The role of adenosine in mediating vasodilatation in mesenteric circulation of the rat in acute and chronic hypoxia

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1. We have compared the roles of adenosine in mediating dilator responses to acute hypoxia in mesenteric microcirculation of control, normoxic (N) rats and in chronically hypoxic (CH) rats kept in an hypoxic chamber at 10% O_2 for 3–4 weeks.

2. In fifteen N rats, acute hypoxia (breathing 6% O_2 for 3 min) induced mean increases in the diameter of arterial vessels of mesentery (whose internal diameter was 10–350 μm) of 8·0 ± 1·9% (mean ± S.E.M.) and of venous vessels (whose internal diameter was 12–360 μm) of 10·4 ± 2·6%. These diameter changes were reduced by ~30% when the adenosine receptor antagonist 8-sulpho-phenyltheophylline (8-SPT, 10^{-3} M) was applied topically to the mesentery.

3. In a further six N rats, topical application of graded concentrations of adenosine (10^{-7}–10^{-3} M) to the mesentery evoked graded increases in the diameter of all arterial and venous vessels, maximum increases with 10^{-3} M being 12·5 ± 3·3 and 8·4 ± 4·3%, respectively; these responses were abolished by 8-SPT.

4. By contrast, in fourteen CH rats, the smaller change in inspirate from 10 to 8% O_2 induced increases in diameter of arterial and venous vessels which had control diameters that were comparable to those of N rats, of 14·1 ± 2·4 and 12·9 ± 2·7%, respectively, and which were virtually equivalent to the responses induced by topical application of 10^{-3} M adenosine (13·3 ± 1·3 and 16·3 ± 2·0% in arterial and venous vessels, respectively). The changes induced by acute hypoxia were abolished by 8-SPT, as were those induced by adenosine.

5. These results suggest that in the intestinal mesentery, where the blood vessels have negligible tissue parenchyma around them, locally released or synthesized adenosine makes a substantial contribution to the dilatation that is evoked in arteriolar vessels by acute hypoxia and to the active dilatation, or passive distension of the venous vessels. The results also suggest that this contribution is accentuated in chronic hypoxia either by greater release of adenosine or greater vascular sensitivity to it.

By using the techniques of intravital microscopy on the anaesthetized rat we have recently provided evidence that during acute systemic hypoxia, the arteriolar and venous vessels of the intestinal mesentery are affected by a local vasodilator influence (Langdown & Marshall, 1995). Since the vessels of the mesentery have negligible tissue parenchyma around them, it seems this dilator influence must reflect either the direct action of hypoxia upon the vascular smooth muscle, or the action of a vasodilator substance that is released from the structures of the blood vessel walls, namely vascular endothelium or vascular smooth muscle, or from structures that are very close to them, for example nerve fibres.

A series of studies upon skeletal muscle vasculature in the rat have demonstrated that the muscle vasodilatation induced by acute systemic hypoxia is largely mediated by locally released adenosine (Neylon & Marshall, 1991; Mian & Marshall, 1991*b*; Marshall, Thomas & Turner, 1993; Thomas, Elnazir & Marshall, 1994). We have proposed that adenosine is released by the skeletal muscle fibres and that it stimulates adenosine receptors on those muscle fibres to activate ATP-sensitive K^+ channels and cause the release of K^+ which then acts as a vasodilator (Marshall *et al.* 1993). However, our direct observations on muscle microcirculation indicate that adenosine is also released from the blood vessel walls themselves, or from very close by and that this acts more directly on the arterial and venous vessels to produce dilatation (Mian & Marshall, 1991*b*). In skeletal muscle, 5′ nucleotidase, the enzyme that synthesizes adenosine from AMP, has been visualized within the blood vessel walls and closely adjacent to the blood vessels, the highest density being at the level of the terminal vessels (Rubio, Berne & Dobson, 1978). Experiments on pig aorta have indicated that hypoxia can

cause vascular smooth muscle to synthesize and release adenosine (Van Harn, Rubio & Berne, 1977). Moreover, it is known that adenosine can be rapidly taken up and released by the vascular endothelium (Olsson & Pearson, 1990) and in the heart at least, the release of adenosine from the endothelium can be stimulated by hypoxia (Deussen, Moser & Schrader, 1986). Thus, there is the possibility that the local vasodilator influence we have identified in mesenteric microcirculation in acute hypoxia is mediated by adenosine.

In respiratory and cardiovascular disorders and at high altitude, hypoxia occurs on a chronic time scale. Very little is known of the effects of chronic hypoxia upon the cardiovascular system. However, there is evidence that reflex vasoconstriction is impaired in patients who are chronically hypoxic (Heistad & Abboud, 1988) and that vascular responses to α-adrenoreceptor stimulation are reduced in chronically hypoxic rats (Doyle & Walker, 1991; Mian & Marshall, 1994). This might be explained if the actions of adenosine and other local dilator influences of adenosine on blood vessel walls are enhanced in chronic hypoxia and counteract the influence of noradrenaline. The mesenteric circulation offers the opportunity of testing this possibility without the complicating influence of substances that might be released from surrounding tissue parenchyma.

