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K_{ATP}-channel activation: effects on myocardial recovery from ischaemia and role in the cardioprotective response to adenosine A_1-receptor stimulation

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1 Optimization of myocardial energy substrate metabolism improves the recovery of mechanical function of the post-ischaemic heart. This study investigated the role of K_{ATP}-channels in the regulation of the metabolic and mechanical function of the aerobic and post-ischaemic heart by measuring the effects of the selective K_{ATP}-channel activator, cromakalim, and the effects of the K_{ATP}-channel antagonist, glibenclamide, in rat fatty acid perfused, working hearts *in vitro*. The role of K_{ATP} channels in the cardioprotective actions of the adenosine A_1-receptor agonist, N^6-cyclohexyladenosine (CHA) was also investigated.

2 Myocardial glucose metabolism, mechanical function and efficiency were measured simultaneously in hearts perfused with modified Krebs-Henseleit solution containing 2.5 mM Ca^{2+}, 11 mM glucose, 1.2 mM palmitate and 100 mu l^{-1} insulin, and paced at 300 beats min^{-1}. Rates of glycolysis and glucose oxidation were measured from the quantitative production of ^3H_2O and $^{14}CO_2$, respectively, from [5-^{3}H/U-^{14}C]-glucose.

3 In hearts perfused under aerobic conditions, cromakalim (10 μM), CHA (0.5 μM) or glibenclamide (30 μM) had no effect on mechanical function. Cromakalim did not affect glycolysis or glucose oxidation, whereas glibenclamide significantly increased rates of glycolysis and proton production. CHA significantly reduced rates of glycolysis and proton production but had no effect on glucose oxidation. Glibenclamide did not alter CHA-induced inhibition of glycolysis and proton production.

4 In hearts reperfused for 30 min following 30 min of ischaemia, left ventricular minute work (LV work) recovered to 24% of aerobic baseline values. Cromakalim (10 μM), administered 5 min before ischaemia, had no significant effect on mechanical recovery or glucose metabolism. CHA (0.5 μM) significantly increased the recovery of LV work to 67% of aerobic baseline values and also significantly inhibited rates of glycolysis and proton production. Glibenclamide (30 μM) significantly depressed the recovery of mechanical function to <1% of aerobic baseline values and stimulated glycolysis and proton production.

5 Despite the deleterious actions of glibenclamide *per se* in post-ischaemic hearts, the beneficial effects of CHA (0.5 μM) on the recovery of mechanical function and proton production were not affected by glibenclamide.

6 The data indicate that the cardioprotective mechanism of adenosine A_1-receptor stimulation does not involve the activation of K_{ATP}-channels. Furthermore, in rat fatty acid perfused, working hearts, stimulation of K_{ATP}-channels is not cardioprotective and has no significant effects on myocardial glucose metabolism.

Keywords: Cardioprotection; myocardial ischaemia; K_{ATP}-channels; adenosine A_1-receptors; glycolysis; glucose oxidation; cromakalim; glibenclamide; N^6-cyclohexyladenosine

Introduction

Myocardial energy substrate preference is an important determinant of outcome after ischaemia-reperfusion injury. The oxidation of free fatty acids, levels of which are elevated in human plasma after myocardial infarction (Lopaschuk *et al*., 1994), is the major source of energy for ATP generation in normoxic hearts (Saddik & Lopaschuk, 1991). However, fatty acids impair the recovery of post-ischaemic mechanical function, in part by altering glucose metabolism (Lopaschuk *et al*., 1990). The oxidation of pyruvate derived from glycolysis is inhibited by acetyl CoA arising from the β-oxidation of fatty acids (Lopaschuk & Stanley, 1997). As a result, rates of glucose oxidation are reduced and the coupling between glycolysis and glucose oxidation is impaired. Under these conditions, the hydrolysis of ATP derived from glucose metabolism becomes a significant source of proton production (Lopaschuk *et al*., 1990).

During reperfusion, the accumulation of protons from ischaemia, together with a continued uncoupling of glycolysis from glucose oxidation, accelerate H^+/Na^+ exchange increasing Na^+ accumulation that ultimately induces Ca^{2+} overload, via activation of the Na^+/Ca^{2+} exchanger (Lazdunski *et al*., 1985; Tani & Neeley, 1989). Myocyte Ca^{2+} overload is an important determinant of ischaemia-reperfusion damage and impaired mechanical function during reperfusion (Bourdillon & Poole-Wilson, 1981; Nayler, 1981; Meissner & Morgan, 1995).

Modulation of myocardial energy substrate metabolism, either by increasing glucose oxidation (McVeigh & Lopaschuk, 1990) or by inhibiting glycolysis (Finegan *et al*., 1993), enhances the recovery of post-ischaemic mechanical function.

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Beneficial alterations in glucose metabolism (Finegan *et al*., 1993) may contribute to the mechanism of cardioprotection associated with adenosine A_1-receptor stimulation (Lasley *et al*., 1990; Lasley & Mentzer, 1992; Thornton *et al*., 1992). In addition, it has been suggested that adenosine A_1-receptor agonists activate K_{ATP}-channels (Kirsch *et al*., 1990) which may contribute to their cardioprotective mechanism (Toombs *et al*., 1993; Van Winkle *et al*., 1994). A number of studies have demonstrated that direct activation of K_{ATP}-channels is cardioprotective (Gross & Auchampach, 1992; Opie, 1993; Grover, 1994; Grover *et al*., 1995), but the relationship between K_{ATP}-channels and energy substrate metabolism has not been clearly defined.

