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Effects of pH on responses to adenosine, CGS 21680, carbachol and nitroprusside in the isolated perfused superior mesenteric arterial bed of the rat

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1 The receptors mediating the vasodilator responses to adenosine in the isolated mesenteric arterial bed of the rat were identified by use of selective agonists and antagonists and the involvement of the endothelium was examined.

2 Adenosine-mediated dilatation of the mesentery was potentiated by the nitric oxide synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME, 100 μM), but in contrast, removal of the endothelium substantially reduced the responses to adenosine.

3 The order of potency of adenosine receptor agonists was: 5'-N-ethylcarboxamidoadenosine (NECA) > 2-p-(-2-carboxyethyl)phenethylamino-5'-N-ethylcarboxamidoadenosine (CGS 21680) >2-chloro-N^6-cyclopentyl-adenosine (CCPA) ⩾ adenosine, suggesting the presence of A_{2A} receptors.

4 Adenosine-mediated dilatation was inhibited by the non-selective adenosine receptor antagonist, 8-phenyltheophylline (3 μM) and by the A_{2A} receptor antagonist 8-(3-chlorostyryl)caffeine (500 nM), but was unaffected by the A_1 receptor antagonist, 1,3-dipropyl-8-cyclopentylxanthine (DPCPX; 10 nM).

5 Reducing the pH of the perfusate to 6.8 potentiated the actions of both CGS 21680 and adenosine, but the vasodilator effects of carbachol were the same at both pH values. The adenosine response at the lower pH as at pH 7.4, was unaffected by DPCPX. The actions of the nitrovasodilator, sodium nitroprusside, were also potentiated at pH 6.8 relative to those at the higher pH value but smaller responses were obtained at the lower pH value with forskolin, a stimulator of adenylyl cyclase, than at pH 7.4.

6 It is concluded that the adenosine receptor mediating dilatation of the rat mesenteric arterial bed is of the A_{2A} subtype, that the response, under the conditions used, is apparently partly dependent on the endothelium (but not due to the release of nitric oxide), and that the response to activation of this receptor is potentiated by a reduction in pH which is similar to that seen in ischaemic conditions.

Keywords: Adenosine; adenosine A_{2A} receptors; superior mesenteric arterial bed; endothelium; L-NAME; pH; CGS 21680; forskolin; nitric oxide; EDHF

Introduction

Adenosine is ubiquitous within the body and has widespread effects as a vasodilator which are mediated through P_1 purinoceptors (Fredholm *et al.*, 1994). For example, it regulates coronary artery dilatation, partly through the action of an adenosine A_2 receptor which is probably of the A_{2A} subtype (Martin *et al.*, 1993; Vials & Burnstock, 1993). Its vascular actions are not restricted to the coronary circulation since it causes vasodilatation in most vascular beds, although vasoconstriction may also occur in the kidney (Rossi *et al.*, 1987). Vuorinen *et al.* (1992, 1994) recently reported that rat mesenteric artery rings were relaxed by adenosine and that this action was blocked by the non-selective A_1 and A_2 receptor antagonist, 8-phenyltheophylline (8-PT). Removal of the endothelium in these rings slightly attenuated the action of adenosine.

Adenosine is thought to be important in regulating vascular tone, particularly by bringing about vasorelaxation during periods of ischaemia when there is an increase in its extracellular concentration which will cause activation of its tissue and cytoprotective effects (Newby, 1984). During hypoxic and ischaemic episodes the extracellular pH also decreases considerably and this appears to be of direct relevance to the adenosine system since lowering the pH below 7.4 amplifies the cardiovascular effects of adenosine (Merrill *et al.*, 1978). This action might be mediated partly by an increase in affinity of A_1 and A_{2A} receptors for adenosine, which appears to be due to protonation of conserved histidine residues in the ligand binding sites of the receptors (Allende *et al.*, 1993; Askalan & Richardson, 1994). Indeed, Askalan (1994) has shown that the vasorelaxant potencies of adenosine and the A_2 adenosine receptor agonist, 2-phenylaminoadenosine (CV 1808) are increased in the isolated aorta of the guinea-pig when the pH of the bathing solution is reduced.

