8-PT) was initially dissolved in 0.1 M NaOH, adenosine was dissolved in 0.05 M NaOH and both were from Sigma Chemical Company, Poole, UK. Levcromakalim (a generous gift from SmithKline Beecham, Surrey, U.K.), was dissolved in 70% (v.v) ethanol. Sodium nitroprusside (Sigma) was dissolved in 0.9% saline and protected from light. All drugs were then diluted to the required concentrations in the Krebs-Henseleit solution.

## Results

### *Effects of glibenclamide on coronary flow*

In 6 preparations, perfused at constant pressure, basal coronary flow was $8.78 \pm 0.76$ ml $min^{-1}$ $g^{-1}$ (wet weight) and the pressure-rate product was $21,487 \pm 2,577$ mmHg $min^{-1}$ (Figures 1, 2a and d). Following the addition of glibenclamide (10 $\mu$M) coronary flow was significantly ($P<0.001$) decreased to $3.89 \pm 0.59$ ml $min^{-1}$ $g^{-1}$ and the mechanical performance was also significantly ($P<0.01$) depressed with a pressure-rate product (PRP) of $6,950 \pm 1,104$ mmHg $min^{-1}$. The reduction in mechanical performance was due to a reduction in DLVP ($P<0.01$; Figure 1 & 2b) and also bradycardia ($P<0.01$; Figure 2c). The reduction in coronary flow was fully reversed by the addition of sodium nitroprusside (100 $\mu$M) when it was $9.20 \pm 0.80$ ml $min^{-1}$ $g^{-1}$, while the mechanical performance was only partially restored (PRP = $14,315 \pm 884$ mmHg $min^{-1}$ (Figures 2a and d).

In 3 vehicle/time control experiments baseline coronary flow was 9.5 ml $min^{-1}$ $g^{-1}$ and the pressure-rate product was $9.51 \pm 0.53$ $23,429 \pm 2,079$ mmHg $min^{-1}$. These variables were not significantly influenced by the addition of DMSO (0.001v/v) or the time course of perfusion. In these experiments the subsequent addition of sodium nitroprusside 100 $\mu$M increased coronary flow to $10.9 \pm 1.1$ ml $min^{-1}$ $g^{-1}$ and the pressure-rate product was $28,607 \pm 3,458$ mmHg $min^{-1}$.

### *Effects of 8-phenyltheophylline on coronary flow*

In 6 different preparations basal coronary flow was $10.4 \pm 0.6$ ml $min^{-1}$ $g^{-1}$ and the PRP was $24,740 \pm 1,350$ mmHg $min^{-1}$ (Figure 3a and d). In the presence of 8-PT 10 $\mu$M these values were significantly ($P<0.001$) reduced to $6.32 \pm 0.60$ ml $min^{-1}$ $g^{-1}$ and $13,290 \pm 1,728$ mmHg $min^{-1}$ respectively (Figure 3a and d), with there being a marked ($P<0.001$) reduction in DLVP and modest bradycardia (Figure 3b and c). The subsequent administration of glibenclamide (10 $\mu$M), in the presence of 8-PT, brought about further decreases in these vaiables to $3.05 \pm 0.55$ ml $min^{-1}$ $g^{-1}$ ($P<0.001$) and $6,085 \pm 1,267$ mmHg $min^{-1}$ ($P<0.001$) with

