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The role of adenosine in rat coronary flow regulation during respiratory and metabolic acidosis

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Abstract

The role of adenosine in rat coronary flow regulation during acidosis was evaluated in isolated, perfused, Langendorff rat heart preparations exposed to brief periods of hypercapnic or metabolic acidosis. Acidosis resulted in increases in coronary flow rate, in conjunction with decreases in ventricular contractile tensions. Heart rates were non-significantly increased. Two non-selective adenosine antagonists, caffeine and 8-phenyltheophylline, markedly attenuated the increases in coronary flow during hypercapnic acidosis without affecting the decline in contractile tension or the heart rate. ZM 241385 (4-(2-[7-amino-2-(2-furyl)[1,2,4]triazolo[2,3-*a*]triazin-5-yl amino]ethyl)phenol), a selective adenosine A_{2A} receptor antagonist, also blocked hypercapnic acidosis-evoked coronary flow rate increases. The adenosine A_1 selective antagonist, 8-cyclopentyl-1,3-dipropylxanthine, did not affect flow rate increases during hypercapnic acidosis. SCH 58261 (5-amino-7-(2-phenyl ethyl)-2-(2-furyl)pyrazolo-[4,3-e]-1,2,4-triazolo[1,5-*c*] pyrimidine, a selective adenosine A_{2A} receptor antagonist, blocked the increases in coronary flow rate evoked by metabolic acidosis. An adenosine transport inhibitor, dipyridamole, doubled coronary flow rates during hypercapnic acidosis. When taken in conjunction with previous reports that acidosis enhances adenosine release from cardiac preparations, these results suggest that adenosine is a significant contributor to acidosis-evoked increases in coronary flow.

Keywords: Coronary flow; Acidosis; Hypercapnia; Adenosine; Adenosine receptor; ZM 241385; SCH 58261; Dipyridamole

1. Introduction

Coronary blood flow is known to be regulated by several factors including coronary perfusion pressure together with neural, hormonal and metabolic mediators. Berne (1963, 1974) proposed that adenosine, a potent endogenous coronary vasodilator, may play a major role in the metabolic regulation of coronary blood flow in response to increases in the rate of tissue metabolism or decreases in blood flow. Thus, ischemia or hypoxia have been shown to elevate myocardial adenosine levels, indicating an increased production of the purine when oxygen supply is inadequate (Bacchus et al., 1982). Cardiac adenosine levels are also elevated when heart rate or the force of contraction are increased. The results of pharmacological attempts to validate the Berne hypothesis have been somewhat inconsistent (Collis, 1991); but in general there has been a consensus that adenosine receptor antagonists reduce the reactive hyperemia following short periods of ischemia evoked by coronary artery occlusion or during hypoxia (Curnish et al., 1972; Giles and Wilken, 1977; Radford et al., 1984; Bache et al., 1988). Similar reductions in the magnitude of the reactive hyperemia were observed when adenosine deaminase was administered into the coronary arteries of hearts exposed to coronary artery occlusion or hypoxia (Saito et al., 1981; Merrill et al., 1986).

Increases in coronary flow have also been observed during hypercapnia (Gonzalez et al., 1968; Clancy and Gonzalez, 1975; Alexander and Liu, 1976; Ely and Sawyer, 1982) and metabolic acidosis (Wang and Katz, 1965; Clancy and Gonzalez, 1975; Merrill et al., 1978; Mustafa and Mansour, 1984). As the increase in coronary blood flow during acidosis is associated with elevated levels of adenosine in the coronary perfusate (Degenring, 1976), Mustafa and Mansour (1984) suggested that the effects of hydrogen ions on coronary flow might be due to adenosine release.