The purpose of this study was to use the rat mesenteric vascular bed to see if the pH of the perfusing fluid might also regulate the actions of adenosine in this region of the vasculature. The identity of the adenosine receptors regulating mesenteric vascular resistance was first determined and then the effects of decreasing pH, from 7.4 to 6.8, on the function of these receptors were investigated. In order to determine if the observed effects of the decreased pH were specific to adenosine, they were compared with the effects of changing pH on the responses to carbachol, an endothelium-dependent dilator, sodium nitroprusside (SNP), an endothelium-independent nitrovasodilator, and forskolin, an activator of adenylyl cyclase. A preliminary account of this work was presented at the Fifth International Symposium on Adenosine and Adenine Nucleotides (Richardson *et al.*, 1994).

Methods

Isolated, perfused, superior mesenteric arterial bed of the rat

Adult male Wistar rats (250-400 g; Tucks Ltd, Rayleigh, Essex) were given a single i.p. injection of sodium pentobarbitone (60 mg kg^{-1}: Sagatal; RMB Animal Health Ltd, Dagenham) and heparin (1000 u kg^{-1}; Paines & Byrne,

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Greenford, Middlesex). The abdomen was opened and the superior mesenteric artery cleaned of adherent tissue before cannulation with polythene tubing (i.d. 0.58 mm, o.d. 0.96 mm; Portex, Hythe, Kent) to allow perfusion of the mesenteric arterial bed essentially as described by McGregor (1965) using a Harvard type 1203A peristaltic pump. The bed was perfused at 37 ± 1°C and 4 ml min^{-1} with oxygenated (95% O_2:5% CO_2) Krebs-Henseleit solution of the following composition (mM): NaCl 118, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25.0, $CaCl_2$ 2.5 and D-glucose 5.5. The perfused mesentery was separated from the gastrointestinal tract, placed on a Perspex block (maintained at 37°C) and covered with Nescofilm (Nippon Shoji Kaishi Ltd, Osaka, Japan). When examining the effects of perfusion at pH 6.8, the concentration of $NaHCO_3$ in the perfusion fluid was reduced to 5 mM, isotonicity being maintained by increasing the concentration of NaCl. Preparations used at the lower pH were equilibrated for 10 min at pH 7.4 before changing to pH 6.8.

A T-piece was placed in the perfusion circuit near the point of insertion of the cannula into the mesenteric artery to allow the perfusion pressure to be measured by a Bell & Howell type 4-422-0001 pressure transducer coupled to a Grass model 79D polygraph. The pressure was set to zero at zero flow so that, since the flow rate was kept constant, the recorded changes in perfusion pressure were directly proportional to changes in vascular resistance.

All preparations were tested for the presence of a functional endothelium by showing that 0.1 μg acetylcholine opposed the vasoconstriction induced by 10 μg noradrenaline (Randall *et al.*, 1989). When required, the endothelium was destroyed by perfusion of the preparation for 120 s with a 0.3% (w/v) solution of CHAPS (3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulphonate; Sigma Chemical Co., Poole, Dorset). Functional destruction of the endothelium was confirmed by showing that acetylcholine no longer opposed the vasoconstriction caused by noradrenaline.

In order to show vasorelaxation, tone was induced by infusion of methoxamine into the preparation to give a final concentration of 100 μM in the perfusate; this concentration was reduced to 10 μM in those experiments in which L-NAME was also present in the perfusate in order to maintain similar increases in perfusion pressure. The mean increases in perfusion pressure given by methoxamine were: 41.2 ± 2.6 mmHg (n = 60) at pH 7.4 in the absence of L-NAME and without CHAPS pretreatment; 49.0 ± 5.2 mmHg (n = 14) at pH 7.4 in the presence of L-NAME; 37.2 ± 6.0 mmHg (n = 8) at pH 7.4 after CHAPS pretreatment; and 37.2 ± 3.4 mmHg (n = 46) at pH 6.8 in the absence of L-NAME and without CHAPS pretreatment. Adenosine and other agonists were given as bolus injections into the perfusion circuit in volumes of 30 μl or less, except for the highest doses given which were in volumes of 100 μl; none of these volumes of the vehicle had any vascular effects. Antagonists were perfused through the preparation for 15 min before determination of responses to an agonist.

Data analysis

The vasodilator responses to adenosine and other agonists are expressed as a percentage relaxation of the methoxamine-induced tone. Values are given as the mean ± s.e.mean. The values of n show the number of preparations in which the individual doses used to construct a dose-response curve were examined. Statistical analyses were made with Student's paired or unpaired t-test (InStat version 2.0, GraphPad Software, San Diego, CA, U.S.A.) of by analysis of variance (StatView 4.0, Abacus Concepts, Berkeley, CA, U.S.A.) as indicated in the text. P values < 0.05 were considered to be significant.