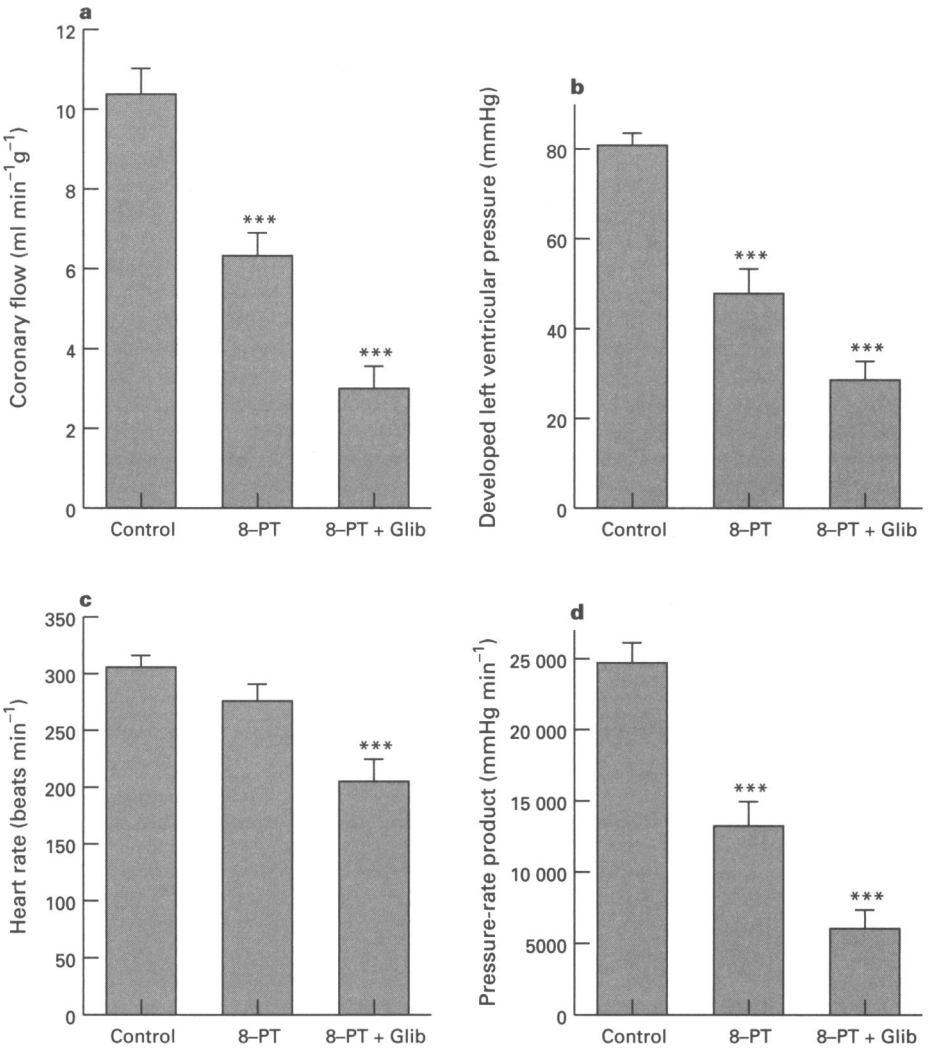


**Figure 3** Cardiac variables in the isolated buffer perfused rat heart under control conditions, in the presence of 8-phenyltheophylline 10 $\mu$M (8-PT) alone and in the presence of both glibenclamide 10 $\mu$M (Glib) and 8-phenyltheophylline 10 $\mu$M (8-PT). (a) Shows the effects on coronary flow, (b) developed left ventricular pressure, (c) heart rate and (d) mechanical performance assessed by the pressure-rate product. ***$P<0.001$ indicate statistically significant differences from the controls. The data were obtained from 6 preparations and are given as means ± s.e.mean (indicated by the vertical bars).

there being both a reduction in developed pressure and bradycardia (Figure 3b and c). All of these values were similar to those obtained in the presence of glibenclamide alone.

### *Effects of levcromakalim on coronary flow*

In 10 hearts perfused under constant pressure conditions coronary flow was $12.3 \pm 1.4$ ml $min^{-1}$ $g^{-1}$, and the PRP was $20,093 \pm 1,520$ mmHg $min^{-1}$. In these preparations levcromakalim gave rise to dose-related increases in flow described by an $ED_{50}$ of $2.31 \pm 1.29$ nmol and a maximum increase in flow of $3.43 \pm 0.09$ ml $min^{-1}$ $g^{-1}$ (Figure 4).

