Adenosine and hypoxic dilation of rat coronary small arteries: roles of the ATP-sensitive potassium channel, endothelium, and nitric oxide

F. M. Lynch, C. Austin, A. M. Heagerty, and A. S. Izzard
Cardiovascular Research Group, Department of Medicine, Manchester Royal Infirmary, Manchester, United Kingdom

Submitted 30 March 2005; accepted in final form 13 October 2005

Lynch, F. M., C. Austin, A. M. Heagerty, and A. S. Izzard. Adenosine and hypoxic dilation of rat coronary small arteries: roles of the ATP-sensitive potassium channel, endothelium, and nitric oxide. Am J Physiol Heart Circ Physiol 290: H1145–H1150, 2006. First published October 21, 2005; doi:10.1152/ajpheart.00314.2005.—The aims of the study were to examine the roles of the ATP-sensitive potassium (K<sub>ATP</sub>) channel, the endothelium, and nitric oxide (NO) in the responses of rat coronary small arteries to adenosine and hypoxia. Segments of rat coronary vessel were investigated in vitro using pressure myography; all vessels studied developed stable spontaneous myogenic tone during equilibration. Glibenclamide (a K<sub>ATP</sub> channel inhibitor) reversed pinacidil but not 2-deoxyglucose-induced dilation. Both adenosine and hypoxia diluted the vessels, and glibenclamide did not reverse these responses. Endothelial removal or N<sup>ω</sup>-nitro-l-arginine methyl ester (l-NAME) inhibited the dilation to adenosine by ~50%; subsequent addition of glibenclamide was without effect. Hypoxic dilation was completely inhibited by endothelial removal or l-NAME. We conclude that adenosine- and hypoxia-induced dilation of rat coronary arteries does not appear to involve the K<sub>ATP</sub> Channel. Adenosine-induced dilation is partial and hypoxic dilation is completely dependent on endothelial-derived NO.

Autoregulation serves to maintain nearly constant blood flow with changes in perfusion pressure; metabolic dilation matches blood flow to the demands of the myocardium. These local mechanisms regulate the diameter of the coronary resistance vessels (6, 17). Myogenic tone is determined by the transmural pressure and is considered to play a major role in coronary autoregulation. Out of several candidates, adenosine and hypoxia are considered to be the most likely mediators of metabolic dilation (5, 26).

Several studies suggest that the ATP-sensitive potassium (K<sub>ATP</sub>) channel is involved in coronary vessel dilation to both adenosine and hypoxia because the dilations are blocked by the K<sub>ATP</sub> channel inhibitor glibenclamide. Therefore, the K<sub>ATP</sub> channel is considered to be a major mediator of coronary metabolic dilation (5, 21). Further support for this contention has been provided by electrophysiological studies and reports immunolocalizing K<sub>ATP</sub> channels in coronary smooth muscle (4, 19). However, functional studies have been conflicting in attempting to implicate the K<sub>ATP</sub> channel in coronary metabolic dilation. Several reports demonstrated a role for endothelium-derived nitric oxide (NO) in the dilatory response to both adenosine and hypoxia (9). Also, a study has shown that removal of the endothelium is needed to unmask a glibenclamide-sensitive dilation to adenosine in pig coronary vessels (9). A further report found that hypoxic dilation was both glibenclamide sensitive and endothelium dependent (14).

Therefore, in the current investigation, we have examined these possibilities on rat pressurized small coronary arteries in vitro. First, we wanted to confirm pinacidil [and 2-deoxyglucose (2-DG)] caused dilation that was reversed by glibenclamide. Next, we studied the effects of adenosine and hypoxia on the tone of rat coronary arteries and the effect of glibenclamide on responses to these stimuli. Finally, the effects of endothelial disruption and/or N<sup>ω</sup>-nitro-l-arginine methyl ester (l-NAME) on the responses to adenosine and hypoxia were studied, plus the subsequent addition of glibenclamide. All experimental protocols were carried out on coronary arteries that had developed a stable level of spontaneous myogenic tone because this is considered to be an important determinant of tone in the coronary vasculature.