Thus, the aims of the present study were to establish whether the local vasodilator effect of acute hypoxia on mesenteric microcirculation is mediated by adenosine and to begin a study on the effects of chronic hypoxia upon the cardiovascular system by testing whether in this condition the local vasodilator influence of hypoxia, specifically that of adenosine, is enhanced.

METHODS

Experiments were performed on two groups of male Wistar rats: Group 1 were reared in air and served as control, normoxic (N) animals; Group 2 were made hypoxic in an hypoxic chamber for 3–5 weeks prior to the acute experiment and served as chronically hypoxic (CH) animals. Group 1 rats were housed in standard rat cages that were placed on top of the hypoxic chamber. The rats weighed 250–300 g at the time of the acute experiment. Group 2 rats were housed in standard rat cages within a chamber in which the O_2 concentration was maintained at 10% O_2 by a servo-controlled system. The chamber and its control system have been described in detail before (Thomas & Marshall, 1995). The gas in the system was continuously circulated by a pump, air or N_2 being added as required. Humidity was controlled by a condensation unit and silica gel. Carbon dioxide was removed by sodium hydroxide and other contaminants were removed by a molecular sieve. The chamber was open to the atmosphere for approximately 20 min every 3 days to allow cleaning of the cages and food and water to be replenished. The chronically hypoxic rats weighed 250–350 g at the time of the acute experiment.

Both Group 1 and Group 2 rats were anaesthetized as described before (Marshall & Metcalfe, 1988): anaesthesia was induced with a nitrous oxide–oxygen mixture (2 : 3) containing halothane (2–3%) and maintained by continuous infusion of Saffan (Pitman-Moore, Uxbridge, UK; 7–12 mg kg^{-1} h^{-1} I.V.). The trachea was cannulated and from then on Group 1 (N) rats routinely breathed air, either directly, or delivered via an air pump through tubing connected at right angles to the side-arm of the tracheal cannula. Group 2 (CH) rats routinely breathed 10% O_2, delivered in the same manner from a gas bag. The femoral artery was cannulated to allow continuous recording of arterial pressure and heart rate as described before and a brachial artery was cannulated to allow 130 ml samples to be removed for blood gas analysis (see Mian & Marshall, 1991*a*). These samples and the hypoxic gas mixtures delivered to the animals (see below) were analysed using a Nova Stat Profile 3 Analyser (V. A. Howe, Waltham, MA, USA).

The animal was then transferred to the modified stage of a microscope. A small loop of intestine was gently pulled out of the abdominal cavity via a mid-line incision and arranged for transillumination of the mesentery. The mesenteric microcirculation was viewed on-line on a video-monitor and recorded on a video-recorder so that analysis of vessel diameter could be made off-line (Mian & Marshall, 1991*a*).

A field of view was chosen for observation which ideally contained at least three or four different categories of arterial and/or venous vessels (see Results); this field of view was maintained throughout the experiment. During preparation the mesentery was superfused with a modified Krebs solution (Mian & Marshall, 1991*a*). When the field of view had been selected and until the end of the experiment the mesentery and the attached loop of intestine was covered with Saran Wrap (Dow Chemical Company, Indianapolis, IN, USA), which has negligible permeability to O_2 and CO_2, Langdown & Marshall, 1995), except when it was briefly removed to allow topical application of a drug (see below). Both Group 1 and Group 2 rats were allowed an equilibration period of 30 min before the protocol began. During this period, the level of anaesthesia was stabilized such that pinching of the paw evoked a slight, or no withdrawal reflex, with little or no accompanying change in arterial pressure; there was no sign of the autonomic components of the defence response (see Marshall & Metcalfe, 1988; Mian & Marshall, 1991*a*; Langdown & Marshall, 1995). This level of anaesthesia was deeper than that used in our recent study on mesenteric microcirculation (Langdown & Marshall, 1995) and would have enhanced the local vasodilator influences of hypoxia and depressed the neurally mediated reflex responses (Marshall & Metcalfe, 1988; see Discussion).

In fifteen rats of Group 1, the diameters of individual mesenteric vessels were measured during air breathing and during two 3 min periods of breathing 6% O_2 before and after topical application to the mesentery of the adenosine receptor antagonist 8-sulphophenytheophylline (8-SPT, 10^{-3} M in Krebs solution). The mean of three measurements made at the beginning of the protocol when breathing air served as the control value. Further measurements were made at 1, 2 and 3 min of each 3 min period of breathing 6% O_2, the maximum change being used for analysis (Mian & Marshall, 1991*a*). Measurements of arterial pressure and heart rate for analysis were taken during air breathing and at the second minute of breathing 6% O_2. Samples for blood gas analysis were taken during air breathing and at the end of the second minute of breathing 6% O_2.