### *Effects of glibenclamide on vasorelaxant responses to adenosine*

For the 6 preparations perfused under constant flow conditions the cardiac variables are given in Table 1. In these preparations adenosine (11 pmol–112 nmol) caused dose-related reductions in coronary perfusion pressure described by an $R_{max} = 30.7 \pm 3.9$ mmHg and an $ED_{50} = 977 \pm 813$ pmol (Figure 5). In these preparations the addition of glibenclamide (10 $\mu$M) to the perfusion fluid caused a significant ($P < 0.01$) increase in perfusion pressure of $44.7 \pm 7.2$ mmHg. Glibenclamide caused a significant ($P < 0.05$) rightward shift in the dose-response curve for the vasorelaxant properties of adenosine ($ED_{50} = 13.5 \pm 3.8$ nmol) and also a depression ($P < 0.05$) of the maximum response ($16.3 \pm 2.4$ mmHg) (Figure 5). A different dose-response curve for the effects of adenosine in the presence of glibenclamide was also constructed under a level of tone equivalent to that in the controls (Table 1). The dose-response curve under these conditions was similar to that previously obtained in the presence of glibenclamide at the normal flow rate ($R_{max} = 21.2 \pm 3.6$ mmHg and $ED_{50} = 18.6 \pm 6.8$ nmol), as it was also shifted to the right with a depression of the maximum (Figure 5).

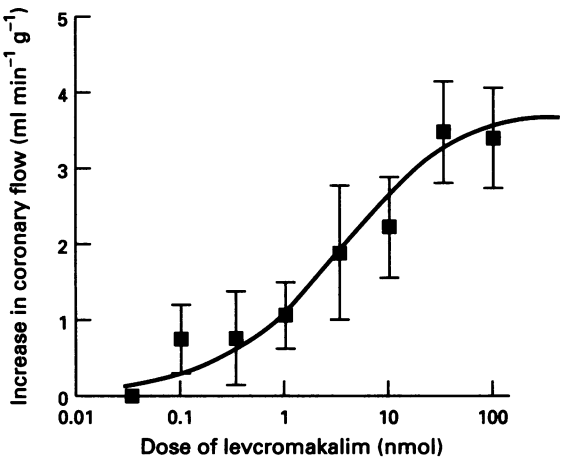


**Figure 4** Dose-response curve for increase in coronary flow in the isolated perfused rat heart in response to levcromakalim ($n = 10$). The vertical bars indicate s.e.mean.

## Discussion

The findings of the present investigation clearly indicate that a glibenclamide-sensitive mechanism and endogenous adenosine make major contributions towards the regulation of basal coronary flow in the isolated perfused heart of the rat. It thus seems reasonable to assume that $K_{ATP}$ plays an important role in mediating these effects. Additionally, a link between adenosine and $K_{ATP}$ activation has been identified in this vascular bed, which indicates that a significant portion of the vaso-regulatory role of adenosine is probably mediated through these channels, at least in the rat.

The participation of $K_{ATP}$ in coronary flow regulation is indicated by the observation that glibenclamide reduced coronary flow by approximately 55%. A similar observation was

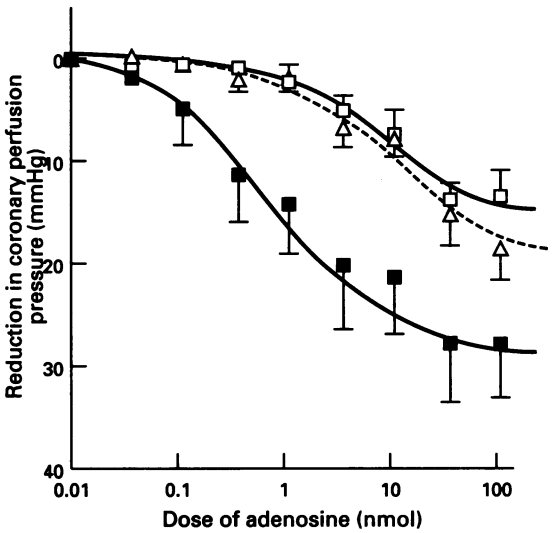


**Figure 5** Dose-response curves for the reductions of coronary perfusion pressure by adenosine in the rat isolated heart, perfused under constant flow conditions. (■) Indicates control data, (□) the responses to adenosine in the presence of glibenclamide 10 $\mu$M at equal flow rate and (△) indicates the effects of glibenclamide on responses to adenosine at equivalent tone. The vertical bars indicate s.e.mean ($n = 6$).