Drugs

All solutions were made up freshly on the day of the experiment. Adenosine, sodium nitroprusside (SNP), acetylcholine, noradrenaline, methoxamine, N^G-nitro-L-arginine methyl ester (L-NAME), carbachol (all from Sigma), and 5'-N-ethylcarboxamidoadenosine (NECA; Research Biochemicals International, Natick, MA, U.S.A.) were dissolved in distilled water; SNP solutions were protected from light before use. 8-Phenyltheophylline (8-PT; Sigma) was dissolved in 10 mM NaOH and then diluted in Krebs-Henseleit solution to give a final concentration of 3 μM. 8-(3-Chlorostyryl)caffeine (CSC;RBI) and forskolin (Sigma) were dissolved in dimethyl sulphoxide as stock solutions of 0.1 and 10 mM, respectively; CSC solutions were stored in the dark before being diluted in Krebs-Henseleit solution for use at a final concentration of 500 nM. 2-Chloro-N^6-cyclopentyladenosine (CCPA), 1,3-dipropyl-8-cyclopentyl-xanthine (DPCPX) and 2-*p*-(-2-car-boxyethyl)-phenethylamino-5'-N-ethylcarboxamidoadenosine (CGS 21680; all from RBI) were dissolved in ethanol to give solutions of 1 (CCPA and CGS 21680) or 10 mM (DPCPX) which were then diluted to give the desired final concentrations in Krebs-Henseleit solution.

Results

Adenosine caused dose-related decreases in the tone of the arterial bed established by 100 μM methoxamine, but the solubility limitations of the nucleotide were such that a clearly defined maximal response could not be reached (Figure 1). The vasodilatation caused by adenosine was attenuated by destruction of the endothelium with CHAPS, such that pretreatment of the mesentery with this zwitterionic detergent reduced the potency of adenosine by up to 10 fold. On the other hand, inhibition of nitric oxide synthesis with L-NAME in endothelium-intact preparations did not reduce the relaxations to adenosine, but rather enhanced them by approximately 4 fold (Figure 1).

Figure 2 shows that the non-selective adenosine receptor agonist, NECA, the A_{2A} receptor-selective agonist, CGS 21680, and the A_1 receptor agonist, CCPA, also caused dose-related reductions in mesenteric vascular resistance. All these agonists caused greater degrees of relaxation than adenosine over the available dose-ranges which were again restricted by solubility limitations. It can be seen that NECA was the most potent of the agents used, being approximately 3 times more potent than CGS 21680, which was in turn 3 times more potent

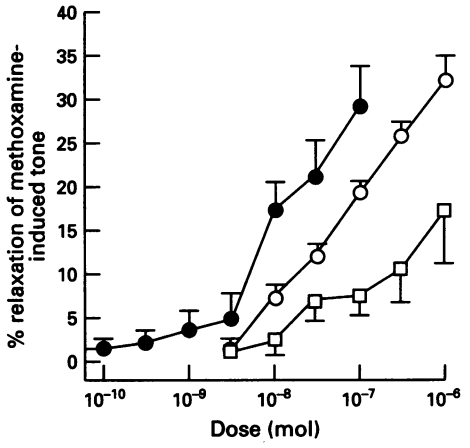

Figure 1. Adenosine-mediated vasodilatation of the superior mesenteric arterial bed of the rat preconstricted with 100 μM methoxamine. The results are shown as the percentage reduction of the methoxamine-induced tone established by adenosine (○, n=4), adenosine in the presence of 100 μM L-NAME (●, n=6), and adenosine after the removal of the endothelium by perfusion of the preparation with 0.3% CHAPS (□, n=8). The symbols show the means with s.e.mean, from the number of preparations given in parentheses.