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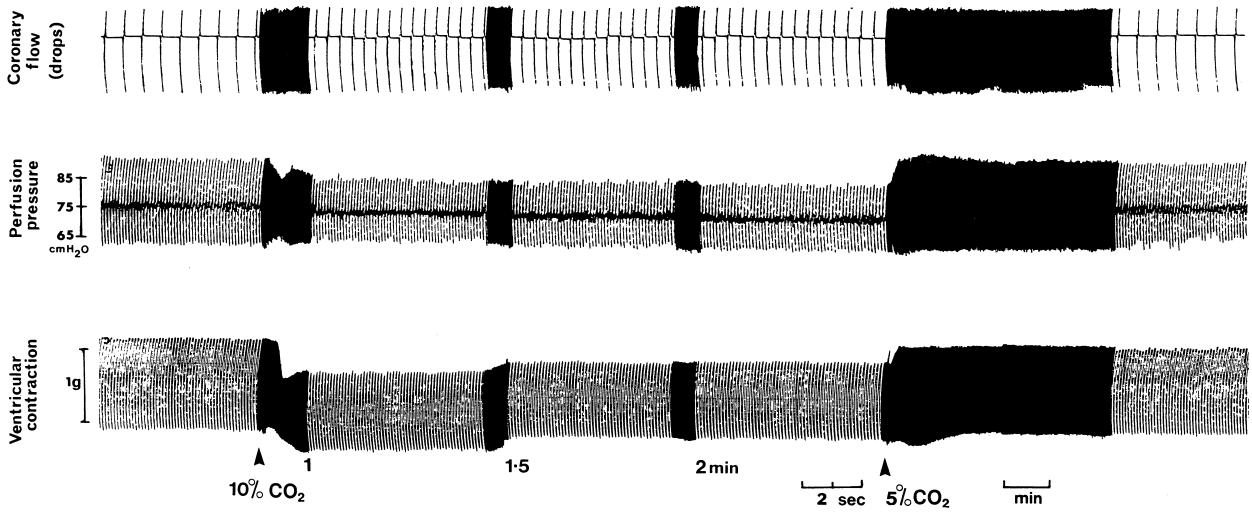

Fig. 1. Original tracings comparing rates of coronary flow (as drops) and ventricular contractions before, during and after a 2 min period of exposure to intracoronary hypercapnic acidosis (10% CO_2). The aortic perfusion pressure record shows the fluctuations caused by ventricular contraction superimposed on a 75 cmH_2O hydrostatic pressure. The contraction record demonstrates a reduction in the force, but not rate, of ventricular contractions during exposure to acidosis. Responses were recorded at two chart paper speeds, as shown by the two time bars beneath the ventricular contraction record.

The present experiments were undertaken to examine the role of adenosine in the coronary vascular effects of hypercapnia-induced and metabolic acidosis using both selective and non-selective adenosine receptor antagonists and dipyridamole, an adenosine transport inhibitor. The results demonstrate that the reduction in coronary vascular

