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Adenosine- and hypoxia-induced dilation of human coronary resistance arteries: evidence against the involvement of K_{ATP} channels

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1 The ATP-sensitive potassium (K_{ATP}) channel may be an important mediator of metabolic dilation in the human coronary circulation. As adenosine and hypoxia are considered to be major mediators of metabolic dilation in the coronary circulation, we investigated the effect of glibenclamide (a K_{ATP} channel blocker) on adenosine and hypoxic dilation of human coronary resistance arteries, with myogenic tone, *in vitro*.

2 Vessels were dissected from the atrial appendage from consenting patients and studied *in vitro* using a pressure arteriograph system. Segments of coronary resistance artery were pressurized to 60 mmHg and the vessels studied developed spontaneous myogenic tone.

3 The K_{ATP} opener pinacidil (final conc. 5×10^{-6} M) resulted in dilation, which was completely reversed by 5×10^{-6} glibenclamide (84 ± 14 vs $-10 \pm 9\%$, pinacidil and pinacidil plus glibenclamide, respectively, $P = 0.009$, $n = 5$).

4 Adenosine (final conc. 10^{-5} M) resulted in dilation, glibenclamide (5×10^{-6} and 10^{-5} M) was without effect (118 ± 12 vs $104 \pm 16\%$ adenosine and adenosine plus 10^{-5} glibenclamide, respectively, n.s., $n = 4$).

5 Hypoxia (8 ± 3 mmHg O_2) resulted in a dilation that reversed when normoxic conditions were restored (60 ± 9 vs $3 \pm 11\%$ hypoxia and post-hypoxia, respectively, $P = 0.014$, $n = 3$). The hypoxic dilation was not affected by glibenclamide (63 ± 14 vs $55 \pm 6\%$ hypoxia and hypoxia plus glibenclamide, respectively, n.s., $n = 4$). In a further series of experiments, vessels were incubated with glibenclamide (5×10^{-6}) prior to a hypoxic challenge; again, glibenclamide was without effect on the hypoxic dilation (-0.008 ± 2 vs $95 \pm 3\%$ glibenclamide and glibenclamide plus hypoxia, respectively, $P = 0.0005$, $n = 3$).

6 These data demonstrate that glibenclamide is without effect on both adenosine and hypoxic dilation of human coronary resistance arteries with myogenic tone, from the right atrial appendage *in vitro*. Our findings suggest that the K_{ATP} channel is unlikely to be a major mediator of metabolic dilation in these arteries.

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Abbreviations: K_{ATP} channel, ATP-sensitive potassium channel; PSS, physiological saline solution

Introduction

The human coronary microcirculation must be able to respond rapidly to changes in metabolic and hypoxic demand. A failure to do so will be associated inevitably with ischaemic episodes and disturbances of rhythm. In this context, both adenosine and hypoxia are important mediators of coronary resistance vessel dilation and may play a critical role in matching coronary blood flow to the demands of the myocardium. Daut *et al.* (1990) first demonstrated that hypoxic dilation was blocked by glibenclamide, a blocker of ATP-sensitive potassium (K_{ATP}) channels, in the intact guinea-pig heart. Subsequently, several studies suggest that K_{ATP} channels are likely mediators of metabolic dilation in the coronary circulation (for a review, see Quayle *et al.*, 1997). However, adenosine-induced relaxation of isolated human coronary arteries preconstricted with a thromboxane A_2 mimetic has been shown to be unaffected by glibenclamide (Kemp & Cocks, 1999). A recent study using pressurized human coronary arteries, with a mixture of myogenic and agonist-induced tone, also found that glibenclamide was without effect on adenosine-induced dilation (Sato *et al.*, 2005). However, using the same experimental set-up, the same group have shown that hypoxic dilation is blocked by glibenclamide (Miura *et al.*, 2003).

The present experiments were carried out to assess the effect of glibenclamide on adenosine and hypoxic dilation of pressurized human coronary resistance arteries, with only myogenic tone, *in vitro*. We report a lack of effect of glibenclamide on both adenosine and hypoxic dilation; this suggests that the K_{ATP} channel is unlikely to be a major mediator of metabolic dilation in the atrial appendage.