**Table 1** The cardiac variables from the isolated buffer-perfused heart in the absence and presence of glibenclamide 10 $\mu$M at both equal flow rate and equivalent tone

| *Treatment* | *Control* | + *Glibenclamide* | + *Glibenclamide at equivalent tone* |
|---|---|---|---|
| Coronary flow (ml $min^{-1}$ $g^{-1}$) | $13.8 \pm 0.9$ | $13.8 \pm 0.8$ | $7.71 \pm 0.64$ (***) |
| Coronary perfusion pressure (mmHg) | $90.1 \pm 3.5$ | $135 \pm 7$ (**) | $94.8 \pm 6.1$ |
| DLVP (mmHg) | $108 \pm 11$ | $97.7 \pm 10.9$ | $67.3 \pm 5.2$ (*) |
| Heart rate (beats $min^{-1}$) | $278 \pm 9$ | $271 \pm 20$ | $241 \pm 20$ |
| Pressure-rate product (mmHg $min^{-1}$) | $30,084 \pm 3,123$ | $26,133 \pm 2,945$ | $16,324 \pm 2,015$ (**) |

*$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ indicate statistically significant differences between the controls and the treatments. The data are given as mean ± s.e.mean ($n = 6$).

obtained in the rat heart by Miwa *et al.* (1994), although it should be noted that they used a much lower concentration of glibenclamide (700 nM) and correspondingly observed a smaller decrease (10%). In the guinea-pig perfused heart glibenclamide 10 $\mu$M produces a 24% reduction in coronary flow (Niya *et al.*, 1994) and this provides evidence for $K_{ATP}$ playing an important regulatory role in this vascular bed. The reduction in flow in the present study was accompanied by a depression of cardiac mechanical performance as shown by reductions in both DLVP and heart rate. The reduction in mechanical performance may have been partially due to myocardial underperfusion, as a result of the reduction in coronary flow, and would therefore indicate that $K_{ATP}$ activity also has an indirect effect on cardiac performance. The effects of glibenclamide on coronary flow were reversed by the nitrovasodilator sodium nitroprusside suggesting that the sulphonylurea was having a specific, reversible action on vascular control as opposed to inducing vasospasm through tissue damage. However, the effects of glibenclamide on mechanical performance were only partially reversed by the restoration of coronary flow. This may indicate that either glibenclamide has a myocardial depressant effect independent of its vascular actions or that the underperfusion resulted in tissue damage. The former would seem unlikely as cardiac $K_{ATP}$-activation, rather than inhibition, is thought to be responsible for a protective myocardial suppressant effect (Noma, 1983). A myocardial depressant effect of sodium nitroprusside may be ruled out as there was no impairment of mechanical performance when sodium nitroprusside was administered in the absence of glibenclamide.

The effects of glibenclamide on vascular regulation were independent of the mode of perfusion as under constant flow conditions this agent caused appreciable increases in vascular tone through the inhibition of $K_{ATP}$. Hence, the involvement of $K_{ATP}$ in vascular regulation occurs in both the presence (i.e. under constant pressure perfusion) and the absence (constant flow mode) of intrinsic autoregulatory mechanisms.

The magnitude of the reduction in coronary flow in response to glibenclamide indicates a substantial role for $K_{ATP}$ and is comparable to the role played by nitric oxide in the rat heart, as chronic inhibition of nitric oxide synthase is associated with a 40% reduction in coronary flow (Constantin-Teodosiu *et al.*, 1995). Despite this important contribution towards vascular regulation the $K_{ATP}$ would not appear to be maximally activated under basal conditions as the potassium channel activator levcromakalim was able to cause appreciable vasodilatation of basal tone in the present study and also in the study by Miwa *et al.* (1994).