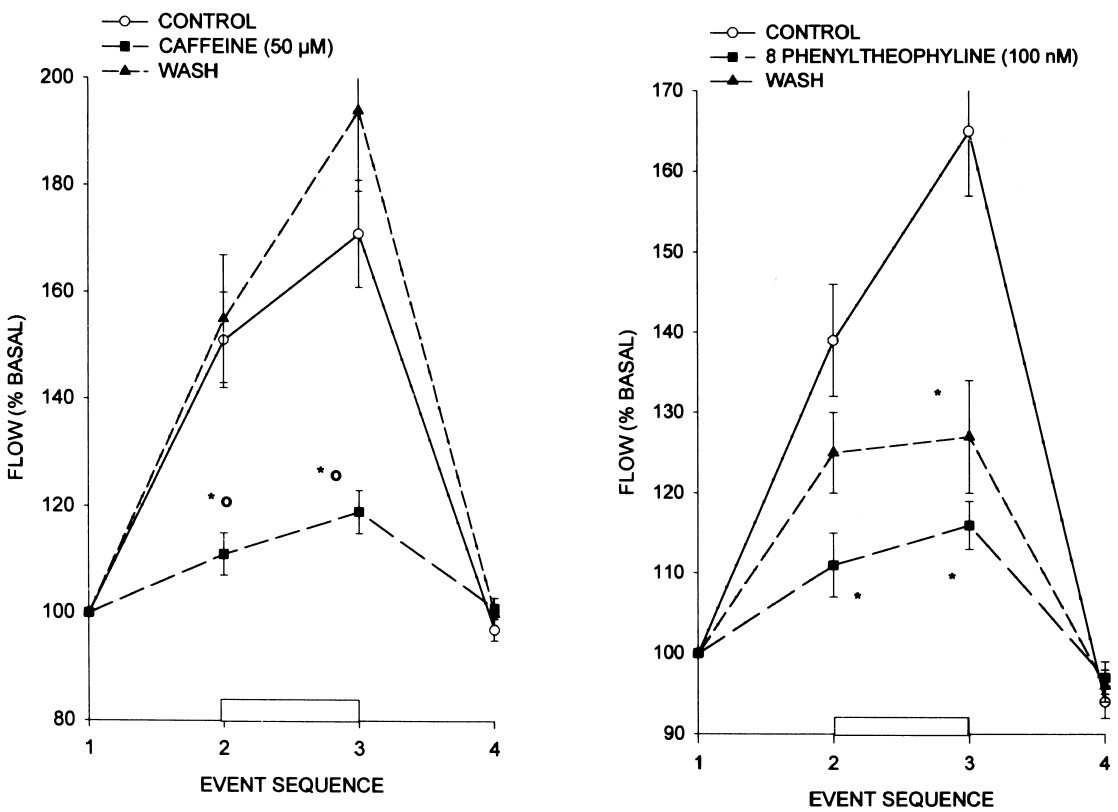

Fig. 2. Percentage changes in basal coronary flow rate during intracoronary hypercapnic acidosis and the effects of prior exposure to caffeine (50 μM) and 8-phenyltheophylline (100 nM) on the flow rates. The event sequences (1–4) indicate flow rates recorded prior to (1) exposure to the acidotic perfusate; at 1 and 2 min after contact with acidotic perfusate (2, 3); and after recovery (4). Exposure to caffeine (50 μM) significantly attenuated the acidosis-evoked increases in flow rate with full recovery 20 min after onset of reperfusion with normal Krebs–Henseleit buffer. 8-Phenyltheophylline (100 nM) significantly attenuated the hypercapnia-evoked flow increases with only partial recovery at 20 min. $* P < 0.05$ vs. original controls; $^{\circ} P < 0.05$ vs. recovery after wash-out of drug solution.

resistance during acidosis is mediated, at least in part, by locally released adenosine.

2. Materials and methods

All experiments were performed in accordance with the 'Guide for the Care and Use of Laboratory Animals' (NIH Publication No. 85-23, revised 1996) and were approved by the Animal Investigation Committee of Wayne State University.

2.1. Heart preparation

Male Sprague–Dawley rats (275–350 g wt.) were anaesthetized with pentobarbital sodium (50 mg/kg intraperitoneal) and heparinized (1000 IU, kg^{-1}) via a femoral vein. The chest was then opened and the heart freed of its connections and submerged in cold Kreb's–Henseleit buffer (KHB). Perfusion was initiated by tying the end of the aortic segment around a perfusion cannula after which the Langendorff preparation was suspended in an enclosed water jacket and perfused retrogradely via the aorta. Perfusion with KHB was carried out using a constant pressure (75 cmH_2O) perfusion system. The KHB consisting of (in mM concentrations): NaCl, 118.0; KCl, 4.7 mM; $CaCl_2$, 2.9 mM; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25; glucose, 11.0, was equilibrated with a gas mixture of 95% O_2 + 5% CO_2 (pH 7.38 ± 0.04; P_{CO_2}, 38.2 ± 4.0) and maintained at 37°C. Changes in the pH of the perfusion solution were obtained either by equilibrating the KHB with a mixture of 90% O_2 + 10% CO_2 (pH 6.99 ± 0.02; P_{CO_2}, 98.3 ± 3.8; hypercapnic acidosis) or by changing the $NaHCO_3$ and NaCl content ($NaHCO_3$, 11.0 mM, NaCl, 132.0 mM) to achieve a pH of 6.97 ± 0.02 with a P_{CO_2} of 40.6 ± 2.6 when equilibrated with 95% O_2 + 5% CO_2 (metabolic acidosis). There were no significant differences in the oxygen partial pressures of the normocapnic, hypercapnic and metabolic acidotic perfusing solutions.

Heart rate and intra-aortic pressure changes were recorded via a Statham P23Dc pressure transducer attached to a side arm of the aortic cannula. The apex of the heart was connected by means of a thread to a force displacement transducer (Grass FT .03) for continuous recording of the rate and force of ventricular contraction. Coronary flow rate was measured with a calibrated drop counter placed beneath the water jacket. The outputs of all three transducers were connected to a Grass Model 7 polygraph.