Although there are no data available on the direct effects of K_{ATP}-channel activators on glucose metabolism in aerobic or post-ischaemic myocardium, a link between K_{ATP}-channel antagonists, such as glibenclamide, and insulin secretion and glucose homeostasis in pancreatic islet cells is well established (Feldman, 1985). A role for K_{ATP}-channels in the regulation of glucose metabolism is also suggested by data showing that K_{ATP}-channel activation is associated with increases in ATP production from glycolysis (Tan *et al*., 1985; Schaffer *et al*., 1985). In addition, K_{ATP}-channels are also present on mitochondrial membranes (Garlid *et al*., 1996) where they may influence energy substrate oxidation.

The aim of this study was to determine the effects of K_{ATP}-channel activation and blockade on glucose metabolism and mechanical function in aerobic and reperfused hearts. As adenosine A_1-receptor stimulation has been linked to K_{ATP}-channel activation, the role of K_{ATP}-channel activation in the metabolic and cardioprotective actions of adenosine A_1-receptor activation by CHA was also investigated.

Methods

Heart perfusions

Male Sprague-Dawley rats (250–400 g), that had been fed *ad libitum* were anaesthetized with sodium pentobarbitone. The heart was rapidly excised and placed in ice-cooled Krebs-Henseleit solution. The aorta was cannulated and a Langendorff perfusion was initiated with Krebs-Henseleit solution (pH 7.4, gassed with a 95%O_2/5%CO_2 mixture) at a hydrostatic pressure of 60 mmHg. Each heart was then trimmed of excess tissue and the pulmonary artery and left atria were cannulated. After a 10 min Langendorff perfusion, hearts were switched to working mode by clamping the aortic inflow line from the Langendorff reservoir and opening the left atrial inflow line. Working hearts were electrically paced at 300 beats min^{-1} (6 to 8 V, Grass S88 stimulator) during periods of aerobic perfusion. Working hearts were perfused in a closed recirculating system at 37°C using an oxygenator with a large surface area in constant contact with the 95%O_2/5%CO_2 gas mixture. The perfusate (volume 100 ml) was a modified Krebs-Henseleit solution containing 2.5 mM Ca^{2+}, 11 mM glucose, 1.2 mM palmitate pre-bound to 3% bovine serum albumin (BSA, Fraction V, hsp) and 100 mu l^{-1} insulin. Perfusions were performed at a constant left atrial preload of 11.5 mmHg with the afterload hydrostatic pressure set to a column height equivalent to 80 mmHg.

Measurement of mechanical function

Heart rate and systolic and diastolic aortic pressures (mmHg) were measured using a Gould P23 pressure transducer connected to the aortic outflow line. Cardiac output (CO) and aortic flow (ml min^{-1}) were measured using ultrasonic flow probes (Transonic T206) placed in the left atrial inflow and aortic outflow lines, respectively; coronary flow was calculated as the difference between CO and aortic flow. Aortic developed pressure (mmHg) was calculated as the difference between systolic and diastolic pressure. O_2 content of the coronary effluent was measured using an oxygen probe (YSI 5331), placed in the pulmonary artery outflow line and connected to a YSI oxygen meter (Model number 5300). Coronary effluent was then returned to the perfusate reservoir. Myocardial oxygen consumption (MVO_2, μmol min^{-1}) was calculated as the product of coronary flow and the difference in oxygen content between the left atrial inflow and the coronary effluent. Left ventricular minute work (LV work, mmHg l min^{-1}) was calculated as (systolic pressure – preload pressure) $\times$ CO, and myocardial efficiency was calculated as LV work (joules) expressed as a percentage of the total potential work (joules) based on MVO_2. Coronary vascular conductance (CVC) was calculated as coronary flow (ml min^{-1})/mean aortic pressure (mmHg).

Measurement of rates of glycolysis and glucose oxidation during aerobic conditions and during reperfusion following ischaemia

Glycolysis and glucose oxidation were measured simultaneously by the quantitative collection of ^3H_2O (liberated at the enolase step of glycolysis) and $^{14}CO_2$ (liberated at the level of pyruvate dehydrogenase complex (PDC) and in the citric acid cycle) from hearts perfused with buffer containing tracer amounts of [5-^{3}H]-glucose and [U-^{14}C]-glucose. Samples of perfusate were taken at 10 min intervals during aerobic perfusion, stored under liquid paraffin oil, and used to determine metabolic rates.

To measure glycolysis, the ^3H_2O in perfusate samples was separated from [^{3}H]-glucose and [^{14}C]-glucose using columns containing Dowex 1-X4 anion exchange resin, as described previously (Finegan *et al*., 1993). Glycolysis rates are expressed as μmol glucose metabolized min^{-1} g^{-1} dry wt. The closed perfusion system allowed the collection of gaseous $^{14}CO_2$ by means of a hyamine trap (volume 40 ml). Samples of hyamine, containing trapped $^{14}CO_2$, were taken at the same time as the perfusate samples. $^{14}CO_2$ trapped as bicarbonate in the perfusate together with $^{14}CO_2$ trapped in the hyamine were measured and glucose oxidation determined as previously described (Finegan *et al*., 1993). Rates of glucose oxidation are expressed as μmol glucose metabolized min^{-1} g^{-1} dry wt.