METHODS

Female Wistar rats (250–350 g) were killed by stunning and cervical dislocation in accordance with our institutional guidelines and the United Kingdom (Scientific Procedures) Act of 1986. Experiments were performed with the approval of the Review Board of the University of Manchester and the Home Office. The hearts were removed and immediately placed in cold (4°C) physiological saline solution (PSS) consisting of (in mM) 119 NaCl, 4.7 KCl, 25 NaHCO<sub>3</sub>, 1.17 KH<sub>2</sub>PO<sub>4</sub>, 1.17 MgSO<sub>4</sub>, 0.026 EDTA, 1.6 CaCl<sub>2</sub>, and 5.5 glucose. Coronary septal arteries (150–250 m<sup>2</sup>) were dissected free and placed in an organ chamber bath (Living Systems Instrumentation, Burlington, VT) containing cold PSS. In the organ chamber bath, one end of the artery was cannulated with a glass micropipette filled with PSS and secured with a nylon suture. With low perfusion, any remaining blood products were flushed from the lumen, and the other end of the artery was cannulated with a second micropipette and tied with nylon. The artery was pressurized to 60 mmHg and checked for leaks. A stable pressure recording while the pressure servosystem is turned to manual, which turns off any automatic maintenance of pressure, confirmed the absence of any leaks. Only arteries without leaks were included in this study. The inner diameter was continually monitored using a video dimension analyzer (Living Systems) connected to a data-acquisition program (Windac) on a PC. The bath chamber was superfused with PSS (pH 7.4) and gassed with 5% CO<sub>2</sub> balanced in air at a superfusion rate of 20 ml/min. The temperature of the bath was maintained at 37°C using a circulating water heater. Arteries were left to equilibrate for a period of 1–2 h, during which time myogenic tone developed.

Experimental protocol. When a stable diameter was achieved, the experimental protocols were performed and changes in diameter were recorded.

To confirm that activation of glibenclamide-sensitive K<sub>ATP</sub> channels in the isolated pressurized rat coronary artery causes dilation, we...
cumulatively added pinacidil, a KATP channel opener, to the circulating solution (1–5 μM, final bath concentrations), and the diameter was allowed to stabilize after each addition. Subsequently, glibenclamide, a KATP channel blocker (1–5 μM), was cumulatively added; again the diameter was allowed to stabilize before addition of subsequent concentrations. The effects of inhibition of glycolysis were also studied. The glucose content in PSS was substituted with 5 mM 2-DG and 0.5 mM glucose (3) and perfused through the bath for ~10 min before the addition of glibenclamide (5 μM). The influence of the putative metabolic dilator adenosine on myogenic tone was investigated by making cumulative additions (1–100 μM) of the drug to the circulating solution. To investigate whether KATP channels were involved in the observed response, we added glibenclamide (1–5 μM) once again. The effects of acute severe hypoxia on arterial tone were also investigated. Hypoxia (<10 mmHg PO2) was induced by switching to a 95% N2-5% CO2 gas mixture. Oxygen levels were monitored using an oxygen meter (Strathclyde Instruments) connected to a needle probe (Linton Instrumentation) placed directly into the bath chamber in close proximity to the cannulated artery. Arteries were maintained in hypoxia for a period of ~10 min before returning to normoxic (>120 mmHg PO2) conditions. To investigate the repeatability of any response, a second hypoxic challenge was carried out once stable levels of myogenic tone had been regained. The involvement of KATP channels was once again examined by addition of glibenclamide (5 μM). Finally, arteries were returned to normoxic conditions.

The role of the endothelium in responses to adenosine or hypoxia was assessed by functionally impairing endothelial function in some experiments. This was achieved by gently rubbing the lumen of the artery with a glass cannula or by passing air bubbles through the lumen of the artery with successful disruption being taken as a dilator response to ACh (100 μM) of <20% of maximal and without loss of myogenic tone. To specifically investigate the involvement of NO and prostaglandins on responses, experiments were performed with responses to adenosine (100 μM) or hypoxia being carried out in the absence, and subsequent presence, of the NO synthase (NOS) inhibitor l-NAME (10 μM) or cyclooxygenase inhibitor indomethacin (1 μM). Arteries were incubated with the inhibitors for a period of 20 min. At the end of the second adenosine intervention, the role of KATP channels was examined by adding glibenclamide (5 μM).

At the end of the experiments, the arteries were superfused with Ca2+-free PSS containing 1 mM EGTA for 20 min to obtain the maximal passive diameter of the artery.

Solutions and drugs. All drugs were obtained from Sigma. Stock solutions of pinacidil, adenosine, and glibenclamide were made in DMSO before dilution in PSS to the final bath concentration. DMSO itself had no effect on myogenic tone. All other stocks and solutions were dissolved in ultradistilled water. All reported concentrations are mean ± SE.