After an initial 20 min stabilization period, flow to the hearts was switched to a reservoir containing either the hypercapnic or metabolic acidotic perfusates for 2 min exposures with 10 min intervening periods of perfusion

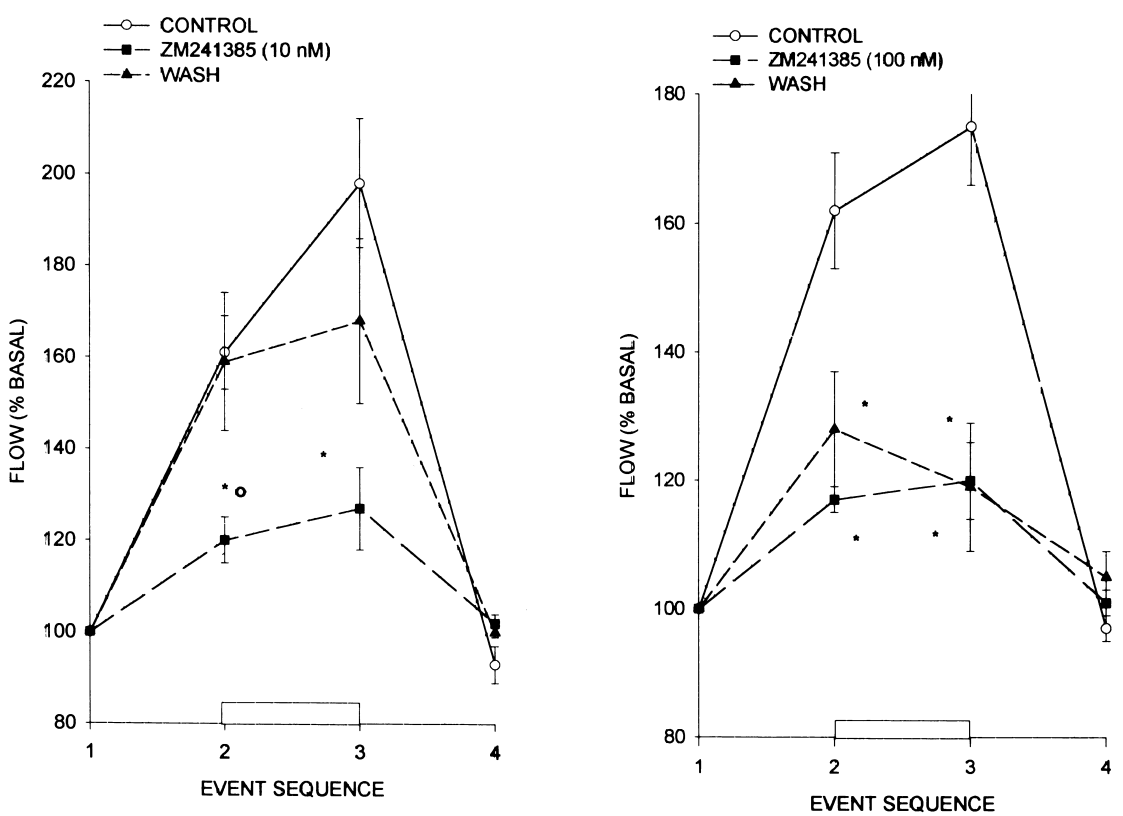

Fig. 3. The adenosine A_{2A} receptor selective antagonist ZM 211385 (10 nM, 100 nM) significantly reduced hypercapnic acidosis-evoked increases in coronary flow rate. Recovery occurred after wash-out of the lower drug concentration. * $P < 0.05$ vs. original control flow; ° $P < 0.05$ vs. recovery after wash-out of drug solution.

with normal KHB. Once two consecutive stable responses to the acidotic perfusates had been recorded, the preparations were exposed to regular KHB containing the agent to be tested for 20 min. The appropriate acidotic perfusate, also containing the agent, was then applied twice for a 2 min period with a 10 min interval between these exposures. Flow with normal (agent-free) KHB was then resumed and testing with acidotic perfusates continued after a 20 min wash-out period with 2×2 min exposures at 10 min intervals.