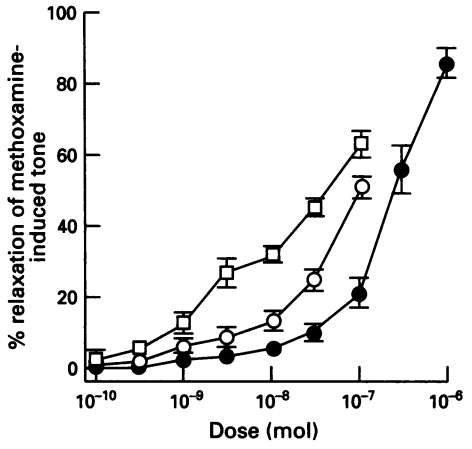

Figure 2. Adenosine receptor agonist-mediated vasodilatation of the superior mesenteric arterial bed. The results are shown as the percentage reductions in the vascular tone, established by infusion of 100 μM methoxamine, given by NECA (□, $n=6$), CGS 21680 (○, $n=7-8$), and CCPA (●, $n=6$). The symbols show the means with s.e.mean from the number of preparations given in parentheses.

than CCPA. CCPA and adenosine were equipotent at the lower doses used but above 100 nmol, CCPA gave greater responses than the parent nucleoside.

Figure 3a shows that the relaxations induced by adenosine were inhibited by 3 μM 8-PT ($P<0.01$ by analysis of variance), showing that the receptor mediating the response is xanthine-sensitive, although the selective A_1 receptor antagonist, DPCPX (10 nM) had no significant effect on the responses (Figure 3b; $P>0.05$, analysis of variance). It may also be seen from Figure 3a that the responses to adenosine were antagonized by CSC (500 nM), a selective antagonist at the A_{2A} receptor ($P<0.01$, analysis of variance).

Reducing the pH of the perfusate to 6.8 significantly ($P<0.01$, analysis of variance) enhanced the dilator responses to adenosine (Figure 3b) by 10 fold and these responses were still insensitive to DPCPX (Figure 3b). Indeed, in the presence of DPCPX the decrease in pH resulted in a similar increase in potency for adenosine to that seen in the absence of the antagonist. It is interesting to note that the increased effectiveness of adenosine as a vasodilator at the lower pH was reflected in a greater response being seen at the highest dose that could be used ($P<0.01$; Student's unpaired t test).

Figure 4 shows that CGS 21680 also gave greater responses at this reduced pH ($P<0.05$; analysis of variance). As with adenosine, there was a parallel shift of the log dose-response curve to the left but the magnitude of the shift (representing a doubling of potency) was less than that seen with adenosine.

In contrast, the vasodilatation induced by the cholinoceptor agonist, carbachol, was unaffected by a change in pH (Figure 5a), though the responses to the nitrovasodilator SNP were considerably potentiated at pH 6.8 (Figure 5b; $P<0.001$, analysis of variance). The shift to the left of the log dose-response curve for SNP was not parallel since that obtained at the lower pH was steeper than that seen at pH 7.4; the approximate increases in potency seen ranged up to 50 fold at responses representing 50% or more reduction of the methoxamine-induced tone. It should be noted that carbachol was more potent than any of the adenosine agonists and a full log dose-response curve was obtained at both pH values. At both pH 7.4 and 6.8 the maximal reversal of methoxamine-induced tone observed with the cholinoceptor agonist was greater than 80% which suggests that changing the pH alone does not change the maximal responsiveness of the vasculature to vasodilators. The maximum response to SNP was seen at the lower pH and was 69.9±0.4%, though a true maximum could not be defined at pH 7.4. Finally, Figure 5c shows that the reduction of the pH of the perfusate to 6.8 significantly diminished the responses to forskolin ($P<0.01$, analysis of variance) such that, although complete reversal of the methoxamine-induced tone was obtained at pH 7.4 with a dose of 100 nmol, the relaxation at this same dose at the lower pH was reduced by 40%.