Data analysis. A single artery was used from each rat. Internal diameters were recorded at steady-state conditions. Data are presented as mean active diameter (±SE) or mean change in active diameter (±SE). Comparison of a response before and after an intervention in a single artery was analyzed with a paired t-test. Comparisons between different groups of arteries were made using unpaired Student t-tests. Differences were considered significant at a P value < 0.05.

RESULTS

The mean passive diameter of arteries used was 227 ± 6 μm (n = 58). Vessels developed a stable myogenic tone within 1 h of cannulation. The mean active diameter after tone developed was 169 ± 0.6 μm.

Effects of pinacidil. Pinacidil (1–5 μM) produced a concentration-dependent dilation of pressurized rat coronary arteries with myogenic tone (Fig. 1) reaching a maximal dilation at 5 μM (n = 7). In two arteries, addition of higher concentrations of pinacidil did not further increase the dilation (data not shown). A concentration of 1 μM glibenclamide had little effect on this change in diameter. However, increasing the concentration of glibenclamide to 5 μM rapidly reversed the pinacidil-induced change in diameter (Fig. 1).

Effects of 2-DG. Addition of the 2-DG solution caused a slowly developing dilation of the rat coronary artery (n = 3). The increase in diameter was 41 ± 13 μm, which was not significantly altered by the addition of glibenclamide (5 μM). However, as the effects of 2-DG were irreversible, glibenclamide was also incubated before application of 2-DG (n = 3). This produced an increase in diameter of similar magnitude (39 ± 7 μm) to that produced in the previous experiment.

Effects of adenosine. Addition of adenosine (1–100 μM) produced a concentration-dependent dilation of the rat coronary arteries (n = 8, Fig. 2). In a single experiment, 1,000 μM adenosine was added, but this did not augment the response further (data not shown). Addition of glibenclamide, at concentrations up to 10 μM, had no effect on the adenosine-induced dilation. The effects of adenosine were readily reversible on washout.

Role of the endothelium in mediating the adenosine-induced dilation. To assess the potential role of the endothelium in mediating the adenosine-induced dilation, we performed a series of experiments in the presence (n = 6) and absence of a functional endothelium (n = 7). Figure 3A shows a reproduction of an original recording taken from an endothelium-intact vessel. The dilation produced by adenosine (100 μM) was significantly reduced in the presence of l-NAME (Fig. 4). Glibenclamide did not alter this response (Fig. 3A). Neither

---

Fig. 1. Effects of pinacidil and glibenclamide on rat coronary arterial diameter. A: reproduction of an original recording of the response of a pressurized rat coronary artery with myogenic tone to pinacidil and glibenclamide. B: mean (±SE) data demonstrating that pinacidil produced a significant increase in mean diameter (*P < 0.05, n = 7, paired t-test, at 3, 4, and 5 μM). This effect was almost completely reversed by 5 μM glibenclamide.
endothelial impairment nor incubation with L-NAME had any significant effect on the basal tone of the arteries. Incubation with indomethacin had no effect on basal tone or on responses to adenosine in endothelium-intact arteries, increases in diameter being 44 ± 11 μm in the absence and 48 ± 2 μm in the presence of the inhibitor, respectively (n = 3).

A second series of experiments were performed in endothelium-impaired arteries (ACh dilation of 20% maximal). Figure 3B shows a reproduction of an original recording taken from a typical experiment. Endothelial impairment significantly reduced the magnitude of the adenosine-induced dilation. It was not further reduced by the presence of L-NAME (Fig. 4) or glibenclamide (Fig. 3B). The magnitude of the adenosine dilation in endothelium-impaired arteries (with or without L-NAME) was similar to that observed in endothelium-intact arteries after incubation with L-NAME. There were no significant differences in the responses of endothelium-impaired arteries, with or without L-NAME, compared with endothelium-intact arteries in the presence of L-NAME, to hypoxia (Fig. 8). Once again, incubation with L-NAME had no significant effect on basal levels of tone. The active diameters between endothelium-intact and endothelium-impaired groups were not significantly different.

DISCUSSION

This study demonstrates for the first time that adenosine and hypoxia dilate pressurized rat coronary arteries with myogenic tone in vitro. We also confirm that the K<sub>ATP</sub> channel opener pinacidil caused dilation of pressurized rat coronary arteries and that this was fully reversed by glibenclamide; this finding is in agreement with a previous study (3). Thus activation of endothelial impairment nor incubation with L-NAME had any significant effect on the basal tone of the arteries.

Incubation with indomethacin had no effect on basal tone or on responses to adenosine in endothelium-intact arteries, increases in diameter being 44 ± 11 μm in the absence and 48 ± 2 μm in the presence of the inhibitor, respectively (n = 3).