2.2. Chemicals

Results with five adenosine antagonists and one transport inhibitor are presented. These were: the relatively non-selective receptor antagonists caffeine (Sigma, St. Louis, MO, USA, 50 μM) and 8-phenyltheophylline (Sigma, 100 nM); an adenosine A_1 selective receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (Sigma, 10 nM); two adenosine A_{2A} selective receptor antagonists, ZM 241385 (4-(2-[7-amino-2-(2-furyl)[1,2,4]-triazolo[2,3-*a*]triazin-5-yl amino]ethyl) phenol) (Zeneca Pharmaceuticals, Cheshire, UK), 10 and 100 nM) and SCH 58261 (5-amino-7-(2-phenyl ethyl)-2-(2-furyl)pyrazolo-[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine) (Schering–Plough Institute, Milan, Italy, 10 nM); and the adenosine transport inhibitor dipyridamole (Sigma, 1 μM). Each drug was tested, for any given concentration, on four hearts.

2.3. Statistical analysis

To correct for variability in basal values, changes in both flow rate and heart rate were calculated as a percent of the initial basal values for each heart. Statistical differences between pre-drug, drug and post-drug conditions were determined by One-way ANOVA followed by Scheffe's test. A probability of < 0.05 was accepted as denoting a significant difference.

3. Results

The effects of hypercapnic acidosis (pH 6.99 ± 0.02) on the heart are shown in Fig. 1. Basal coronary flow rates prior to the CO_2 challenges were 6.57 ± 0.46 (S.E.M.) ml/min; during challenges flow increased to 10.59 ± 0.37 ml/min and then returned to 6.28 ± 0.45 ml/min. The ventricular contraction records revealed that tension decreased over the initial 60 s of perfusion with the hyper-