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Methods

Patients gave their consent and the experiments were carried out according to ethics committee guidelines. Human right atrial appendage, which is routinely removed for cannulation during cardiopulmonary bypass procedures, was harvested during elective surgery. The tissue was immediately placed in cold (4°C) physiological saline solution (PSS) consisting of (mM) 119 NaCl, 4.7 KCl, 25 $NaHCO_3$, 1.17 KH_2PO_4, 1.17 $MgSO_4$, 0.026 EDTA, 1.6 $CaCl_2$ and 5.5 glucose. Resistance arteries (70–180 μm in diameter) were dissected free and placed in a pressure arteriograph (Living Systems Instrumentation, Burlington, VT, U.S.A.) containing cold PSS (Halpern *et al*., 1984; Izzard *et al*., 1996). Each end of the vessel segment was slipped onto a glass micropipette filled with PSS and secured with a nylon suture. Intraluminal pressure was then set to 60 mmHg and the vessel checked for leaks; only leak-free vessel segments were included in this study. The inner diameter and wall thickness were continually monitored using a video dimension analyser (Living Systems Instrumentation, Burlington, VT, U.S.A.) connected to a data acquisition program on a computer. The bath chamber was superfused with PSS gassed with 5% CO_2 in air at a superfusion rate of 20 ml min^{-1}. The temperature of the bath was maintained at 37°C using a circulating water heater and arteries were left to equilibrate for a period of 1–2 h, during which time myogenic tone developed.

Experimental protocol

Once the arteries had developed a stable level of myogenic tone, pinacidil (10^{-6} and 5×10^{-6} M) or adenosine (10^{-6} and 10^{-5} M) was added to the circulating PSS and the diameter allowed to stabilize for 10 min. Then glibenclamide (10^{-6} and 5×10^{-6} M) was added. To investigate the effect of hypoxia, gassing conditions were switched to 95% N_2/5% CO_2 for 10 min and oxygen levels were monitored by placing a needle probe (Diamond General, Ann Arbor, MI, U.S.A.) directly into the bath chamber in close proximity to the cannulated artery. Glibenclamide (5×10^{-6} M) was added to the circulating PSS before returning to normoxic conditions. We also examined the effects of preincubation of glibenclamide on the response to adenosine and hypoxia. Glibenclamide (5×10^{-6} M) was added to the circulating PSS 20 min prior to the addition of adenosine or induction of hypoxia.

At the end of the studies, vessels were superfused with Ca^{2+}-free PSS containing 1 mM ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid for 20 min to obtain the maximum diameter.

Solutions and drugs

All drugs were obtained from Sigma (Poole, Dorset, U.K.). Pinacidil, adenosine and glibenclamide were dissolved in DMSO. All other stocks and solutions were dissolved in ultradistilled water. All reported concentrations are final molar concentrations in the vessel chamber.

Data analysis

Data are expressed as mean ± s.e.m. Dilations are expressed as a per cent maximal dilation (Ca^{2+}-free PSS). Statistical comparisons of the dilations before and after glibenclamide were carried out using a paired Student's *t*-test; $P<0.05$ was considered significant.

Results

Out of the approximately 100 arteries dissected from the atrial appendage and mounted in the pressure arteriograph, 21 were leak free and developed stable spontaneous myogenic tone. The demographic data of these 21 patients are shown in Table 1. The mean diameter of the arteries with myogenic tone was 94 ± 5 and 119 ± 5 μm in Ca^{2+}-free PSS.

Pinacidil (n = 5)

A concentration of 5×10^{-6} M pinacidil caused a dilation that was reversed by 5×10^{-6} M glibenclamide (84 ± 14 vs −10 ± 9%, pinacidil and pinacidil plus glibenclamide, respectively, $P = 0.009$, $n = 5$; Table 2). A representative recording is shown in Figure 1.

Adenosine (n = 6)

A concentration of 10^{-5} M adenosine caused a dilation that was unaffected by 5×10^{-6} and 10^{-5} glibencamide (118 ± 12 vs 104 ± 16% adenosine and adenosine plus 10^{-5} glibenclamide,

Table 1 Profile of patient demographics

Sex	
Male	19
Female	2
Age (years) (mean ± s.e.m.)	64 ± 4
Surgical procedure	
CABG	21
AVR	2
MVR	1
Underlying disease	
Hypertension	7
Diabetic	5
Coronary artery disease	5
Hypercholesterolaemia	7
None of the above	3

AVR: atrial valve replacement; CABG: coronary artery bypass graft; MVR: mitral valve replacement.

Table 2 Effect of glibenclamide on pinacidil-, adenosine- and hypoxia-induced dilations

Manoeuvre	*Control*	*Dilation (% maximal)* *Plus glibenclamide* (M) 10^{-6}	5×10^{-6}	10^{-5}
Pinacidil (M) (n = 5)				
10^{-6}	−7 ± 12			
5×10^{-5}	84 ± 14	100 ± 16	−10 ± 9*	
Adenosine (M) (n = 4)				
10^{-6}	40 ± 14			
5×10^{-6}	118 ± 18	107 ± 14	95 ± 16	104 ± 16
Hypoxia (n = 4)	63 ± 14		55 ± 6	

Data are expressed as % maximal (Ca^{2+}-free PSS).
*$P<0.05$.