Calculation of the rate of proton production from glucose metabolism

When the rates of glycolysis and glucose oxidation are identical, the net production of protons from glucose metabolism is zero. However, if the rate of glycolysis exceeds that of glucose oxidation, there is a net production of two protons per molecule of glucose that passes through glycolysis that is not subsequently oxidized (Dennis *et al*., 1991). Consequently, the rate of proton production attributable to the hydrolysis of ATP arising from glucose metabolism can be calculated as 2 $\times$ (rate of glycolysis – rate of glucose oxidation).

Experimental protocols

Hearts were randomly assigned to untreated or one of four drug treatment groups; cromakalim (10 μM), CHA (0.5 μM),

glibenclamide (30 μM) or CHA (0.5 μM) in combination with glibenclamide (30 μM). Hearts from each group were subjected to either an aerobic or an ischaemia-reperfusion protocol (Figure 1).

Aerobic perfusion protocol

Glycolysis, glucose oxidation, proton production and mechanical function were measured at 10 min intervals throughout 80 min of aerobic perfusion. Following 45 min of perfusion (Baseline), hearts were assigned to either untreated or drug-treated groups and were perfused for a further 35 min (Treatment) either in the absence (untreated) or presence of cromakalim (10 μM), CHA (0.5 μM), glibenclamide (30 μM) or CHA (0.5 μM) in combination with glibenclamide (30 μM). The direct effects of drug treatment on glycolysis, glucose oxidation, proton production and mechanical function were determined by comparing values obtained during Treatment with those obtained during Baseline as well as with values measured in time-matched untreated, or glibenclamide-treated hearts.

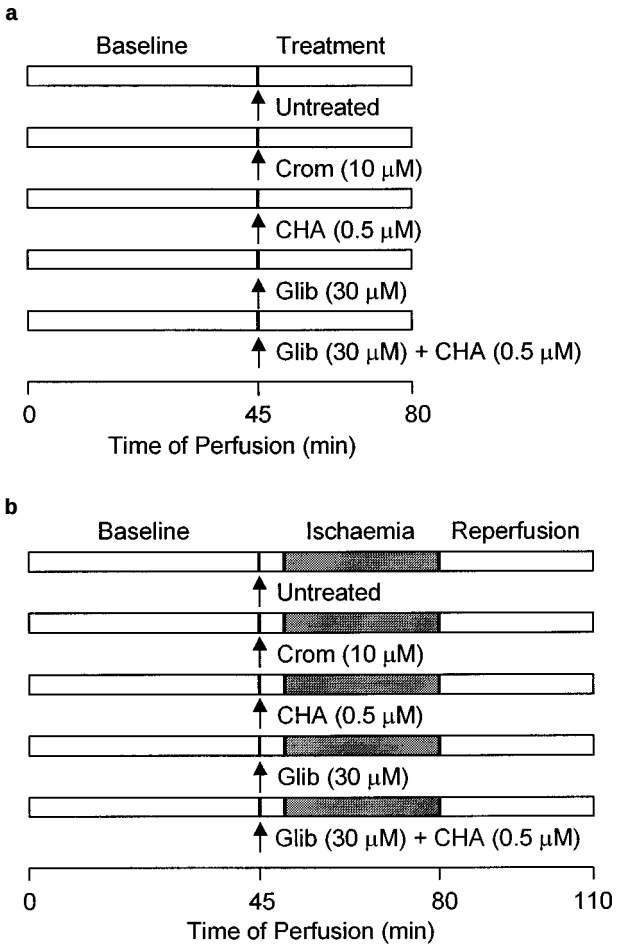

Figure 1 Aerobic and ischaemia reperfusion protocols. All hearts were initially perfused and equilibrated in Langendorff (non-working) mode for 10 min. Thereafter, they were switched to working mode (Time 0 min) and perfused aerobically for 45 min (baseline). At 45 min, hearts were either untreated or were exposed to perfusate containing cromakalim (10 μM, Crom), N^6-cyclohexyladenosine (0.5 μM, CHA), glibenclamide (30 μM, Glib) or a combination of glibenclamide and N^6-cyclohexyladenosine (Glib + CHA). In the aerobic perfusion protocol (a), hearts were perfused under aerobic conditions in the presence of the drugs for a further 35 min (treatment). In the ischaemia reperfusion protocol (b), drugs were allowed to equilibrate for 5 min after which hearts were subjected to normothermic global no-flow ischaemia (ischaemia) for 30 min. At 80 min flows were re-established and hearts were reperfused in working mode with drug-containing perfusate for a further 30 min period (reperfusion).

Ischaemia-reperfusion protocol

Following 50 min of aerobic perfusion (Pre-ischaemia), hearts were subjected to 30 min of global, no-flow ischaemia and pacing was discontinued. After 30 min of ischaemia, hearts were reperfused and pacing (300 beats min^{-1}) re-established for a further 30 min (Reperfusion). Interventions to defibrillate the hearts were not employed. Mechanical function and glucose metabolism were determined at 10 min intervals during the pre-ischaemic and reperfusion periods. Comparisons of glycolysis, glucose oxidation, proton production and mechanical function were made between pre-ischaemic and reperfusion periods for each group and between values obtained during reperfusion for drug-treated and time-matched untreated or glibenclamide-treated hearts.