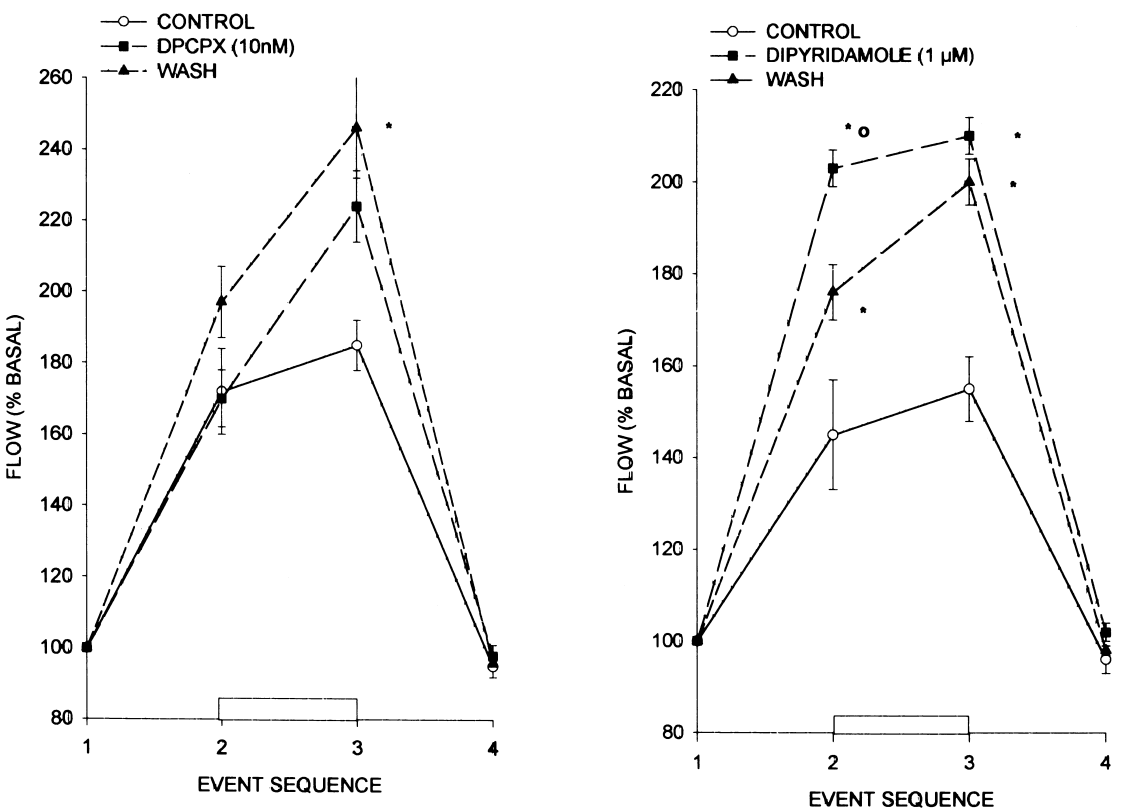

Fig. 4. The adenosine A_1 receptor selective antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX, 10 nM) did not block the hypercapnia-evoked increases in coronary flow. Twenty minutes after wash-out the flow response was actually enhanced. Dipyridamole (1 μM), an adenosine transport inhibitor, significantly enhanced hypercapnic acidosis-evoked increases in coronary flow. Partial recovery was evident after a 20 min wash-out period. $* P < 0.05$ vs. original pre-drug controls; $^{\circ} P < 0.05$ vs. recovery after wash-out of drug solution.

capnic perfusate. Developed tension remained constant for the next 60 s and then recovered rapidly during the initial 20 s of reperfusion with normal KHB. The intra-aortic pressure recording shows the ventricular contraction-elicited pressure fluctuations superimposed on the sustained background pressure of 75 cmH_2O. During acidosis the pressure pulse was frequently attenuated reflecting the reductions in ventricular contractility shown in the lower trace. Exposure to hypercapnic perfusates caused non-significant increases in heart rate. The effects of exposure to metabolic acidosis were comparable to those observed with hypercapnic acidosis. Coronary flow rates increased, ventricular contractility was depressed and intra-aortic pressure fluctuations were attenuated, albeit at a slightly slower rate than with hypercapnic acidosis.

The effects of two relatively non-selective adenosine receptor antagonists are illustrated in Fig. 2. Caffeine (50 μM) markedly attenuated the increases in coronary flow during exposure to hypercapnic acidotic perfusion. Following reperfusion with caffeine-free perfusate the flow responses to acidosis recovered and were non-significantly greater than in the pre-drug controls. This result confirmed initial observations of the persistence of the CO_2-evoked increases in coronary vascular flow during the course of an experiment. 8-phenyltheophylline (100 nM), a more potent although relatively non-selective adenosine receptor antagonist, also significantly attenuated the increase in coronary flow during exposure to hypercapnic acidosis. In this instance, recovery was only partial following a 20 min wash-out period. Neither agent affected basal coronary flows or heart rates. This lack of effect was also observed with the selective receptor antagonists.

ZM 241385 is a potent, competitive, non-xanthine adenosine A_{2A} selective adenosine receptor antagonist (Poucher et al., 1995). At a 10 nM concentration ZM 241385 caused a significant attenuation (Fig. 3) of the CO_2-evoked levels of coronary perfusate flow. Partial recovery had occurred after a 20 min wash-out. ZM 241385 (100 nM) had comparable effects to the 10 nM concentration on CO_2-evoked coronary vascular flow but in this instance there was no recovery of flow after a 20 min wash-out. The similarity between the effect of the two concentrations of this agent on CO_2-evoked increases in coronary flow suggests that a maximal response was already present at the 10 nM concentration and that the residual increase in coronary flow may have been mediated via non-adenosine A_{2A} receptor mechanisms, including the activation of adenosine A_{2B} receptors coupled to K_{ATP} channels in the vascular smooth muscle (Mutafova-Yambolieva and Keef, 1997), although the similarity of the degree of block with ZM 241385 and caffeine, a non-selective adenosine receptor antagonist, appears to preclude the latter possibility. Alternatively, it is possible that ZM 241385 failed, at both concentrations, to block a reserve of 'spare' adenosine A_{2A} receptors which would still have been accessible to adenosine.