The involvement of $K_{ATP}$ in the regulation of coronary flow identified in the present investigation accords with similar findings in the canine coronary vasculature (Samaha *et al.*, 1992; Imamura *et al.*, 1992; Billman *et al.*, 1993) and resistance beds in the hamster (Jackson, 1993). Furthermore, haemodynamic data from conscious rats indicate that glibenclamide produces regional increases in vascular resistance (Moreau *et al.*, 1994), which suggests that $K_{ATP}$ also regulates vascular tone *in vivo* and in beds other than the coronary vasculature. Despite these observations there are several studies which do not support a role for $K_{ATP}$ in the control of basal vascular tone under normal conditions (for reviews see Nichols & Lederer, 1991; Nelson & Quayle, 1995), and only contribute during metabolic impairment (Randall, 1994).

The reduction in coronary flow in response to the non-selective adenosine antagonist was to be expected as adenosine is an established coronary vasodilator and has a well-defined role in coronary flow regulation (Berne, 1963; 1980). It is interesting to note that the subsequent addition of glibenclamide in the presence of 8-PT was similar to the addition of glibenclamide alone and may suggest that there is a degree of overlap between the vasodilator actions of adenosine and $K_{ATP}$ activation. In this respect the coupling of adenosine receptor activation and $K_{ATP}$ opening is reasonably well established. Specifically, patch clamp studies using membrane patches of rat cultured ventricular myocytes (Kirsch *et al.*, 1990), and whole-cell current recordings from porcine isolated coronary vascular smooth muscle cells (Dart & Standen, 1993), have indicated that adenosine $A_1$ receptors may be positively coupled, via a G-protein, to $K_{ATP}$. The possibility that adenosine is coupled to $K_{ATP}$ receives further functional support from evidence that adenosine $A_1$, but not $A_2$ receptor agonists cause sulphonylurea-sensitive vasorelaxation of porcine coronary vessels (Merkel *et al.*, 1992). Furthermore, in the rabbit ear vasculature adenosine $A_1$ receptor activation augments the vasorelaxant potency of the potassium channel opener levcromakalim, perhaps suggesting modulation at the level of the $K_{ATP}$ (Randall *et al.*, 1994). To test the hypothesis that adenosine may be acting via $K_{ATP}$ in the rat coronary vasculature the effects of glibenclamide on the vasorelaxant responses to adenosine were examined under constant flow conditions. In these experiments glibenclamide caused non-competitive inhibition of adenosine responses, whether compared at the same flow rate or equivalent tone. A non-specific inhibitory effect of glibenclamide on vasodilatation may be excluded as the vasodilator responses to sodium nitroprusside were unaffected by its presence. These experiments therefore provide evidence that in the rat coronary vasculature, adenosine may cause $K_{ATP}$ activation, either directly or indirectly. In addition to acting via $K_{ATP}$ there was a glibenclamide-insensitive component to adenosine-mediated vasorelaxations. This accords with work of von Beckerath *et al.* (1991) and Niya *et al.* (1994), which similarly identified both glibenclamide-sensitive and -insensitive components in the guinea-pig perfused coronary vasculature. This difference was related to the different receptor subtypes involved. Interestingly, the non-vascular actions of adenosine on guinea-pig ventricular tissue are glibenclamide-insensitive (Xu *et al.*, 1994).

The contribution made by $K_{ATP}$ towards coronary flow regulation was appreciably greater than that made by endogenous adenosine. Hence, adenosine release cannot fully explain the activation of the $K_{ATP}$. It is therefore possible that the endogenous release of vasoactive agents such as prostanoids may also lead to $K_{ATP}$ activation (Bouchard *et al.*, 1994). Alternatively, under basal conditions a significant proportion of $K_{ATP}$ could be in the open state in the absence of agonist-stimulation or in the context of a highly metabolic organ such as the heart activated as a consequence of metabolic stress.

In the present investigation important roles have been identified for both $K_{ATP}$ and adenosine in the regulation of coronary flow. Furthermore, it is likely, at least to some degree, that the action of adenosine is transduced through $K_{ATP}$ activation. It is suggested that the 'adenosine hypothesis' (Berne, 1980) and the 'ATP hypothesis' (Nichols & Lederer, 1991) of blood flow regulation are not mutually exclusive.