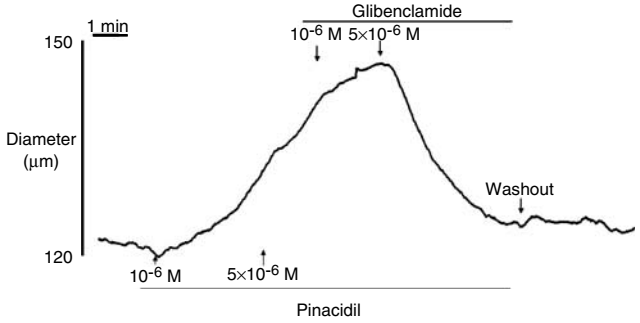

Figure 1 A recording of the response of a pressurized human coronary artery with myogenic tone to pinacidil followed by glibenclamide.

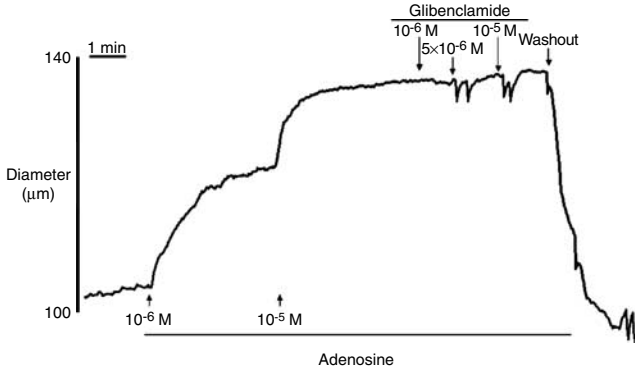

Figure 2 A recording of the response of a pressurized human coronary artery with myogenic tone to adenosine followed by glibenclamide.

respectively, n.s., $n=4$; Table 2). A representative recording is shown in Figure 2. Preincubation with 5×10^{-6} glibenclamide for 20 min prior to the addition of 10^{-5} M adenosine did not alter the dilator response ($n=2$, data not shown).

*Hypoxia (*n = *10)*

Hypoxic conditions reduced the O_2 tension from 150 ± 13 to 8 ± 3 mmHg ($P<0.001$). Hypoxia caused a dilation that reached a plateau in 4.6 ± 0.9 min and return to normoxic conditions caused a reversal of the dilation in 4.1 ± 1.3 min (60 ± 9 vs $3\pm11\%$ hypoxia and post-hypoxia, respectively, $P=0.014$, $n=3$). Hypoxic dilation was unaffected by 5×10^{-6} glibenclamide (63 ± 14 vs $55\pm6\%$ hypoxia and hypoxia plus glibenclamide, respectively, n.s., $n=4$; Table 2). A representative recording is shown in Figure 3. Preincubation with 5×10^{-6} glibenclamide for 20 min prior to and during the hypoxic challenge did not block the hypoxia-induced dilation (-0.008 ± 2 vs $95\pm3\%$ glibenclamide and glibenclamide plus hypoxia, respectively, $P=0.0005$, $n=3$).

Discussion

In this study, we find that both adenosine and hypoxic dilation human coronary arteries with myogenic tone *in vitro* are not affected by glibenclamide. These results argue against a major role for K_{ATP} channels in these responses. Conversely, pinacidil

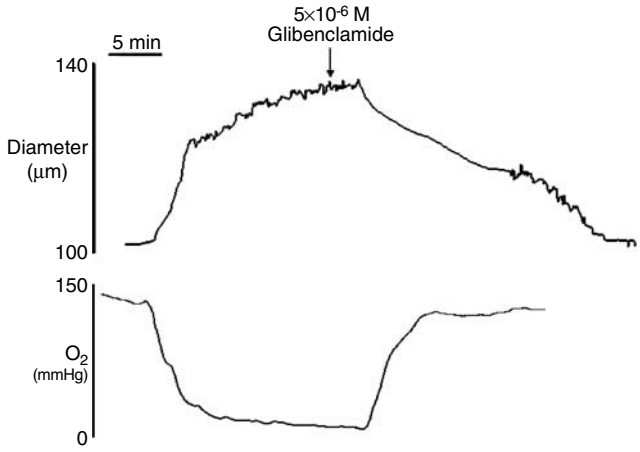

Figure 3 A recording of the response of a pressurized human coronary artery with myogenic tone to hypoxia followed by glibenclamide.