At the end of each perfusion protocol, heart ventricles were frozen with Wollenberger clamps cooled to the temperature of liquid N_2 for the later determination of their total dry weight.

Drugs and reagents

D-[5-^{3}H]-glucose and D-[U-^{14}C]-glucose (5 mCi $mmol^{-1}$) were purchased from Dupont Canada Inc (Ontario). Hyamine hydroxide was purchased from ICN Biomedicals Inc (Ohio). Bovine serum albumin (BSA fraction V, hsp) was obtained from Boehringer Mannheim (IN). Insulin (Regular, CZI) was obtained from Connaught Novo (Ontario). Dowex 1-X4 anion exchange resin (200–400 mesh, chloride form) was obtained from Bio-Rad Laboratories (CA). Cromakalim and glibenclamide were purchased from Sigma Chemical Co. (MO) and N^6-cyclohexyladenosine (CHA) was purchased from Research Biochemicals International (MA).

Statistical analysis

Data are expressed as mean ± s.e.mean. Multiple groups of data were compared using a one-way analysis of variance supported by the Bonferroni Multiple Comparison test for inter-group differences. Where only two groups of data were compared, a Student's t test was utilized. For paired comparisons, a paired Student's t test was used. Differences were considered significant when $P<0.05$.

Results

Aerobic perfusions

Mechanical function of all hearts was stable during aerobic perfusion, and after 80 min LV work was depressed by only 9 ± 2% relative to values at 45 min (Table 1). Cromakalim (10 μM) had no effect on LV work, but caused a small, but significant, increase in CVC. CHA (0.5 μM) had no effect on LV work or CVC. In the untreated group, glycolysis and proton production were similar in the baseline and treatment periods (Figure 2). However, glucose oxidation increased gradually during aerobic perfusion and was significantly higher during the treatment period relative to the baseline period. When compared with the untreated, time-matched group, cromakalim had no significant effect

on glycolysis, glucose metabolism or proton production, whereas CHA significantly inhibited glycolysis and proton production but had no significant effect on glucose oxidation (Figure 2).

When compared with the untreated, time-matched group, glibenclamide (30 μM) had no significant effect on LV work or CVC (Table 1), but significantly increased glycolysis and proton production (Figure 2). When compared to values obtained in the time-matched group perfused in the presence of glibenclamide (30 μM), CHA (0.5 μM) in combination with glibenclamide had no significant effects on LV work, CVC or glucose oxidation. However, in the presence of glibenclamide, the CHA-induced inhibition of glycolysis and proton production was unaffected (Figure 2).

Table 1 Mechanical function during baseline and treatment periods for hearts perfused under aerobic conditions

	Untreated ($n=11$)		*CHA* ($n=10$)		*Cromakalim* ($n=7$)		*Glibenclamide* ($n=9$)		*Glibenclamide + CHA* ($n=8$)	
	B	*T*	*B*	*T*	*B*	*T*	*B*	*T*	*B*	*T*
Diastolic pressure (mmHg)	65±1	67±1	64±2	65±1	67±2	67±2	65±2	66±2	67±2	68±2
Systolic pressure (mmHg)	117±2	114±2	124±3	121±3	121±2	115±3	118±4	114±4	121±3	115±3
Coronary flow (ml min^{-1})	23±1	22±2	23±1	25±1	27±2	28±2	26±2	25±2	24±1	24±1
LV work (mmHg l^{-1} min^{-1})	7.4±0.1	6.8±0.2	8.1±0.3	7.5±0.4	7.6±0.4	6.8±0.4	7.5±0.4	6.7±0.4	7.7±0.3	6.6±0.3
HR × PSP (10^{-3} mmHg $mmHg^{-1}$)	35.2±0.5	34.3±0.5	37.4±0.9	36.5±0.8	36.3±0.6	34.5±0.8	35.6±1.2	34.1±1.3	36.3±0.8	34.5±0.7
CVC (10^{-1} ml min^{-1} $mmHg^{-1}$)	2.8±0.2	2.6±0.2	2.7±0.1	3.0±0.1	3.2±0.3	3.4±0.2*	3.2±0.2	3.1±0.3	2.9±0.1	2.9±0.2
Efficiency (%)	15.4±0.8	15.3±0.8	15.6±0.6	14.7±0.9	13.2±1.4	13.2±1.6	13.8±1.6	13.5±1.8	13.7±0.3	13.1±0.4

Values are expressed as means±s.e.mean of *n* hearts. All hearts were untreated during the initial aerobic baseline (B) period. During the treatment (T) period, hearts were either untreated or treated with CHA (0.5 μM), cromakalim (10 μM), glibenclamide (30 μM) or glibenclamide (30 μM) in combination with CHA (0.5 μM). Data are shown for diastolic and systolic aortic pressures, coronary flow, left ventricular minute work (LV work), heart rate-peak systolic pressure product (HR × PSP), coronary vascular conductance (CVC) and myocardial efficiency (LV work per MVO_2). *Indicates that a value during the treatment period is significantly different ($P<0.05$) from its respective baseline value. There were no significant differences between values in the drug-treated groups and their respective, time-matched, untreated group.