This study was funded by a project grant from The British Heart Foundation (PG/94060) and an equipment grant from The Royal Society. The author thanks Professor S.M. Gardiner for helpful comments made during the preparation of the manuscript.

## References

AVERSANO, T., OUYANG, P. & SILVERMAN, H. (1991). Blockade of the ATP-sensitive potassium channel modulates reactive hyperemia in the canine coronary circulation. *Circ. Res.*, **69**, 618–622.

BELLONI, F.L. & HINTZE, T.H. (1991). Glibenclamide attenuates adenosine-induced bradycardia and coronary vasodilatation. *Am. J. Physiol.*, **261**, H720–H727.

BERNE, R.M. (1963). Cardiac nucleotides in hypoxia: possible role in regulation of coronary blood flow. *Am. J. Physiol.*, **204**, 317–322.

BERNE, R.M. (1980). The role of adenosine in the regulation of coronary blood flow. *Circ. Res.*, **47**, 807–813.

BILLMAN, G.E., AVERDANO, C.E., HALLIWALL, J.R. & BURROUGHS, J.M. (1993). The effects of the ATP-dependent potassium channel antagonist, glyburide, on coronary blood flow and susceptibility to ventricular fibrillation in unanesthetized dogs. *J. Cardiovasc. Pharmacol.*, **21**, 197–207.

BOUCHARD, J-F., DUMONT, E. & LAMONTAGNE, D. (1994). Evidence that prostaglandins $I_2$, $E_2$, and $D_2$ may activate ATP sensitive potassium channels in the isolated rat heart. *Cardiovasc. Res.*, **28**, 901–905.

CLAPP, L.H. & GURNEY, A.M. (1992). ATP-sensitive $K^+$ channels regulate resting membrane potential of pulmonary arterial smooth muscle cells. *Am. J. Physiol.*, **262**, H916–H920.

CONSTANTIN-TEODOSIU, D., GREENHAFF, P.L., GARDINER, S.M., RANDALL, M.D., MARCH, J.E. & BENNETT, T. (1995). Creatine favourably affects distressed myocardial energy metabolism in Brattleboro rats caused by chronic inhibition of nitric oxide synthase *Br. J. Pharmacol.*, (in press).

DART, C. & STANDEN, N.B. (1993). Adenosine-activated potassium current in smooth cells isolated from the pig coronary artery. *J. Physiol.*, **471**, 767–786.

IMAMURA, Y., TOMOIKE, H., NARISHIGE, T., TAKAHASHI, T., KASUYA, H. & TAKESHITA, A. (1992). Glibenclamide decreases basal coronary blood flow in anesthetized dogs. *Am. J. Physiol.*, **263**, H399–H404.

JACKSON, W.F. (1993). Arteriolar tone is determined by activity of ATP-sensitive potassium channels. *Am. J. Physiol.*, **265**, H1797–H1803.

KIRSCH, G.E., CODINA, J., BIRNBAUMER, L. & BROWN, A.M. (1990). Coupling of ATP-sensitive $K^+$ channels to $A_1$ receptors by G-proteins in rat ventricular myocytes. *Am. J. Physiol.*, **259**, H820–H826.

KOMARU, T., LAMPING, K.G., EASTHAM, C.L. & DELLSPERGER, K.C. (1991). Role of ATP-sensitive potassium channels in coronary microvascular autoregulatory responses. *Circ. Res.*, **69**, 1146–1151.

LAMPING, K.G., BLOOM, E.N. & HARRISON, D.G. (1994). Regulation of native coronary collateral vessel dilation after coronary occlusion in the dog. *Am. J. Physiol.*, **266**, H769–H778.

LANGENDORFF, O. (1895). Untersuchungen am überlebenden Säugethierherzen. *Pflügers Arch.*, **61**, 291–332.

MERKEL, L.A., LAPPE, R.W., RIVERA, L.M., COX, B.F. & PERRONE, M.H. (1992). Demonstration of vasorelaxant activity with an $A_1$-selective adenosine agonist in porcine coronary artery-involvement of potassium channels. *J. Pharmacol. Exp. Ther.*, **260**, 437–443.