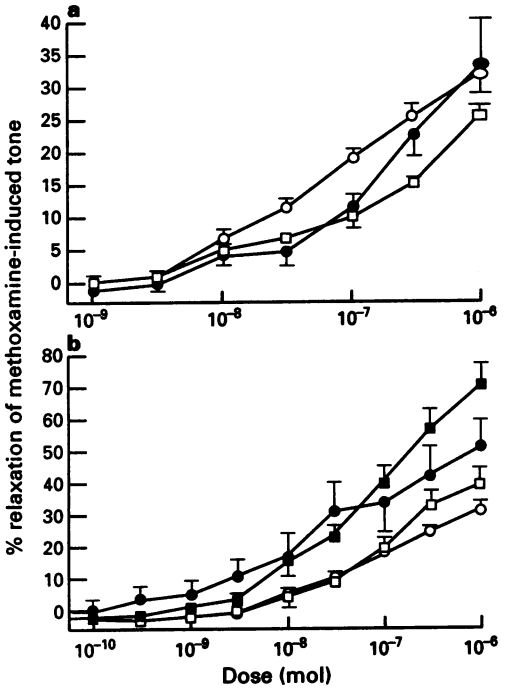

Figure 3. The effects of antagonists, and of reducing perfusate pH to 6.8, on adenosine-mediated vasdilatation of the superior mesenteric arterial bed of the rat. Responses are shown as a percentage reduction in the methoxamine-induced tone. Panel (a) shows the responses to adenosine (○, $n=4$) and adenosine in the presence of either 3 μM 8-PT (●, $n=6$) or 500 nM CSC (□, $n=6$) at pH 7.4. Panel (b) shows the responses obtained to adenosine at pH 7.4 (□, $n=6$; ○, $n=4$) and pH 6.8 (■, $n=4-6$; ●, $n=4$) in the presence (□,■) and absence (○,●) of 10 nM DPCPX. The data for adenosine at pH 7.4 in the absence of either antagonist are the same as those shown in Figure 1 and are included here for clarity. The symbols show the means with s.e. mean, from the number of preparations given in parentheses.

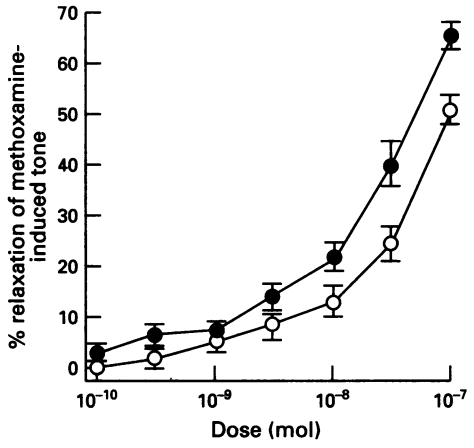

Figure 4. Vasodilator responses to CGS 21680 in the superior mesenteric arterial bed of the rat at pH 7.4 (○, $n=7-8$) and 6.8 (●, $n=6$). Responses, shown as a percentage reduction in the tone induced by 100 μM methoxamine, are given as the means±s.e. mean from the number of preparations shown in parentheses. The data for pH 7.4 are those also shown in Figure 2 and are repeated here for ease of comparison.

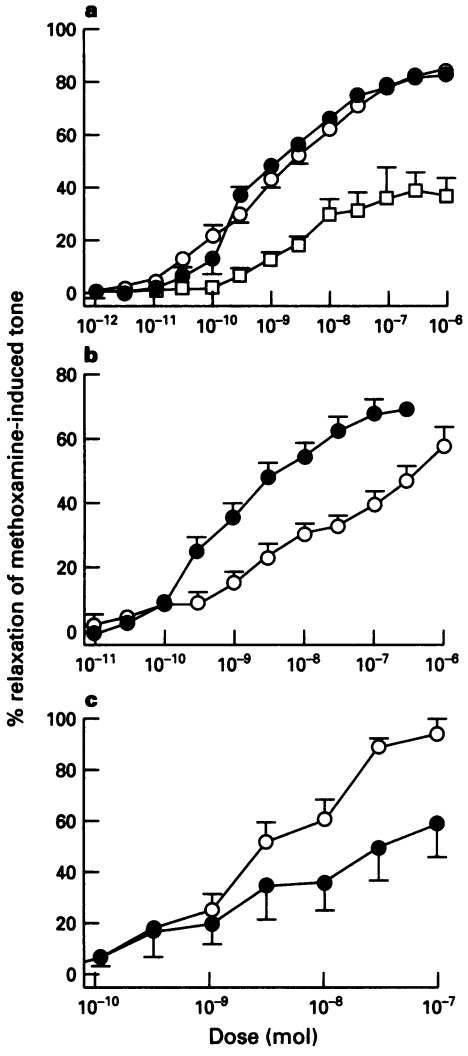

Figure 5. The effect of changing pH of the perfusate on the vasodilator responses to (a) carbachol alone (○,●) and in the presence of L-NAME (□); (b) SNP and (c) forskolin in the superior mesenteric arterial bed of the rat: (○, □) show the responses obtained at pH 7.4 and (●) those recorded at pH 6.8. Responses are shown as a percentage reduction in the tone induced by methoxamine and are given as the mean with s.e. mean, from 6-8 preparations.