(a K_{ATP} channel opener) dilates these vessels, and this effect is fully reversed by glibenclamide. This finding is in agreement with a recent study, which showed that aprikalim (a K_{ATP} channel opener) dilated pressurized human coronary resistance vessels and this was antagonized by glibenclamide (Miura *et al*., 2003). Furthermore, in the aforementioned study, inwardly rectifying Kir 6.1 protein was identified, and K_{ATP} subunit expression detected in the human coronary vasculature. Therefore, it seems that K_{ATP} channels are present in human coronary resistance vessels and their activation leads to dilation.

Previously, it has been proposed that adenosine is a physiologically important dilator in the coronary circulation, matching blood flow to the demands of the myocardium (Rubio & Berne, 1969). We find that adenosine-induced dilation of human coronary resistance arteries, with myogenic tone, is not reversed by glibenclamide. This finding is in agreement with previous studies employing human vessels from the atrial appendage using a wire myograph, where the arteries have been preconstricted with a thromboxane A_2 mimetic, and using pressurized arteries, where myogenic tone was enhanced with acetylcholine or endothelin-1 (Kemp & Cocks, 1999; Sato *et al*., 2005). Thus, the apparent lack of involvement of the K_{ATP} channel in the adenosine-induced dilation of human coronary arteries *in vitro* is unlikely to be a consequence of the nature of the coronary artery tone employed. Recent *in vivo* studies employing glibenclamide also suggest that K_{ATP} channels are not essential for adenosine-induced hyperaemia in the coronary circulations in man, consistent with these *in vitro* observations (Farouque *et al*., 2002).

We find that hypoxia results in a reversible dilation human coronary resistance arteries, which is in agreement with a previous study using similar techniques (Miura *et al*., 2003). However, there is a major difference between the two investigations with respect to the effect of glibenclamide. We find glibenclamide to be without effect on hypoxic dilation, whereas Miura *et al*. (2003) observed significant inhibition of the hypoxia-induced dilation. Glibenclamide, at a concentration that fully reversed the pinacidil-induced dilation, was without effect on hypoxic dilation in the current

study. The only differences between the aforementioned study and ours that may conceivably account for this discrepancy as far as we can ascertain are (1) all our investigations were carried out on vessels that had developed a stable level of spontaneous myogenic tone and did not possess additional agonist-induced tone and (2) in our preparation, the O_2 tension was reduced to an average of $\sim$8 mmHg with hypoxia, compared with 23 mmHg in the study of Miura *et al*. (2003). Although the results of the current study suggest that the mode of constriction is not a factor influencing the effect of glibenclamide on adenosine-induced dilation of human coronary resistance arteries from the atrial appendage, this may not necessarily be the case with hypoxic dilation. Our aim was to study vessels with spontaneous myogenic tone because we considered this to be a more physiological situation. Nevertheless, with our samples, we found that vessels that do not develop myogenic tone responded poorly to exogenous vasoconstrictors. In the study of Miura *et al*. (2003), the inhibitory effect of glibenclamide was significant after 15 min of hypoxic exposure; however, the hypoxic dilation took more than 10 min to fully develop. Thus, inhibition of the dilation was not significant at 5 and 10 min. In our study, hypoxic dilation reached a plateau within 5 min; this may be because the hypoxia was more severe, as discussed above. Nevertheless, it is conceivable that in our study the arteries were not exposed to glibenclamide for a sufficient time to reverse the hypoxic dilation, even though dilation to pinacidil was fully reversed within this time frame. However, this is unlikely, as we found that a 20 min prior incubation with glibenclamide did not inhibit the hypoxic dilation.

It is possible that the pathological state of the patients from which the atrial appendages were obtained has influenced the results concerning the role of K_{ATP} channels in adenosine and hypoxic dilation. Miura *et al*. (2003) found that diabetes mellitus reduces the activity of the K_{ATP} channel, although this was not associated with an increased non-K_{ATP} component of hypoxic dilation. Left ventricular hypertrophy has been shown to reduce the glibenclamide-sensitive component of α2-adrenergic dilation of coronary resistance arteries from the pig (Gendron *et al*., 2004), although in the dog, left ventricular hypertrophy is associated with an increase reliance on K_{ATP} channels to augment flow during exercise (Melchert *et al*., 1999).

In conclusion, the adenosine and hypoxic dilation human coronary resistance arteries, with spontaneous myogenic tone, from the atrial appendage are not affected by glibenclamide. These data suggest that K_{ATP} channels may not play a major role in metabolic dilation of these vessels.

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