Table 2 Mechanical function during baseline and reperfusion periods for hearts subjected to 30 min of global ischaemia

	Untreated ($n=11$)		*CHA* ($n=8$)		*Cromakalim* ($n=8$)		*Glibenclamide* ($n=9$)		*Glibenclamide + CHA* ($n=9$)	
	B	*R*	*B*	*R*	*B*	*R*	*B*	*R*	*B*	*R*
Diastolic pressure (mmHg)	67±1	37±8*	64±2	62±7	70±1	46±11	65±1	17±1†	65±4	71±3#
Systolic pressure (mmHg)	124±1	52±14*	127±2	104±13†	125±2	61±16	122±2	19±1†	123±10	110±7#
Coronary flow (mmHg)	26±1	10±4*	23±3	24±3†	22±1	12±4	24±1	2±1	25±4	27±5#
LV work (mmHg l^{-1} min^{-1})	8.0±0.3	1.9±0.8*	7.8±0.4	5.2±0.9†	7.4±0.3	1.5±0.6	7.9±0.3	0.01±0.01†	7.8±1.3	5.3±1.1#
HR × PSP (10^{-1} $mmHg^{-1}$ $beat^{-1}$)	37.1±0.4	15.8±4.1*	38.1±0.6	31.1±3.8†	37.5±0.7	18.3±4.7	36.6±0.7	5.6±0.4†	36.9±3.0	33.1±2.2#
CVC (10^{-1} ml min^{-1} $mmHg^{-1}$)	3.0±0.2	1.5±0.3*	2.7±0.2	2.8±0.3†	2.5±0.3	2.4±0.4	2.8±0.2	1.0±0.2†	3.0±0.4	3.2±0.6#
Efficiency (%)	14.7±0.8	3.9±1.5*	15.1±1.1	10.3±1.8†	15.1±0.9	4.4±2.3	14.7±1.0	0.3±0.1†	13.6±3.1	10.3±2.5#

Values are expressed as means±s.e.mean of *n* hearts. All hearts were perfused under aerobic baseline (B) conditions for 50 min. After 45 min of aerobic perfusion, hearts either remained untreated or were treated with CHA (0.5 μM), cromakalim (10 μM), glibenclamide (30 μM) or glibenclamide (30 μM) in combination with CHA (0.5 μM). After a further 5 min period to facilitate drug equilibration within the perfusion system, hearts were subjected to 30 min of global ischaemia followed by 30 min of aerobic reperfusion. (R). Drugs were present throughout the periods of ischaemia and reperfusion. Data are shown for diastolic and systolic aortic pressures, coronary flow, left ventricular minute work (LV work), heart rate-peak systolic pressure product (HR × PSP), coronary vascular conductance (CVC) and myocardial efficiency (LV work per MVO_2). *Indicates that a value during the reperfusion period is significantly different ($P<0.05$) from its respective baseline value; †indicates that a value is significantly different ($P<0.05$) from its respective untreated, time-matched group, and #indicates that a value is significantly different ($P<0.05$) from its respective glibenclamide-treated group.

Ischaemia and reperfusion

During reperfusion following 30 min of global ischaemia, the recovery of mechanical function in untreated hearts was significantly depressed and by the end of the 30-min period of reperfusion, LV work had recovered to only 24±1% of pre-ischaemic values (Figure 3). Rates of glycolysis and proton production were significantly elevated during reperfusion compared with pre-ischaemic values (Figure 4). Glucose oxidation recovered during reperfusion to a rate that was similar to pre-ischaemic values. When compared with untreated hearts, cromakalim (10 μM) had no significant effect on the recovery of mechanical function during reperfusion and LV work recovered to 20±8% of pre-ischaemic values (Figure 3). Also, cromakalim had no effect on rates of glycolysis, glucose oxidation and proton production when compared with the untreated, time-matched group (Figure 4). In contrast, CHA (0.5 μM) significantly enhanced the recovery of mechanical function during reperfusion compared with the untreated, time-matched group (Figure 3) and increased the recovery of LV work from 24±1 to 70±11% of pre-ischaemic values. CHA inhibited glycolysis and proton production rates during reperfusion when compared with those observed in the untreated, time-matched group, but did not affect glucose oxidation (Figure 4).

When compared with the untreated, time-matched group, glibenclamide (30 μM) significantly impaired the recovery of mechanical function during reperfusion (Figure 4) and LV work recovered to less than 1% of pre-ischaemic values (Figure 3). However, glibenclamide did not significantly affect any of the indices of glucose metabolism during reperfusion when compard with the untreated, time-matched group (Figure 4). Despite the marked deleterious effects of glibenclamide *per se*, hearts treated with a combination of CHA (0.5 μM) and glibenclamide (30 μM) recovered to a significantly greater extent than hearts treated with glibenclamide alone (Figure 3). The recovery of LV work during reperfusion increased to 73±7% of pre-ischaemic values. Similarly, glibenclamide failed to alter the beneficial effects of CHA on glucose metabolism. The combination CHA and