MIWA, A., JINNO, Y., YOKOYAMA, T., IZAWA, T. & OGAWA, N. (1994). Pharmacological analysis of the effects of KRN2391 on coronary vasculature in perfused rat heart. *Gen. Pharmacol.*, **25**, 471–474.

MOREAU, R., KOMEICHI, H., KIRSTETTER, P., YANG, S., AUPETIT-FAISANT, B., CAILMAIL, S. & LEBREC, D. (1994). Effects of glibenclamide on systemic and splanchnic haemodynamics in conscious rats. *Br. J. Pharmacol.*, **112**, 649–653.

NAKAE, I., QUAN, L., SUGIMOTO, Y., TSUTAMOTO, T. & KINOSHITA, M. (1994). Glibenclamide-induced oscillation of canine coronary artery is independent of myocardial ischemia. *J. Cardiovasc. Pharmacol.*, **23**, 473–479.

NARISHIGE, T., EGASHIRA, K., AKATSUKA, Y., KASUDA, Y., NUMAGUCHI, K., SAKATA, M. & TAKESHITA, A. (1993). Glibenclamide, a putative ATP-sensitive $K^+$ channel blocker, inhibits coronary autoregulation in anesthetized dogs. *Circ. Res.*, **73**, 771–776.

NELSON, M.T. & QUAYLE, J.M. (1995). Physiological roles and properties of potassium channels in arterial smooth muscle. *Am. J. Physiol.*, **268**, C799–C822.

NICHOLS, C.G. & LEDERER, W.J. (1991). Adenosine triphosphate-sensitive potassium channels in the cardiovascular system. *Am. J. Physiol.*, **261**, H1675–H1686.

NIYA, K., UCHIDA, S., TSUJI, T. & OLSSON, R.A. (1994). Glibenclamide reduces the coronary vasoactivity of adenosine receptor agonists. *J. Pharmacol. Exp. Ther.*, **271**, 14–19.

NOMA, A. (1983). ATP-regulated $K^+$ channels in cardiac muscle. *Nature*, **305**, 147–148.

RANDALL, M.D. (1994). The involvement of ATP-sensitive potassium channels in the modulation of vascular responses in isolated perfused resistance beds of the rat. *Br. J. Pharmacol.*, **113**, 173P.

RANDALL, M.D. (1995). The role of $K_{ATP}$-channels and adenosine in the regulation of coronary flow in the isolated buffer perfused rat heart. *Br. J. Pharmacol.*, **116**, 173P.

RANDALL, M.D. & MCCULLOCH, A.I. (1995). The involvement of ATP-sensitive potassium channels in $\beta$-adrenoceptor-mediated vasorelaxation in the isolated rat mesenteric arterial bed. *Br. J. Pharmacol.*, **115**, 607–612.

RANDALL, M.D., UJIIE, H. & GRIFFITH, T.M. (1994). Modulation of vasodilatation to levcromakalim by adenosine analogues in the rabbit ear: an explanation for hypoxic augmentation. *Br. J. Pharmacol.*, **112**, 49–54.

SAMAHA, F.F., HEINEMAN, F.W., INCE, C., FLEMING, J. & BALABAN, R.S. (1992). ATP-sensitive potassium channel is essential to maintain basal coronary vascular tone *in vivo*. *Am. J. Physiol.*, **262**, C1220–C1227.

STURGESS, N.C., ASHFORD, M.J.L., COOK, D.L. & HALES, C.N. (1985). The sulphonylurea receptor may be an ATP-sensitive potassium channel. *Lancet*, **ii**, 474–475.

VON BECKERATH, N., CYRYS, S., DISCHNER, A. & DAUT, J. (1991). Hypoxic vasodilatation in isolated, perfused guinea-pig heart: an analysis of the underlying mechanisms. *J. Physiol.*, **442**, 297–319.

XU, J., WANG, L., HURT, C. & PELLEG, A. (1994). Endogenous adenosine does not activate ATP-sensitive potassium channels in the hypoxic guinea-pig ventricle *in vivo*. *Circulation*, **89**, 1209–1216.

*(Received April 11, 1995*
*Revised August 14, 1995*
*Accepted August 18, 1995)*