With the exception of treatment with CHAPS, the various conditions had only limited effects on basal perfusion pressure in the mesenteric arterial bed. When the preparation was perfused at the lower pH of 6.8, there was a 19.9% lower ($P<0.05$; unpaired t test) basal pressure than at the physiologically normal pH of 7.4 (pH 7.4, mean perfusion pressure $=18.7\pm1.1$ mmHg, $n=60$; pH 6.8, mean perfusion pressure $=15.6\pm0.7$ mmHg, $n=46$). It can be seen from Table 1 that none of the adenosine antagonists significantly affected the basal perfusion pressure in the preparation at pH 7.4, but destruction of the endothelium with CHAPS or inhibition of nitric oxide synthesis with L-NAME did significantly increase basal perfusion pressure, by 56% and 10.5% respectively. However, transient increases in pressure were observed after addition of 8-PT (a mean peak perfusion pressure of 22.1 ± 3.3 mmHg was observed before the pressure declined to the stable value given in the table; $n=6$) and L-NAME (mean peak pressure $=32.9\pm8.0$ mmHg; $n=6$). As at 7.4, DPCPX had no significant effect on basal perfusion pressure at the reduced pH value of 6.8.

Table 1 Effects of antagonists and of destruction of the endothelium with CHAPS on basal perfusion pressure in the perfused superior mesenteric arterial bed of the rat

	Basal perfusion pressure before intervention (mmHg)	*Basal perfusion pressure after intervention* (mmHg)	n
pH 7.4			
Treatment with CHAPS	19.2±1.7	30.0±2.0***	8
8-PT (3 μM)	15.0±1.4	15.4±1.6	6
CSC (500 nM)	12.4±1.6	12.3±2.1	8
DPCPX (10 nM)	17.7±3.0	20.2±3.5	6
L-NAME (100 μM)	15.1±1.1	16.7±1.4*	14
pH 6.8			
DPCPX (10 nM)	15.9±1.4	14.0±1.0	6

Statistically different from the pressure before perfusion of the preparation with L-NAME: *$P<0.05$; Student's paired t test.
Statistically different from the pressure before treatment of the preparation with CHAPS: ***$P<0.001$: Student's paired t test.

Discussion

The present results show that adenosine relaxes the preconstricted superior mesenteric arterial bed of the rat, but the lack of effect of adenosine antagonists on basal perfusion pressure suggest that endogenous adenosine has no significant role in maintaining vascular tone in the vessels within the preparation. Thus investigations of the effects of exogenous adenosine are likely to be unaffected by the presence of considerable amounts of endogenous nucleoside.

Previous studies in mesenteric arterial rings showed that the adenosine response was partly inhibited by removal of the vascular endothelium (Vuorinen *et al.*, 1992). Here also, a reduction in the potency of adenosine on removal of the endothelium was seen, although, being up to 30 fold, it was considerably more than the 3 fold reduction observed with the isolated arterial rings (Vuorinen *et al.*, 1992). However, it seems unlikely that adenosine acts by inducing the release of nitric oxide from the endothelium since the nitric oxide synthase inhibitor, L-NAME, potentiated the responses to adenosine (Figure 1) but inhibited the responses to the endothelium-dependent vasodilator, carbachol (Figure 5a). Therefore it seems unlikely that insufficient L-NAME was used to inhibit nitric oxide production. Ralevic & Burnstock (1991) showed that a lower concentration (30 μM) than used here inhibited relaxation to acetylcholine in this preparation. In addition, 100 μM L-NAME (the same concentration as that used in the present study), potentiated the contractile responses to endothelin-1 (as did removal of the endothelium) in this preparation in a manner reversible by excess L-arginine, but not D-arginine (Douglas *et al.*, 1991).

Thus it seems unlikely that the discrepancy between the effects of endothelium removal and of addition of L-NAME on the responses to adenosine can be explained in terms of failure to inhibit nitric oxide synthase and it seems that destruction of the endothelium either by perfusion with CHAPS as used here, or by rubbing (as used in the isolated mesenteric arterial ring by Vuorinen *et al.*, 1992), does not inhibit the relaxation in response to adenosine by preventing release of nitric oxide. An alternative mechanism for endothelium-dependent relaxation is the release of endothelium-derived hyperpolarizing factor (EDHF). Adenosine releases EDHF from porcine coronary artery endothelial cells *in situ* (Olanrewaju *et al.*, 1995) and it has been suggested that EDHF is a significant mediator of small arterial relaxation to muscarinic agonists and histamine in the perfused mesenteric bed (Adeagbo & Triggle, 1993;

Garland *et al.*, 1994). This hypothesis is supported by our observation of incomplete inhibition by L-NAME of the responses to carbachol. Hence, it is possible that the decrease in adenosine-mediated vasorelaxation seen after removal of the endothelium from the mesenteric artery or arterial bed is due to removal of the source EDHF.