A further six rats of Group 1 breathed air throughout and responses evoked in the mesenteric vessels by topical application of 10^{-7}, 10^{-5} and 10^{-3} M adenosine were tested before and after

Table 1. Values of arterial blood gases and pH (pH_a) recorded in Group 1 rats during air breathing and when breathing 6% O_2 before and after topical application of 8-SPT to the mesentery

	P_{a,O_2} (mmHg)	P_{a,CO_2} (mmHg)	pH_a
Before 8-SPT			
Air	92·4 ± 1·6	42·9 ± 1·4	7·22 ± 0·01
6% O_2	34·4 ± 2·4*	44·2 ± 2·9	7·19 ± 0·01
After 8-SPT			
Air	74·2 ± 16·9	43·7 ± 1·4	7·34 ± 0·10
6% O_2	34·3 ± 2·4*	43·8 ± 2·8	7·19 ± 0·02

* Significant difference between values recorded in air and 6% O_2 ($P < 0·05$).

topical application of 8-SPT (10^{-3} M). The peak of the response to adenosine was taken for analysis.

In fourteen CH rats of Group 2 which routinely breathed 10% O_2, tests were made of responses evoked when the inspirate was changed to air for 3 min, to 8% O_2 for 3 min and to topical application of adenosine (10^{-3} M) before and after topical application of 8-SPT. Measurements of arterial pressure, heart rate and vessel diameter and blood gas analyses were made as described above for Group 1. In each of these rats a 90 ml sample of arterial blood was taken at the beginning of the experiment for measurement of haematocrit.

Statistical analyses

All results are expressed as means ± S.E.M. Changes in vessel diameter are expressed as a percentage of the control diameter when breathing air (Group 1) or 10% O_2 (Group 2). In each group, comparisons were made of responses evoked before and after 8-SPT by using Student's paired t test. Comparisons between Groups 1 and 2 were made using Student's unpaired t test. $P < 0·05$ was considered significant.

RESULTS

The control values of arterial blood gases, and those recorded when the inspirate was changed before and after 8-SPT are shown in Table 1 (Group 1) and Table 2 (Group 2).

Group 1 (normoxic)

By the second minute of the first 3 min period of breathing 6% O_2, arterial O_2 pressure (P_{a,O_2}) had fallen substantially, but there was little change in P_{a,CO_2} or arterial pH (Table 1). Arterial pressure fell gradually during hypoxia (from 125 ± 4·5 to 79 ± 6·9 mmHg, $P < 0·05$) reaching its lowest value at approximately the second minute of hypoxia, while heart rate tended to increase (from 412 ± 15 to 422 ± 7·3 beats min^{-1}) as described before (e.g. Mian & Marshall, 1991*a*).

The mesenteric vessels were divided into seven categories on the basis of their internal diameters and anatomical position (Furness & Marshall, 1974). Briefly, the principal arteries (80–350 μm internal diameter) are the arterial vessels that branch from the mesenteric artery and radiate towards the intestine. The small arteries (30–40 μm) branch from the principal arteries into the connective tissue of the mesentery. They supply the terminal arterioles (18–30 μm) which in turn lead into the precapillary arterioles (10–18 μm). The capillaries branch predominantly from the precapillary arterioles but also from the terminal arterioles. The capillaries drain into collecting venules (12–30 μm) which in turn drain into the small veins (10–50 μm). These are the vessels that drain

Table 2. Values of arterial blood gases and pH (pH_a) recorded in Group 2 rats when breathing 10% O_2, air or 8% O_2 before and after topical application of 8-SPT to the mesentery

	P_{a,O_2} (mmHg)	P_{a,CO_2} (mmHg)	pH_a
Before 8-SPT			
10% O_2	52·4 ± 3·5	41·7 ± 3·1	7·17 ± 0·02
Air	86·9 ± 7·8*	42·4 ± 3·8	7·12 ± 0·02
8% O_2	42·3 ± 3·9*	41·8 ± 4·3	7·13 ± 0·03
After 8-SPT			
10% O_2	51·5 ± 3·9*	41·7 ± 3·6	7·18 ± 0·02
Air	89·6 ± 4·5*	43·5 ± 3·3	7·14 ± 0·02
8% O_2	43·4 ± 3·3*	40·1 ± 3·9	7·15 ± 0·03

* Significant difference from values recorded in 10% O_2 ($P < 0·05$).

into the principal veins (100–360 μm) which converge from the intestine and run parallel with the principal arteries.

The first period of breathing 6% O_2 evoked diameter changes in each section of the arterial and venous tree, some vessels showing an increase in diameter and others a decrease, as is indicated by the large error bars in Fig. 1. Overall, more vessels showed an increase in diameter. Thus, of twenty-eight arterial vessels, twenty-three showed an increase in diameter and the remainder showed a decrease, while of twenty-four venous vessels, twenty showed an increase and the remainder a decrease in diameter. In general, the increases in diameter reached a maximum at the second minute of hypoxia when arterial pressure reached its lowest level (see also Mian & Marshall, 1991*a*; Langdown & Marshall, 1995).

Topical application of 8-SPT to the mesentery had no significant effect on the blood gases, arterial pressure and heart rate during air breathing, nor on the values attained during 6% O_2. By 10 min after application of 8-SPT, all vessels used for analysis were at their original control diameter; any that were not were excluded from the study. After 8-SPT, 6% O_2 again induced both increases and decreases in diameter in the mesenteric vessels (Fig. 1). In the small arteries, terminal and precapillary arterioles and in the collecting venules, the mean increases in diameter were smaller after 8-SPT than before, but the differences did not reach statistical significance. However, when the arterial and the venous vessels were grouped according to whether they showed an increase or a decrease in diameter during 6% O_2 before 8-SPT, then it was found that 8-SPT significantly reduced the hypoxia-induced increases in diameter in arterial and venous vessels; 8-SPT had no effect on the hypoxia-induced decreases in diameter (Fig. 1).