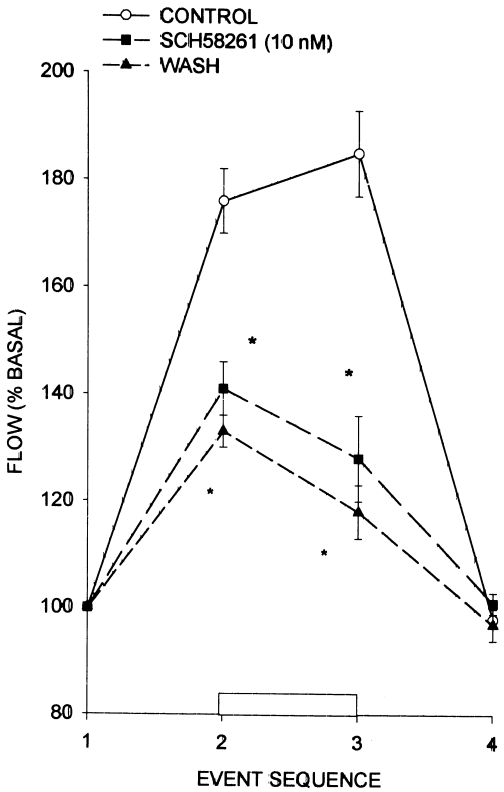

Fig. 5. The adenosine A_{2A} receptor selective antagonist SCH 58261 (5-amino-7-(2-phenyl ethyl)-2-(2-furyl)pyrazolo-4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine; 10 nM) significantly attenuated increases in coronary flow evoked by metabolic acidosis. There was no recovery after a 20 min wash-out of the agent. $* P < 0.05$ vs. pre-drug controls.

SCH 58261, a potent, selective, competitive, non-xanthine adenosine A_{2A} receptor antagonist structurally related to ZM 241385 (Zocchi et al., 1996), binds with high affinity to coronary artery membranes (Belardinelli et al., 1996). This antagonist was selected for experiments to evaluate the role of adenosine A_{2A} receptors in coronary vasodilation evoked by metabolic acidosis. SCH 58261 (10 nM) significantly attenuated the increase in coronary flow (Fig. 5) during metabolic acidosis. There was minimal recovery after a 20 min wash-out period.

8-Cyclopentyl-1,3-dipropylxanthine (10 nM), a highly selective adenosine A_1 receptor antagonist did not block the coronary flow response to hypercapnea (Fig. 4).

The adenosine transport inhibitor, dipyridamole (1 μM) doubled the coronary flow increase during hypercapnic acidosis (Fig. 4). Partial recovery of the CO_2-evoked responses to pre-drug levels was evident after a 20 min wash-out period.

4. Discussion

The purpose of this study was to determine if increases in coronary flow during respiratory or metabolic acidosis are mediated by adenosine, and if so to define the identity

of the adenosine receptors. Adenosine A_1 and A_2 subtypes of cell membrane receptors mediate the known cardiovascular effects of adenosine. Adenosine A_1 receptors are involved in slowing the heart rate, decreasing atrioventricular conduction, reducing atrial contractility, and inhibiting noradrenaline release from sympathetic nerve terminals (Collis, 1991; Mubagwa et al., 1996). Activation of adenosine A_2 receptors causes coronary vasodilation and increases in coronary conductance. Belardinelli et al. (1996, 1998) have recently established that the adenosine A_{2A} receptor plays a major role as the mediator of coronary vasodilation by adenosine.

Several studies have demonstrated increased coronary blood flows during hypercapnia (Gonzalez et al., 1968; Clancy and Gonzalez, 1975; Alexander and Liu, 1976; Ely and Sawyer, 1982) and metabolic acidosis (Wang and Katz, 1965; Merrill et al., 1978; Mustafa and Mansour, 1984). Whether P_{CO_2} or pH exert a direct effect on the smooth muscle of coronary vessels, or if the vascular response is mediated via other mechanisms is presently unresolved.

Adenosine and nitric oxide have been proposed as metabolic mediators of coronary vasodilation and there have been suggestions that their actions may be coupled, with adenosine eliciting vascular dilation by stimulating the release of nitric oxide from the vascular endothelium (Zanzinger and Bassenge, 1993; Abebe et al., 1994; Huckstorf et al., 1994; Maekawa et al., 1994; Li et al., 1995). However, in the isolated guinea pig heart N^6-nitro-L-arginine methyl ester (L-NAME), an inhibitor of nitric oxide synthesis, inhibited only a fraction of the coronary dilator effect of exogenous adenosine (Vials and Burnstock, 1993). L-NAME also failed to inhibit adenosine-evoked dilation of isolated coronary arterioles (Jones et al., 1995) or adenosine-induced decreases in the coronary perfusion pressure of the in situ dog heart (Gurevicius et al., 1995).