However, the endothelial dependence of the responses of the rat perfused mesenteric bed to adenosine remains equivocal as Rubino *et al.* (1995) very recently reported that L-NAME (30 μM) had no significant effect on the responses to 10 nmol adenosine or 300 nmol 2-chloroadenosine. Some of the discrepancies in these investigations of the role of the endothelium could be due to the need to increase perfusion pressure of this preparation in order to be able to observe vasodilatation. The perfusion pressures in the study of Rubino *et al.* (1995) were much higher (basal, 40.6±2.9 mmHg; increase with methoxamine, 80.2±3.7, $n=12$) than those observed here and they used a higher perfusion rate (5 ml min^{-1}). Thus the flow conditions in the beds in the two studies might produce sufficiently different shear and myogenic tone to modify the apparent role of the endothelium in the responses to adenosine. In this respect it should be noted that although the basal perfusion pressure in our study was increased after removal of the endothelium with CHAPS, the perfusion pressures in the presence of methoxamine in preparations with and without endothelium, and in presence of L-NAME, were not significantly different. Thus, within the present study the difference between the effects of removing the endothelium and inhibition of nitric oxide synthase cannot be explained by differences in basal tone.

In the present study, the identity of the receptor mediating the response to adenosine was investigated by the use of three other agonists at adenosine receptors. NECA, CGS 21680 and CCPA, and the antagonists 8-PT, CSC and DPCPX. The non-selective adenosine receptor antagonist, 8-PT reduced the responses to adenosine, most markedly at lower doses, which shows that the receptor, or receptors, involved are xanthine-sensitive and therefore not of the A_3 subtype which is known to be relatively insensitive to this class of antagonists (Fredholm *et al.*, 1994; van Galen *et al.*, 1994). In addition, the insensitivity of the response to DPCPX suggests that the A_1 receptor was not involved. Since the A_{2A} selective agonist CGS 21680 (140 fold selective over the A_1 receptor, Jarvis *et al.*, 1989) caused relaxation of this preparation it would appear that the adenosine A_{2A} receptor mediated the observed relaxation. This is consistent with the similar potency of NECA and CGS 21680 (Fredholm *et al.*, 1994) since, at the A_{2B} receptor in the guinea-pig aorta, NECA is at least 1000 times more potent than CGS 21680 whereas it is almost equipotent at the coronary artery A_{2A} receptor in the same species (Martin, 1992; Alexander *et al.*, 1994). CGS 21680 also has low potency at A_{2B} receptors in guinea-pig cerebral cortex (Alexander *et al.*, 1994) and in *Xenopus* oocytes (Yakel *et al.*, 1993). Hence, the observation that CGS 21680 caused relaxation of the mesenteric bed in the rat suggests that the dilator receptor is of the A_{2A} subtype.

This contrasts with the observations of Rubino *et al.* (1995) who reported no response to CGS 21680 in the mesenteric bed at doses up to 1 μmol and concluded that the P_1-purinoceptor mediating the vasodilator responses to adenosine was of the A_{2B} subtype. There is no obvious explanation for this discrepancy as both groups purchased the compound from the same source. Different solvents were used to make the stock solution (ethanol in the present study, 50% DMSO in the other investigation) but, as CGS 21680 is not known to be particularly unstable in solution, this does not seem likely to be the cause of the differences. In contrast to the study of Rubino *et al.* (1995), the presence of an A_{2A} receptor in the rat mesenteric bed was confirmed in our investigation by the use of CSC to antagonize the responses to adenosine. This compound distinguishes between A_{2A} and A_{2B} receptors in embryonic chick heart cells (Liang & Haltiwanger, 1995) and the approximate 4–6 fold shift (Figure 3a) in the adenosine log dose-response curve found here is close to that which would be expected from the affinity of this antagonist ($K_b=60$nM; Jacobson *et al.*, 1993) at the A_{2A} receptor in rat PC12 cells.