In the six rats that breathed air throughout and in which responses to topical application of adenosine were tested, adenosine produced increases in diameter in all sections of the arterial and venous trees (Fig. 2). These responses were graded with the concentration of adenosine. Considered as a percentage of the control diameter, the terminal and precapillary arterioles amongst the arterial vessels showed the largest increases in diameter to each concentration of adenosine, while principal arterioles showed the smallest increases. On the venous side of the circulation, the small veins and collecting venules showed larger mean changes in diameter than the principal veins. After 8-SPT had been

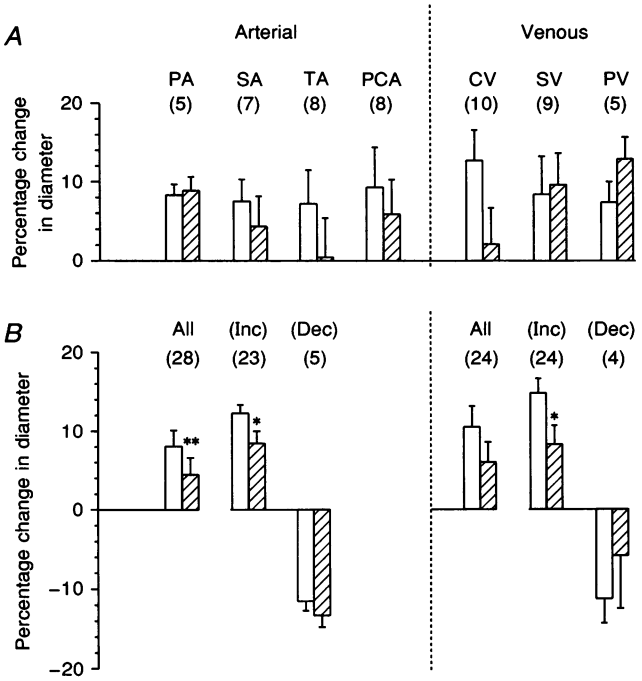

Figure 1. Responses evoked in arterial and venous vessels of mesentery of control rats when the inspirate was changed from air to 6% O_2

The columns represents change in diameter (± S.E.M.) as a percentage of control before (□) and after 8-SPT (▨). *A*, responses evoked in consecutive sections of arterial and venous trees. Abbreviations are: PA, principal arteries; SA, small arteries; TA, terminal arterioles; PCA, precapillary arterioles; CV, collecting venules; SV, small veins; PV, principal veins. *B*, responses evoked in all arterial or all venous vessels grouped together and in those that showed an increase (Inc) or a decrease (Dec) in diameter in response to 6% O_2 before 8-SPT. Numbers in parentheses above columns indicate number of vessels. Significant differences between responses evoked before and after 8-SPT: $*P < 0{\cdot}05$, $**P < 0{\cdot}01$.

topically applied to the mesentery, even the highest concentration of adenosine produced a negligible change in diameter in all sections of the vascular tree.

Group 2 (chronically hypoxic)

As expected, in these chronically hypoxic (CH) animals breathing 10% O_2, P_{a,O_2} was significantly lower than in the normoxic (N) animals of Group 1 when they were breathing air (Table 1 cf. Table 2). Moreover, arterial pressure was significantly lower in CH rats breathing 10% O_2 than in N rats breathing air ($110 \pm 4{\cdot}3$ *vs.* $125 \pm 4{\cdot}5$ mmHg, $P < 0{\cdot}05$). There was no significant difference between the levels of heart rate in CH and N rats when the former were breathing 10% O_2 and the latter air ($440 \pm 4{\cdot}5$ *vs.* 412 ± 15 beats min^{-1}). The haematocrit value in CH rats was $56 \pm 0{\cdot}9$%. The mesenteric vessels chosen for direct observation were selected on the basis of their anatomical position (see above); the control diameters of each vessel type were comparable to those of N rats.

During the 3 min period of breathing 8% O_2, CH rats showed a fall in P_{a,O_2}, but no change in P_{a,CO_2} or arterial pH (Table 2). Meanwhile, arterial pressure showed a gradual fall to $93 \pm 5{\cdot}0$ mmHg ($P < 0{\cdot}05$) and heart rate tended to rise (to $455 \pm 6{\cdot}8$ beats min^{-1}) as in N rats. The levels of P_{a,O_2} and arterial pressure attained in CH rats when breathing 8% O_2 were clearly not as low as in N rats breathing 6% O_2 (Table 2 cf. Table 1). Nevertheless, the hypoxia-induced increases in the diameters of the mesenteric vessels when CH rats breathed 8% O_2 were as great as, if not greater than, those induced when N rats breathed 6% O_2 (Fig. 3 cf. Fig. 1). In particular, when considered as a percentage change from the control diameter, the mean increases in diameter in the terminal and precapillary arterioles of CH rats were about twice as large as those seen in these categories of vessels in N rats. The proportion of the total vessels studied that showed increases in diameter was also greater in CH than in N rats. Thus, of the twenty-two arterial vessels studied in CH rats, twenty showed an increase in diameter and only two showed a decrease, and of the eighteen venous vessels studied in CH rats, sixteen showed an increase and only two a decrease in diameter.