A second series of experiments were performed in endothelium-impaired arteries (ACh dilation of <20% maximal). Figure 3B shows a reproduction of an original recording taken from a typical experiment. Endothelial impairment significantly reduced the magnitude of the adenosine-induced dilation. It was not further reduced by the presence of L-NAME (Fig. 4) or glibenclamide (Fig. 3B). The magnitude of the adenosine dilation in endothelium-impaired arteries (with or without L-NAME) was similar to that observed in endothelium-intact arteries after incubation with L-NAME.

Effects of hypoxia. Changing the gassing mixture to 95% N<sub>2</sub>-5% CO<sub>2</sub> significantly reduced the mean PO<sub>2</sub> from 161 ± 7 mmHg to 10 ± 2 mmHg (Fig. 5). This was accompanied by a significant dilation of the pressurized rat coronary arteries (n = 8, Figs. 5 and 6). The response was rapidly reversible (mean time 10 ± 1 min) on return to normoxia (159 ± 10 mmHg; mean time 2 ± 0.1 min). The response was repeatable as evidenced by the second hypoxic challenge causing a dilation of similar magnitude (Fig. 6). Glibenclamide (5 μM) did not alter the hypoxia-induced dilation.

Role of the endothelium in mediating the hypoxia-induced dilation. In arteries with an intact endothelium, L-NAME almost completely abolished the dilation to hypoxia (n = 6, P < 0.05, Figs. 7A and 8). Endothelial impairment (ACh dilation <20% maximal) almost completely abolished the hypoxic dilation (n = 7, Figs. 7B and 8). There was no additional effect of L-NAME (Fig. 7B and 8). There were no significant differences in the responses of endothelium-impaired arteries, with or without L-NAME, compared with endothelium-intact arteries in the presence of L-NAME, to hypoxia (Fig. 8). Once again, incubation with L-NAME had no significant effect on basal levels of tone. The active diameters between endothelium-intact and endothelium-impaired groups were not significantly different.
KATP channels could act as a mechanism for dilation of rat coronary arteries. However, although both adenosine and hypoxia dilated the coronary arteries, these dilations were unaffected by glibenclamide. The adenosine-induced dilation was partially inhibited (~50%) by endothelial impairment or l-NAME, indicating a partial dependence on NO released from the endothelium; under these conditions, glibenclamide was still without effect. Hypoxic dilation was completely blocked by endothelial impairment or l-NAME, indicating a complete dependence of this response, in rat coronary small arteries with myogenic tone, on endothelium-derived NO.

Our finding that glibenclamide inhibits a pinacidil-induced dilation is also in agreement with several previous studies using systemic as well as coronary arteries (3, 20). KATP channels are by definition K+ channels that are inhibited by intracellular ATP ($k_i = 10^{-10} – 10^{-100}$ mol/l) at the intracellular side of the plasma membrane. Therefore, inhibition of glycolysis in the coronary arteries may lead to a sufficient reduction in ATP for KATP channel opening to occur, a situation that may occur in hypoxia to cause dilation. Consistent with this idea, Conway et al. (3) demonstrated that 2-DG dilated rat small coronary arteries with myogenic tone, and this dilation was reversed by glibenclamide. In an attempt to reproduce this, we approached the experiment in two different ways. Initially, we applied glibenclamide when the 2-DG dilation stabilized. After this, we superfused with PSS but were unable to reverse the dilation. Because this dilation was irreversible, we postulated that even
Adenosine and hypoxic responses of coronary arteries

If glibenclamide were having an effect at the cellular level, this was not being translated to the functional level because the contractility of the artery was compromised by the experimental intervention. Incubating glibenclamide before application of 2-DG did not block the dilation. We find that glibenclamide-sensitive K<sub>ATP</sub> channels are not involved in mediating the 2-DG-induced dilation, and the reason for the discrepancy with the study of Conway et al. (3) is unclear.

This study demonstrates for the first time that adenosine dilates pressurized rat coronary resistance arteries with myogenic tone in vitro. The dilator response to adenosine is well documented in other isolated coronary preparations: porcine arteries with myogenic tone (10, 13), ET1-constricted arteries (8), and in vivo preparations [canine (24, 30), goat (7), and human (28)].