However, CCPA, which is considered to be a selective A_1 receptor agonist, was only 3 fold less potent than CGS 21680. Although this could be interpreted as showing an involvement of A_1 receptors in the vasodilator response to adenosine, the A_1 receptor-selective antagonist, DPCPX, had no antagonist effect on adenosine-mediated dilatation (Figure 3b). The concentration of antagonist used (10 nM) should antagonize A_1 receptor-mediated effects as it gave a dose-ratio of 25 when used to antagonize the effects of CCPA on the rate of beating of rat atria (Monopoli *et al.*, 1994). Furthermore, in a study of induction of cyclic AMP accumulation in guinea-pig cultured coronary artery endothelial cells, the potency of CGS 21680 relative to that of CCPA at this A_{2A} receptor was only 12 fold (Schiele & Schwabe, 1994) which suggests that, despite the high selectivity of CCPA and CGS 21680 in binding studies (CCPA being 10,000 fold selective for the A_1 receptor over the A_{2A}; Lohse *et al.*, 1988), their potency ratio is not as marked in functional studies. It therefore seems reasonable to conclude from the present study that adenosine mediates relaxation in the rat superior mesenteric arterial bed by acting through 8-PT- and CSC-sensitive, but DPCPX-insensitive, A_{2A} receptors.

At the reduced pH of 6.8, the relaxant responses to adenosine were probably also mediated by A_{2A} receptors since they were unaffected by DPCPX, and CGS 21680 caused a reduction in perfusion pressure. It is also apparent from Figures 3 and 4 that the reduction in pH significantly potentiated the relaxations to adenosine and CGS 21680, relative to those obtained at the higher, and physiologically normal, pH of 7.4. There are a number of possible explanations for this effect, including changes in agonist affinity, in the coupling of the adenosine receptor to its effector, or even a more general change in the dilator capacity of the arterial bed. Also, alterations of effector function or adenosine uptake and metabolism could be influential. However, since both adenosine and CGS 21680 exhibited increases in effectiveness, a change in metabolism cannot account for all of the 10 fold increase in the potency of adenosine. An increase in the effectiveness of the adenylyl cyclase system could not account for increased responses to adenosine agonists as the responses to forskolin were less at the lower pH than at pH 7.4. In addition, since the responses to the cholinoceptor agonist, carbachol, were not different at the two pH values, nor was complete reversal of methoxamine-induced tone obtained, it is unlikely that the vasodilator capacity itself was potentiated by the reduction in the pH of the perfusate. A change in the ionization state of the agonists is also unlikely since adenosine agonists bearing the same ionizable groups can show significantly different pH sensitivities in binding assays (Askalan & Richardson, 1994). It will be interesting to determine the mechanisms involved in the increased responsiveness to adenosine agonists at reduced pH values.

In contrast to carbachol, the responses to the nitrovasodilator SNP were, like those to adenosine and CGS 21680, potentiated by reducing the pH of the perfusate. This effect might result from increased liberation of nitric oxide from the nitrovasodilator at the lower pH, or perhaps might be due to a pH-sensitive component in the vasodilator action of nitric oxide. No similar pH sensitivity of the action SNP was seen in the dilatation of the guinea-pig aorta (Askalan, 1994), suggesting that increased nitric oxide liberation was not responsible for the increased activity of SNP. If there is an increase in the effectiveness of nitric oxide as a vasodilator, then this was not reflected in an increase in the response to carbachol. However, as discussed above, the response to carbachol is not entirely mediated through nitric oxide in this preparation and thus there might be opposing effects of reduced pH on nitric oxide-mediated vasodilatation and that given by EDHF.

Since the actions of agonists in the perfused mesenteric bed were determined by the use of bolus dosing, rather than by

equilibrium studies, it is possible that the observed changes in response to the adenosine agonists were the result of changes in the dynamics of drug interaction with the tissue rather than simply from the increases in affinity shown in the binding studies of Askalan (1994) and Askalan & Richardson (1994). However, it is unlikely that endogenous agonists like adenosine would act at equilibrium in physiological circumstances, or in the pathophysiological conditions associated with ischaemia. Therefore the use of bolus dosing for agonists would appear to be justified and the present results suggest that potentiation of the vasodilator effects of endogenous adenosine could occur during naturally-occurring reductions in tissue pH and contribute to improving tissue viability.

In conclusion the observation of increased vasodilatation in response to the adenosine agonists is consistent with the hypothesis that H^+ ions potentiate the effects of adenosine, an effect which might be mediated partly by an ionisable group in the ligand binding site of the A_{2A} receptor and, possibly, by other mechanisms including changes in receptor-effector coupling, effector activity and/or adenosine metabolism.

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