Topical application of 8-SPT to the mesentery had no effect on the changes in blood gases (Table 2), arterial pressure or heart rate that were induced by 8% O_2. However, the hypoxia-induced increases in diameter in the mesenteric arterial and venous vessels were greatly reduced (Fig. 3). When the different categories of vessels were considered separately, then 8-SPT greatly reduced mean increases in diameter in the principal arteries, precapillary arterioles and principal veins, while in the terminal arterioles and small veins, mean increases in diameter were converted to mean decreases. Similar tendencies were apparent for the other vessel types, but the effects did not reach statistical significance. When all the arterial and venous vessels were grouped according to whether they showed an increase or a decrease in diameter during the first period of 8% O_2, then 8-SPT very clearly attenuated the increases in diameter (Fig. 3).

For comparison, it may be noted that topical application of adenosine (10^{-3} M) to the mesentery before 8-SPT produced

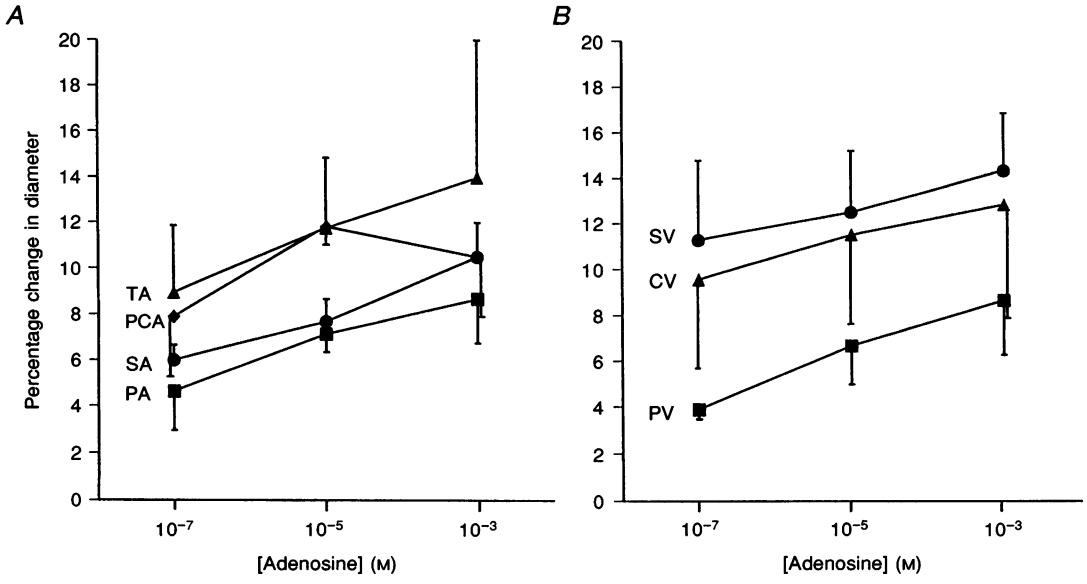

Figure 2. Changes in diameter evoked in arterial and venous vessels of the mesentery of control rats by topical application of graded concentrations of adenosine

A, results from arterial vessels; *B*, results from venous vessels. Each point represents mean (± S.E.M.) percentage change from control diameter. Abbreviations of vessel types as in Fig. 1.