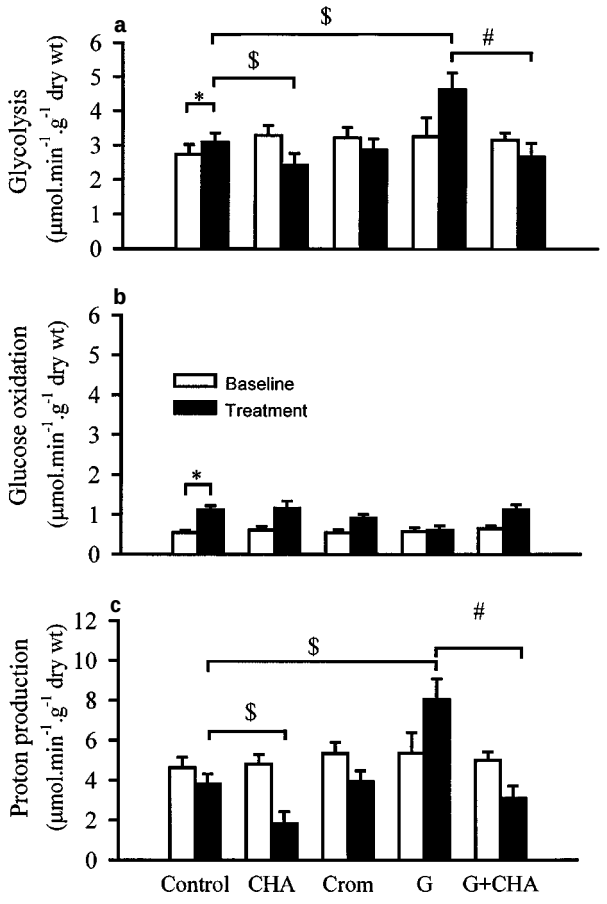

Figure 2 Rates of glucose metabolism (a and b) and proton production (c) in hearts perfused under aerobic conditions. All hearts were untreated during the initial aerobic baseline period. During the treatment period, hearts were either untreated (Control, $n=11$) or treated with CHA (0.5 μM, $n=10$), cromakalim (Crom, 10 μM, $n=7$), glibenclamide (G, 30 μM, $n=9$) or glibenclamide in combination with CHA (G+C, $n=8$). Values are shown as means±s.e.mean of n hearts. *Indicates that values obtained in untreated hearts during the baseline and reperfusion periods are significantly different ($P<0.05$); $indicates that a value is significantly different ($P<0.05$) from its respective untreated, time-matched group, and #indicates that a value is significantly different ($P<0.05$) from its respective glibenclamide-treated group.

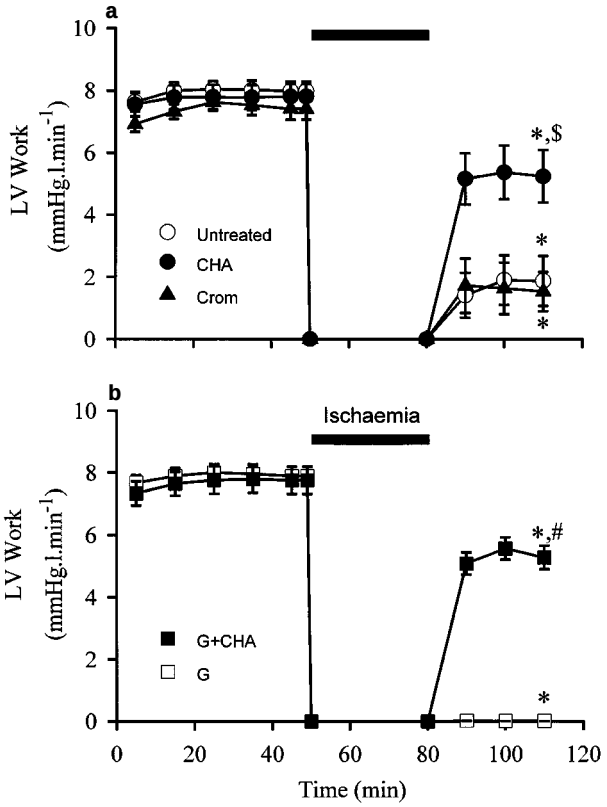

Figure 3 Left ventricular (LV) works in hearts subjected to 30 min of global ischaemia and reperfusion. Values are shown as means of n hearts; vertical lines indicate s.e.mean. All hearts were perfused under aerobic baseline conditions for 50 min. After 45 min of aerobic perfusion, hearts either remained untreated ($n=11$) or were treated with CHA (0.5 μM, $n=8$), cromakalim (Crom, 10 μM, $n=8$), shown in (a), or with glibenclamide (G, 30 μM, $n=9$) or glibenclamide in combination with CHA (G+CHA, $n=9$), shown in (b). After a further 5-min period to facilitate drug equilibration within the perfusion system, hearts were subjected to 30 min of global ischaemia (shown by horizontal bar) followed by 30 min of aerobic reperfusion. Drugs were present throughout the periods of ischaemia and reperfusion. *Indicates that a value at the end of reperfusion is significantly different ($P<0.05$) from its respective baseline value; $indicates that a value at the end of reperfusion is significantly different ($P<0.05$) from its respective untreated, time-matched group, and #indicates that a value is significantly different ($P<0.05$) from its respective glibenclamide-treated group.

glibenclamide significantly attenuated rates of glycolysis and proton production during reperfusion when compared with the time-matched, glibenclamide-treated group (Figure 4). Glucose oxidation during reperfusion was significantly increased in hearts exposed to the combination of CHA and glibenclamide.