Early indications of an association between coronary conductance, hypercapnic acidosis and adenosine were observed by Raberger et al. (1975) who discovered that the dilator action of adenosine administered into the coronary artery was dependent on arterial pH and P_{CO_2}. Degenring (1976) subsequently established that acidosis (pH 7.0) resulted in enhanced levels of adenosine and inosine in guinea pig hearts perfused with KHB equilibrated with 95% oxygen, together with significant increases in the rate of adenosine release into coronary perfusates. Concurrently, there was a small, although non-significant, decline in ATP levels in the heart. Perfusion with KHB equilibrated with 20% oxygen yielded even greater increases in adenosine levels together with significant declines in ATP levels. Merrill et al. (1978) subsequently demonstrated that a hypercapnic acidosis potentiated adenosine-evoked coronary flows in the isolated perfused guinea pig heart. The increased sensitivity to adenosine at lower pH values was attributed to the inhibition of adenosine uptake by heart cells with increased concentrations of the purine to which the coronary resistance vessels would be exposed (Mustafa, 1980; Mustafa and Ghai, 1981).

Adenosine is released from cultured vascular endothelial cells by a process which is enhanced by carbon dioxide (Nees et al., 1980; Nees and Gerlach, 1983). Cultured rat aortic vascular smooth muscle cells also produce and release adenosine and this release can be modulated by changes in pH and P_{CO_2} (Belloni et al., 1986). These authors speculated that the enhanced release from vascular smooth muscle could contribute to greater adenosine levels in the vicinity of the coronary resistance vessels. Endogenous adenosine could thus participate in CO_2's vasodilator action.

We found acidosis, whether respiratory or metabolic, to cause pronounced decreases in ventricular contraction and in left ventricular systolic pressure development. The slower onset of decline in ventricular contractions observed in hearts exposed to metabolic acidosis in comparison to hypercapnic acidosis may be a reflection of the ability of lipid-soluble CO_2 to diffuse across vessel walls more rapidly than H^+. The decreased contractility may have been a result of inhibition of calcium transport in the heart during acidosis (Hess et al., 1984).

Accompanying the fall in cardiac function during acidosis was an increase in coronary vascular flow. In this instance, adenosine release cannot be attributed to an increasing cardiac work load but must, rather, have been a reflection of the change in pH. A possible explanation for the fall in ATP levels and accompanying increase in adenosine levels observed by previous investigators is that intracellular acidification, with enhanced H^+/Na^+ exchange, would result in an accelerated rate of ATP utilization by Na^+–K^+ ATPase (Tani and Neely, 1990; Van Emous et al., 1998).

The effects of selective and non-selective adenosine receptor antagonists observed in these experiments clearly define the adenosine receptor on the coronary vasculature responsible for the acidosis-evoked hyperemia in rat hearts as being the adenosine A_{2A} receptor type. Two non-selective adenosine receptor antagonists attenuated the increase in flow elicited by hypercapnic acidosis, as did the highly selective adenosine A_{2A} receptor antagonist, ZM 241385. Another potent, selective adenosine A_{2A} receptor antagonist, SCH 58261, markedly attenuated the flow response to metabolic acidosis (Fig. 5). Conversely, a potent, selective, adenosine A_1 receptor antagonist, DPCPX, failed to depress the coronary flow response to hypercapnic acidosis. This result is consistent with recent studies by Belardinelli et al. (1996, 1998)) which defined porcine and guinea pig coronary adenosine receptors as being adenosine A_{2A} in type. Further evidence in support of a mediator role for adenosine in acidosis-evoked increases in coronary flow comes from our observation that the transport inhibitor, dipyridamole, enhanced the increase in flow during hypercapnic acidosis.

5. Conclusion

The results reported here provide compelling evidence that activation of adenosine A_{2A} receptors by endogenously released adenosine makes a substantial contribution to the coronary vasodilation elicited by hypercapnic and metabolic acidosis. In that the carbon dioxide and lactic acid produced during periods of enhanced activity can generate an intracellular acidosis, it is likely that similar mechanisms may operate in other tissues to balance vascular supply to metabolic activity in the absence of hypoxia or ischemia.

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