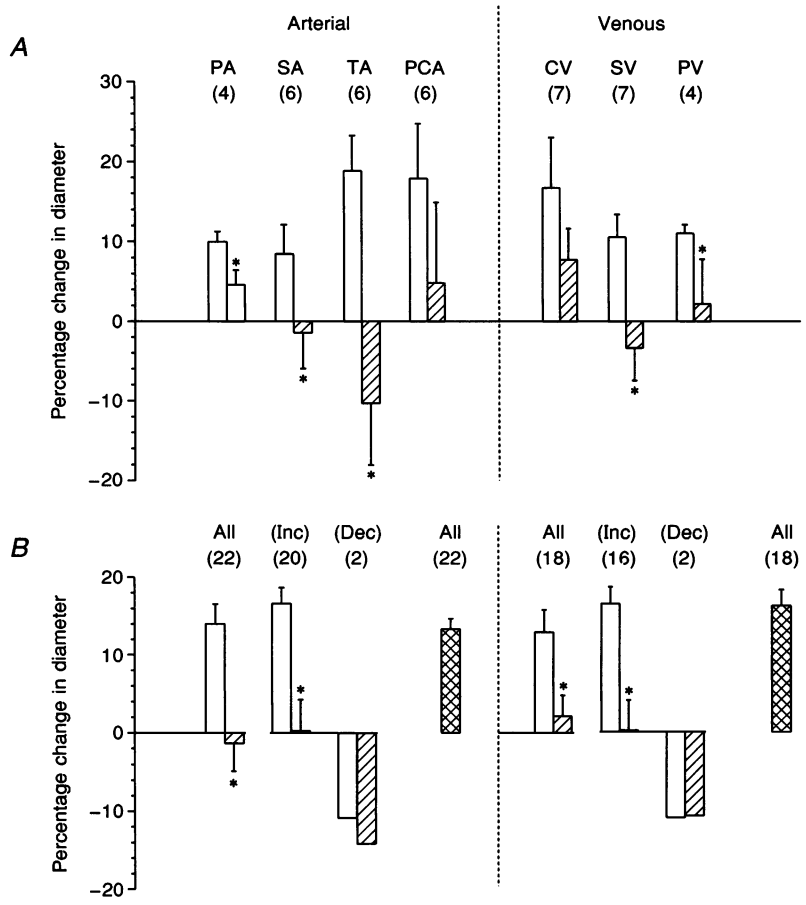

Figure 3. Responses evoked in arterial and venous vessels of the mesentery of chronically hypoxic rats when the inspirate was changed from 10% to 8% O_2

Before (□) and after 8-SPT (▨); ▩, percentage changes (± S.E.M.) in diameter evoked by topical application of adenosine (10^{-3} M) before 8-SPT. Abbreviations as in Fig. 1.

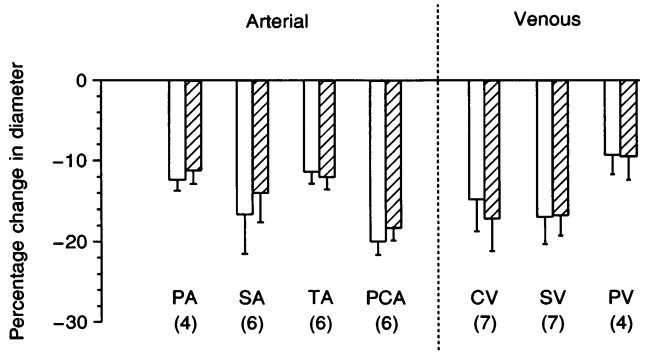

Figure 4. Responses evoked in arterial and venous vessels of the mesentery of chronically hypoxic rats when the inspirate was changed from 10% O_2 to air

Before (□) and after 8-SPT (▨); abbreviations as in Fig. 1.

increases in diameter in each section of the arterial and venous trees that were as large as those induced by 8% O_2 (Fig. 3). Moreover, the mean increases in diameter evoked by adenosine (10^{-3} M) in arterial and venous vessels of CH rats were comparable to those evoked in N rats: 13·3 ± 1·3 and 12·2 ± 3·3% in the arterial vessels of CH and N rats, respectively, and 16·3 ± 2·0 and 12·2 ± 4·3% in the venous vessels of N and CH rats, respectively. The responses evoked by adenosine in CH rats were virtually abolished by 8-SPT as in N rats.

By contrast with the effect of 8% O_2, the 3 min period of air breathing induced a significant rise in arterial pressure to 130 ± 4·0 mmHg ($P < 0·05$), but no change in heart rate, accompanied by the expected increase in P_{a,O_2}; P_{a,CO_2} and arterial pH showed little change (Table 2). Concomitantly, all sections of the arterial and venous trees showed a decrease in diameter (Fig. 4). Topical application of 8-SPT had no effect on the changes in arterial pressure heart rate, or blood gases that were induced by breathing air (Table 2). 8-SPT also had no detectable effect on the changes in vessel diameter induced by air breathing (Fig. 4).

DISCUSSION

The present results suggest that locally released adenosine exerts a substantial dilator influence upon the mesenteric circulation during acute systemic hypoxia and that this influence is even greater in chronic systemic hypoxia.

Acute hypoxia

As expected, when the normoxic animals of Group 1 were given 6% O_2 to breathe, P_{a,O_2} fell substantially and there was a fall in arterial pressure (see Marshall & Metcalfe, 1988; Mian & Marshall, 1991*a*,*b*; Langdown & Marshall, 1995). However, in contrast with our recent study on the mesentery in which the terminal arterioles and some of the precapillary arterioles and small veins consistently showed constriction in response to a similar level of hypoxia (Langdown & Marshall, 1995), these vessels generally showed an increase in diameter, as did all other sections of both the arterial and venous trees. This may be explained by the fact that the level of anaesthesia chosen for the present study was deep, as compared with that of our previous study. Deep anaesthesia would be expected to facilitate expression of the local dilator influences of hypoxia and depress the sympathetically mediated vasoconstrictor influences initiated by peripheral chemoreceptor stimulation and hypoxia of the central nervous system (see Marshall & Metcalfe, 1988). The fact that there was no fall in P_{a,CO_2} at the third minute of hypoxia in the present study also indicates that the level of anaesthesia was sufficiently deep to depress the respiratory response to stimulation of peripheral chemoreceptors (cf. Marshall & Metcalfe, 1988; Langdown & Marshall, 1995).