Discussion

The present study demonstrates that cromakalim, a selective K_{ATP}-channel activator, does not enhance recovery of mechanical function of post-ischaemic working rat hearts. However, glibenclamide, a selective K_{ATP}-channel inhibitor, significantly impaired the recovery of post-ischaemic mechanical function. Furthermore, the inability of glibenclamide to inhibit the beneficial effects of CHA indicates that the mechanism by which CHA, a selective adenosine A_1-receptor agonist, elicits cardioprotection does not involve activation of K_{ATP}-channels.

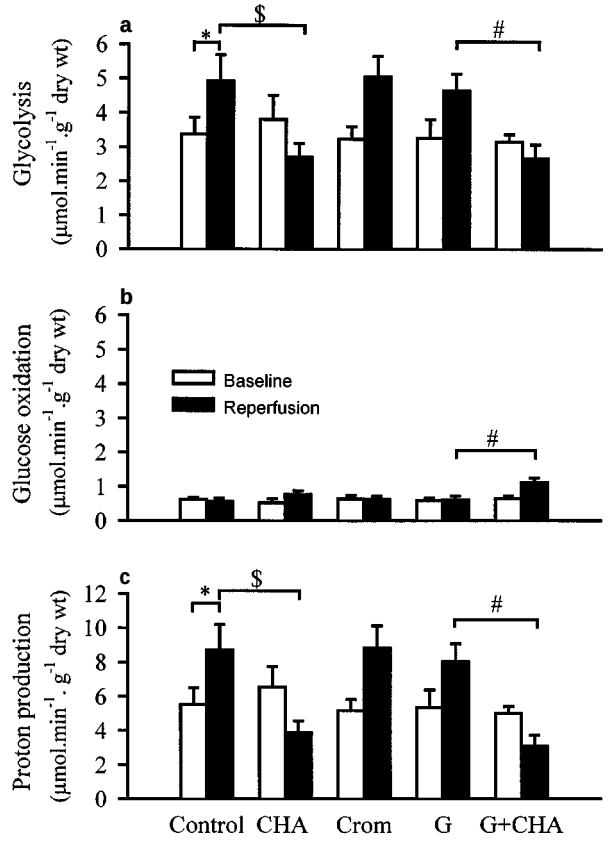

Figure 4 Rates of glucose metabolism (a and b) and proton production (c) in hearts subjected to 30 min global ischaemia and reperfusion. All hearts were untreated during the initial aerobic baseline period. After 45 min of aerobic perfusion, hearts either remained untreated (Control, $n=7$) or were treated with CHA (0.5 μM, $n=7$), cromakalim (10 μM, $n=8$), glibenclamide (30 μM, $n=6$) or glibenclamide in combination with CHA ($n=9$). After a further 5 min period to facilitate drug equilibration within the perfusion system, hearts were subjected to 30 min of global ischaemia followed by 30 min of aerobic reperfusion. Drugs were present throughout the periods of ischaemia and reperfusion. *Indicates that a value at the end of reperfusion is significantly different ($P<0.05$) from its respective baseline value; $indicates that a value at the end of reperfusion is significantly different ($P<0.05$) from its respective untreated, time-matched group, and #indicates that a value is significantly different ($P<0.05$) from its respective glibenclamide-treated group.

K_{ATP}-channels have been the subject of intense research as a potential target in the treatment of ischaemic heart disease and conflicting evidence has been obtained concerning the cardioprotective efficacy of drugs that alter K_{ATP}-channel activity (see reviews by Gross & Auchampach, 1992; Opie, 1993; Weiss & Venkatesh, 1993; Grover, 1994; Terzic *et al*., 1995). It has been shown that both K_{ATP}-channel activation (D'Alonzo *et al*., 1992; Cole, 1993; Yao & Gross, 1994a,b; Hilde *et al*., 1995; Ferdinandy *et al*., 1995) and K_{ATP}-channel blockade (Bril *et al*., 1992; Billman *et al*., 1993; Siegl, 1994; Tosaki & Hellegouarch, 1994) protect the myocardium from ischaemic damage. Paradoxically, it has also been shown that K_{ATP}-channel activation (Tosaki & Hellegouarch, 1994) and blockade (Mitani *et al*., 1991) can also have deleterious effects on ischaemic myocardium. Finally, some authors have reported that modulation of K_{ATP}-channels has no effect on ischaemia-induced myocardial injury (Kitzen *et al*., 1992; Venkatesh *et al*., 1992).

The diversity of results obtained using agents that modify K_{ATP}-channel activity suggests that the methodology used to study these agents is critical to the outcome observed. While the majority of evidence supports the hypothesis that K_{ATP}-channel activation is cardioprotective, the negative results obtained by some investigators warrant further investigation. One possible explanation for the discordant results is an increase in the incidence of fibrillation observed in the presence of K_{ATP}-channel activators (Chi *et al*., 1990; Grover *et al*., 1990a; Ferdinandy *et al*., 1995). Prodysrhythmic activity, caused either by K_{ATP}-channel-mediated shortening (Escande, 1988; Cole, 1993) of an already abbreviated action potential (Penny & Sheridan, 1983; D'Alonzo *et al*., 1992) or by a K_{ATP}-channel-induced loss of intracellular K^+ (Tosaki & Das, 1997), might have masked any potential cardioprotective efficacy (for reviews see Coetzee, 1992; Gross & Auchampach, 1992; Wilde, 1994; Terzic *et al*., 1995). This would appear to be one explanation for the lack of cardioprotection in response to K_{ATP}-channel activation in the present study.