Adenosine receptors have been identified on both the coronary vascular smooth muscle and endothelial cells (22, 23, 27). Adenosine can act by activating A<sub>1</sub> or A<sub>2</sub> receptors to elicit dilation through a receptor-incorporated cAMP pathway. However, patch-clamp studies demonstrate that opening of K<sub>ATP</sub> channels after adenosine A<sub>1</sub> activation may not involve the cAMP pathway (4). In our preparation, inhibition of these channels using glibenclamide had no effect on the response to adenosine. Similarly, others have also shown that adenosine-induced dilation of porcine coronary arteries preconstricted by addition of ET1 was unaltered by the presence of glibenclamide (8). In contrast, adenosine-induced porcine coronary vasodilation is inhibited at least partially by glibenclamide in preparations with myogenic tone (9, 10, 13) and also in vivo preparations (2, 30).

Endothelial impairment significantly attenuated the adenosine response. This observation agrees with studies in coronary arteries from other species, namely canine coronary artery (24) and guinea-pig coronary artery (29). We found that inhibition of NOS using L-NAME attenuated the adenosine-induced dilation to a similar extent as endothelial impairment in the present study (Fig. 4), suggesting that the endothelial factor involved is NO. Inhibition of cyclooxygenase had no effect on responses to adenosine in the present study. The NO dependence of the adenosine response has been observed by others (7, 10, 13, 24, 30). Similarly, lack of response to cyclooxygenase inhibition has also been reported (10, 13). Our results suggest that adenosine exerts its actions by at least two pathways. One acts by releasing NO from endothelial cells, and the other acts directly on the smooth muscle cells. Hein et al. (9) found that endothelial impairment or L-NAME partially inhibited the dilation to adenosine in pressurized pig coronary arteries with myogenic tone and that the residual dilation was nearly abolished by glibenclamide. In contrast, we find that the endothelium-independent component of the dilation to adenosine in rat coronary arteries is insensitive to glibenclamide. The mechanisms through which adenosine acts on vascular smooth muscles cells require further investigation.

We found that hypoxia produced a dilation of pressurized rat coronary resistance arteries with myogenic tone. The degree of hypoxia used in our study is severe (<10 mmHg Po<sub>2</sub>). This level was chosen because in control experiments we did not observe a response to hypoxia at levels greater than 20 mmHg Po<sub>2</sub>. The time course of our hypoxic challenge (10 min) was chosen because the arteries failed to regain myogenic tone after exposure to longer periods of hypoxia.

The hypoxic dilation was unaffected by inhibition of K<sub>ATP</sub> channels using glibenclamide. Kerkhof et al. (12) also found that K<sub>ATP</sub> channels did not participate in the hypoxic relaxation of isolated rat coronary septal arteries constricted with KCl or U-46619. However, several studies have demonstrated that inhibition of these channels can reduce or abolish the effects of hypoxia in human, rabbit, and porcine coronary arteries (5, 14, 19). Although hypoxia is often associated with a fall in intracellular ATP, this is not always the case, and there are reports of unchanged arterial intracellular ATP concentrations during hypoxia (1). Nevertheless, we found that even metabolic inhibition with 2-DG caused a dilation that was unaffected by glibenclamide in rat coronary arteries.

Endothelial impairment completely abolished the hypoxic dilation, an effect that was mimicked by endothelial NOS inhibition. Thus it is evident that the hypoxic dilation in the pressurized rat coronary resistance artery is an endothelium-dependent phenomenon elicited by NO. This observation is in agreement with in vivo studies in dogs (18), isolated guinea-pig hearts (25), and isolated porcine coronary arteries (11). Nevertheless, as reported for adenosine-induced dilations, there are apparent discrepancies in the literature regarding the inhibitory effects of glibenclamide and/or endothelial impairment; this may be related to species differences.

In summary, we have shown that both adenosine and severe acute hypoxia dilate isolated rat coronary arteries with myogenic tone in vitro. Although our functional studies indicate the presence of K<sub>ATP</sub> channels in our preparation, the dilations to both adenosine and hypoxia were unaffected by glibenclamide. Conversely, the responses were found to be partially (adenosine) or completely (hypoxia) inhibited by endothelial impairment or by inhibition of NOS. Our results highlight the important role the endothelium plays in regulating rat coronary vascular responses to adenosine and hypoxia, which are considered to be important mediators of metabolic dilation in the...
coronary circulation. Our preliminary findings in human coronary arteries with myogenic tone also demonstrate dilations to adenosine and hypoxia that are unaffected by the addition of glibenclamide (15, 16); therefore, these findings have pathophysiological implications because coronary metabolic dilator responses may be blunted in conditions associated with endothelial dysfunction, which may further exacerbate perfusion problems within the heart.

GRANTS

We thank the British Heart Foundation and the Wellcome Trust for funding this research.

REFERENCES