In our recent study on the mesentery, the hypoxia-induced vasoconstrictor responses in terminal and precapillary arterioles were attenuated or converted to dilator responses by local blockade of the α-adrenoceptors, while the hypoxia-induced dilator responses were greatly reduced when the local P_{O_2} was kept high during systemic hypoxia (Langdown & Marshall, 1995). We therefore deduce that the dilatation of the terminal precapillary arterioles was mediated either by the direct action of hypoxia upon the vascular smooth muscle, or by the local release of a vasodilator substance. It seems reasonable to propose that the increases in diameter induced in these same sections of the arterial tree vessels and in the small arteries and principal arteries in the present study were also mediated by local dilator mechanisms. However, given that mesenteric arterial vessels can show myogenic responses to changes in intravascular pressure (Lang & Johnson, 1988; Hebert & Marshall, 1988), it is also possible that these more proximal arterial vessels showed myogenic dilatation to the hypoxia-induced fall in systemic arterial pressure.

The hypoxia-induced increases in diameter all along the venous tree might also have been mediated by the local dilator effects of hypoxia (see Langdown & Marshall, 1995). However, given that mesenteric venous vessels respond passively to changes in intravascular pressure (Hebert & Marshall, 1988), they may have been passively distended if the dilatation of the arterial vessels was sufficient to increase the blood flow and pressure transmitted to the venous side of the circulation. These possibilities are given further consideration below.

In the experiments in which adenosine was topically applied to the mesentery all sections of the arterial tree showed graded dilator responses to graded concentrations of adenosine, the largest responses being evoked in distal arterioles, and these responses were blocked by the adenosine receptor antagonist 8-SPT (10^{-3} M). Thus, we can conclude that adenosine receptors are present all along the mesenteric arterial tree and that they can be blocked by the concentration of 8-SPT we used. If we assume that 10^{-3} M adenosine induced a maximal dilatation, then the capacity for dilatation was relatively small in all sections of the arterial tree (9–12% of control diameter). This may be explained by the deep level of anaesthesia: the vessels may have been relatively dilated under our control conditions due to low sympathetic activity (see above). The increases in diameter induced by adenosine in the venous vessels are more difficult to interpret: they could reflect passive distension secondary to dilatation on the arterial side of the circulation, rather than active, venous dilator responses.

Considering the arterial vessels that were dilated by hypoxia, the magnitude of their responses was similar to the maximal dilatation induced by adenosine (see Figs 1

and 3), indicating that the severe hypoxia induced by 6% O_2 (P_{a,O_2}, 34 mmHg) induced maximal arterial dilatation. Our previous time-control studies indicated that responses induced in individual vessels are consistent for at least two repetitions of a given hypoxic stimulus (Mian & Marshall, 1991*a*). Thus the fact that the dilator responses induced by hypoxia in the present study were reduced by about 30% by 8-SPT indicates that they were partly, but not wholly, mediated by adenosine. The sections of the arterial tree whose responses seemed most affected by 8-SPT were the more distal arterial vessels, the very sections that were most affected by topical application of adenosine. As the dilator responses of the principal arteries were not changed by 8-SPT they may have been mediated by some other local effect of hypoxia, or they were myogenic responses as discussed above.

The adenosine that dilated the distal arterial vessels during hypoxia may have originated from the hydrolysis of ATP released from the sympathetic nerve fibres, for this innervation extends to the precapillary arterioles (Furness & Marshall, 1974) and ATP is co-localized with noradrenaline in mesenteric and other vascular beds (Burnstock, 1988). However, this seems unlikely if the sympathetic activation that might be expected during hypoxia were depressed by the relatively deep level of anaesthesia (see above). Similarly, it is unlikely that adenosine induced dilatation by presynaptically inhibiting the release of noradrenaline from the sympathetic fibres (see Fulsgang, Therkildsen & Crone, 1988). Rather, adenosine may have been released as such from the vascular smooth muscle or endothelium by the action of hypoxia; radiolabelled adenosine that was loaded into the endothelial cells of isolated guinea-pig hearts was released into the perfusate when it was equilibrated with 15% rather than 95% O_2 (Deussen *et al.* 1986). Alternatively, adenosine may have been formed from ATP that was released from the endothelium by the local action of hypoxia; ATP was released from isolated guinea-pig hearts when the perfusate was switched from equilibration with 95% O_2 to 0% O_2 (Hopwood, Lincoln, Kirkpatrick & Burnstock, 1989) and ATP was released from the endothelium of isolated rat mesenteric vasculature by an increase in shear stress (Ralevic, Milner, Kirkpatrick & Burnstock, 1992). Adenosine may have produced dilatation either by acting directly on the vascular smooth muscle, or by acting on the endothelium and releasing endothelium-derived relaxing factor (EDRF, or nitric oxide) as in cremaster arterioles (Baker & Sutton, 1993).

The effects of 8-SPT on mesenteric venous vessels can be explained by the two alternative hypotheses discussed above. Thus, the attenuation of the hypoxia-induced increases in venous diameter by 8-SPT could be explained if they were dilator responses mediated by the actions of locally released adenosine or if they reflected passive distension caused by arteriolar dilatation.