An important difference between the present experiments and those of other authors is the inclusion in the perfusate of a relevant concentration of fatty acid, in addition to glucose, as an energy substrate. Although fatty acid oxidation is a major source of adenosine 5′-triphosphate (ATP) in normoxic hearts (Saddik & Lopaschuk, 1991), it has deleterious consequences on the recovery of post-ischaemic mechanical function (Lopaschuk *et al*., 1993). Moreover, free fatty acids reduce the threshold for fibrillation in rat perfused hearts (Makiguchi *et al*., 1991). Thus, in the current model, it is possible that an increased susceptibility of hearts to fibrillate masked any cardioprotective potential of K_{ATP}-channel activation. This interaction may have profound implications for the potential of K_{ATP}-channel activators in the clinical management of ischaemic heart disease as high levels of free fatty acids (equivalent to those used in the present study) are present in human plasma following myocardial ischaemia (Lopaschuk *et al*., 1994). Thus, these results, in combination with those of other studies (Goldberg *et al*., 1988; Fox *et al*., 1991) suggest that K_{ATP}-channel activators would be of limited clinical efficacy and prone to marked adverse effects.

The second part of the study investigated the potential role of K_{ATP}-channel activation in mediating the cardioprotective response of adenosine A_1-receptor agonists. Adenosine A_1-receptor stimulation protects the myocardium against ischaemic injury (Lasley & Mentzer, 1992; Thornton *et al*., 1992; Finegan *et al*., 1996). The mechanism of action may involve beneficial alterations in glucose metabolism (Finegan *et al*., 1996). When hearts are perfused with both fatty acids and

glucose, the coupling between myocardial glycolysis and glucose oxidation is impaired (Lopaschuk *et al*., 1990; Saddik & Lopaschuk, 1991) leading to an acceleration of proton production and acidosis. Inhibition of proton production during reperfusion improves the recovery of mechanical function by reducing H^+/Na^+ and Na^+/Ca^{2+} exchange that ultimately lead to Ca^{2+} overload (Tani & Neely, 1989; Lopaschuk *et al*., 1993). Adenosine A_1-receptor stimulation inhibits glycolysis that improves the coupling between glycolysis and glucose oxidation, thereby reducing proton production and Ca^{2+} overload. Other evidence suggests that cardioprotection arising from adenosine A_1-receptor stimulation is mediated through K_{ATP}-channels (Kirsch *et al*., 1990; Toombs *et al*., 1993; Van Winkle *et al*., 1994). This conclusion is based on experiments where K_{ATP}-channel antagonists such as glibenclamide or 5-hydroxydecanoate inhibit adenosine-mediated cardioprotection.

In the present study glibenclamide was ineffective in antagonizing either the mechanical protection or the beneficial alterations in glucose metabolism induced by A_1-receptor stimulation. This result is in agreement with the findings of Grover *et al*. (1996) who showed that glyburide had no effect on cardioprotection elicited by the adenosine A_1-receptor agonist, **R**(−)N^6-(2-phenylisopropyl)adenosine, in rat Langendorff (non-working) perfused hearts. Furthermore, in agreement with Mitani *et al*. (1991), we showed that glibenclamide *per se* impaired the recovery of post-ischaemic mechanical function and that this effect was independent of alterations in coronary perfusion. Our demonstration that glibenclamide increases rates of glycolysis confirms the data of Schaffer *et al*. (1985), and suggests that the detrimental effect of glibenclamide on the recovery of post-ischaemic function may be due to an increased production of protons that exacerbates Ca^{2+} overload. The direct deleterious effect of glibenclamide raises the possibility that glibenclamide-induced antagonism of the beneficial actions of adenosine receptor agonists (Kirsch *et al*., 1990; Toombs *et al*., 1993; Van Winkle *et al*., 1994), was due to functional, rather than pharmacological antagonism. An additional consideration is the potential interaction of these drugs at the level of the coronary vasculature (Grover *et al*., 1990b). In rat working hearts perfused in the absence of erythrocytes, coronary flow is close to maximal and is unaffected by adenosine agonists. Thus, in the present studies there was no potential for glibenclamide to antagonize the cardioprotective actions of CHA by inhibiting CHA-induced coronary hyperaemia. In other studies where coronary flow may increase in response to adenosine agonists (Randall, 1995), functional antagonism between glibenclamide and CHA on coronary perfusion may have been more pronounced.

In summary, our data demonstrate that direct activation of K_{ATP}-channels failed to induce cardioprotection or influence myocardial glucose metabolism. Lack of efficacy of K_{ATP}-channel activation may be due either to an absence of coronary vasodilatation in the working heart, or to enhanced electrical disturbances caused by a shortening of the cardiac action potential. In addition, our data indicating that glibenclamide possesses direct deleterious effects and, nevertheless, fails to inhibit CHA-induced cardioprotection indicate that the beneficial effect of adenosine A_1-receptor stimulation on post-ischaemic function does not involve activation of K_{ATP} channels.

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