Chronic hypoxia

The chronically hypoxic animals that had been maintained in 10% O_2 for 3–4 weeks and that were studied when breathing 10% O_2 had, as expected, a substantially reduced P_{a,O_2}. The fact that their P_{a,CO_2} value was similar to that of the normoxic animals breathing air suggests that their systemic hypoxia was not serving as a stimulus to increase respiration. This may be explained by the relatively deep level of anaesthesia.

Although we did not measure haematocrit in the normoxic animals of the present study we know from other experiments that in normal rats reared in air, it is ~42% (Davies, Thomas & Marshall, 1994). Thus, the chronically hypoxic animals showed a substantially raised haematocrit (56%) which would have helped to increase the arterial O_2 content despite the reduction in P_{a,O_2}. Nevertheless, there was indication of cardiovascular adjustment to the state of hypoxia in that arterial pressure in the chronically hypoxic animals breathing 10% O_2 was lower than in the normoxic animals breathing air. This may, of course, reflect a reduced cardiac output and/or a reduced peripheral resistance. Our observations on the mesenteric circulation do not allow us to distinguish between these possibilities. In each section of the arterial and venous trees, the vessels we studied in the chronically hypoxic animals breathing 10% O_2 were of comparable diameter to those of the normoxic animals breathing air. We cannot exclude the possibility that overall the diameters of arterial and venous vessels of the chronically hypoxic animals were actually larger and that for some reason we selected vessels that were of comparable diameter in two groups. However, we can state that judging from the responses evoked by a supramaximal concentration of adenosine (10^{-3} M), arterial and venous vessels of the same diameter in the two groups of animals had a similar capacity to increase their diameter.

Since the chronically hypoxic animals showed a rise in arterial pressure on acute return to air and a decrease in diameter in all sections of the mesenteric arterial and venous trees, it might be argued, in contrast to the discussion above, that these animals were under a tonic dilator influence of hypoxia which might interfere with responses evoked by nerve-released or circulating noradrenaline (see introduction). In view of the role that adenosine played in the response to acute hypoxia (see above), it might have been expected that this dilator influence was adenosine. However, the fact that 8-SPT had no effect on the resting diameter of the arterial and venous vessels, nor on their response to air breathing, argues against this possibility. It may be that the return to air removed the influence of a dilator substance other than adenosine, whose generation is oxygen dependent (see Marshall, 1995). However, it might be better to regard the return to air as exposure to hyperoxia, rather than simply the removal of hypoxia, for the arterial O_2 content would have increased to substantially above normal, given the

raised haematocrit. Then, the rise in arterial pressure and constriction of the mesenteric arterial vessels might be attributed to the direct effect of oxygen upon the vascular smooth muscle, or to the increased generation of a vasoconstrictor substance, or substance that interferes with the action of a vasodilator. Oxygen-derived free radicals are likely candidates as they inactivate EDRF (Rubanyi & Vanhoutte, 1986). The decrease in the diameters of the mesenteric venous vessels upon air breathing may have reflected passive collapse secondary to arterial vasoconstriction, rather than active venous constriction.

On the other hand, our results suggest that adenosine did play a major role in the chronically hypoxic animals in the responses induced by a further acute hypoxic challenge. First, it should be noted that the change from breathing 10% O_2 to breathing 8% O_2 produced a fall in P_{a,O_2} (from 52 to 42 mmHg) that was much smaller than that induced in the normoxic animals when they changed from breathing air to breathing 6% O_2 (92 to 34 mmHg), but this was accompanied by increases in the diameter of the great majority of mesenteric arterial vessels that were as large as those evoked in the normoxic animals by 6% O_2. Moreover, the hypoxia-induced dilator responses in the chronically hypoxic animals were as large as the maximal dilator responses induced by topical application of adenosine (10^{-3} M). Thus, it seems that the chronically hypoxic animals had become more sensitive to the dilator effects of an acute hypoxic challenge. Second, whereas in the normoxic animals 8-SPT reduced the hypoxia-induced dilator response by about 30%, in the chronically hypoxic animals it virtually abolished them. Thus, the enhanced susceptibility to the dilator influence of acute hypoxia was almost entirely attributable to adenosine. Moreover, considering the effects of 8-SPT on the different sections of the arterial tree, it seems that in the chronically hypoxic animals, in contrast to the normoxic animals, adenosine was important in dilating the principal arteries as well as the more distal arterial vessels. What we cannot deduce is whether the greater influence of adenosine in the chronically hypoxic animals represents a greater release of ATP or adenosine in response to acute hypoxia (see above), or a greater sensitivity of the arterial vessels to adenosine.

In summary, the present results provide the first evidence that in the mesenteric circulation, adenosine that is locally released by or synthesized by the blood vessel wall can play a functional role in acute systemic hypoxia, by dilating the arteriolar vessels and causing active dilatation, or passive distension, of the venous vessels. By these influences, adenosine would be expected to reduce regional vascular resistance and increase regional blood volume. They also suggest that this role of adenosine is greatly enhanced in chronic hypoxia, either because more adenosine is released in response to an acute hypoxic challenge, or because the vascular sensitivity to adenosine is increased.

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Acknowledgements

This work was supported by the British Heart Foundation.

Received 23 March 1995; accepted 25 May 1995.