The behaviour of muscle microcirculation in chronically hypoxic rats: the role of adenosine

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1. In rats housed in a hypoxic chamber at 12% O_2 for 3–5 weeks (CH) and in normal rats housed in air (N), we directly observed responses of arterial and venous vessels of the spinotrapezius muscle to changes in O_2 concentration in the inspirate. Both CH and N rats were anaesthetized with Saffan. They had haematocrits of 55·0 ± 0·9% (mean ± S.E.M.) and 41·9 ± 0·5%, respectively.

2. In CH rats breathing 12% O_2 and N rats breathing air, arterial and venous vessels from comparable anatomical positions in the vascular tree were of similar internal diameter. They also showed similar maximum dilator responses to topical adenosine (10^{-3} M); 14·1 ± 1·1 and 16·3 ± 1·7% in all arterioles, 15·5 ± 1·2 and 11·5 ± 0·6% in all venules in CH and N rats, respectively.

3. In CH rats, the change from 12% O_2 to air for 3 min induced constriction in all arterioles and venules (−12·9 ± 1·0 and −14·3 ± 1·7%, respectively), whereas in N rats, the change from air to 12% O_2 for 3 min induced net dilatation (3·9 ± 1·8% in arterioles and 4·7 ± 0·8% in venules). Topical application of the adenosine receptor antagonist 8-sulphophenyltheophylline (8-SPT, 10^{-3} M) had no effect on control diameters in CH or N rats, nor on constrictor responses to air in CH, but reversed or reduced dilator responses to 12% O_2 in N rats (to − 2·4 ± 1·3% in arterioles and 2·0 ± 0·9% in venules).

4. In CH rats, the change from 12 to 8% O_2 produced net dilatation as great as that induced in N rats by the larger change from air to 8% O_2: 8·5 ± 2·6 and 5·0 ± 3·7% in arterioles and 10·3 ± 1·8 and 6·4 ± 1·9% in venules, respectively. These responses were similarly reduced by 8-SPT to −4·3 ± 1·9 and −5·2 ± 2·7% in arterioles and to −6·9 ± 2·0 and −1·5 ± 2·0% in venules, respectively.

5. These results indicate that CH rats were acclimated to 12% O_2 such that the resting tone of arterial and venous vessels of muscle was comparable to that of N rats breathing air. They also suggest that adenosine had little tonic dilator influence in CH rats breathing 12% O_2 despite its contribution to the dilatation induced in N rats by acute exposure to 12% O_2. This may reflect the greater haematocrit in CH rats which normalized the O_2 supply to muscle. However, CH rats were more sensitive than N rats to the dilator influence of acute systemic hypoxia and this was largely mediated by adenosine.

Our recent studies on the rat showed that locally released adenosine plays a major part in the vasodilatation induced in skeletal muscle by acute systemic hypoxia (Neylon & Marshall, 1991; Marshall, Thomas & Turner, 1993; Thomas, Elnazir & Marshall, 1994). We concluded that adenosine acts in part by stimulating adenosine receptors on the skeletal muscle fibres that are coupled to ATP-sensitive K^+ (K_{ATP}) channels, so releasing K^+, which then dilates the blood vessels; the remaining vasodilatation is mediated by the more direct influences of adenosine upon the blood vessel walls (Marshall *et al.* 1993). This may include dilatation mediated by receptors on the vascular smooth muscle or endothelium of arterioles and venules and by prejunctional receptors that inhibit the release of noradrenaline from the sympathetic nerve fibres that supply arterioles, but not venules (see Marshall, 1982, 1995). Our direct observations on the microcirculation of the spinotrapezius muscle indicated that during acute hypoxia, adenosine is released from the muscle fibres in immediate proximity to the blood vessels or even from the blood vessels themselves (Mian & Marshall, 1991*c*). This is the location of the 5′-nucleotidase that hydrolyses AMP to adenosine (Rubio, Berne & Dobson, 1973), but adenosine can also be stored and released by the vascular endothelium (Deussen, Moser & Schrader, 1986; see Ralevic, Lincoln & Burnstock, 1992). We proposed that adenosine is released

in proportion to the local P_{O_2} and so preferentially dilates the more distal arterioles and venules and vessels in regions where local P_{O_2} is particularly low (Mian & Marshall, 1991*c*).

We have since developed this hypothesis by experiments on the microcirculation of the intestinal mesentery, where there is negligible tissue parenchyma around the blood vessels (Mian & Marshall, 1995). Thus we established that acute, severe hypoxia induced by breathing 6% O_2 for 3 min under conditions of deep anaesthesia could indeed induce vasodilatation in the arterioles and venous vessels of the mesentery that was reduced by *ca* 30% by topical application of the adenosine receptor antagonist, 8-sulphophenyltheophylline (8-SPT). But, in that same study we found that adenosine played a much greater role in rats that had been made chronically hypoxic in a hypoxic chamber at 10% O_2 for 3–5 weeks. In these animals, under similar conditions of deep anaesthesia, a relatively small change in the inspired gas mixture, from 10 to 8% O_2, induced increases in mesenteric arteriolar and venous diameter that were at least as great as those induced in control rats by switching the inspirate from air to 6% O_2. Moreover, the dilator responses in the chronically hypoxic rats were completely abolished by 8-SPT, suggesting that either adenosine is more readily released from the immediate vicinity of the vessels in the chronically hypoxic state, or the vessels are more sensitive to it (Mian & Marshall, 1995).

As the mesentery is a specialized tissue and because we had created unphysiological conditions of deep anaesthesia and severe hypoxia so as to maximize the effects of adenosine, an obvious question raised by our results is: does adenosine assume a greater functional role in skeletal muscle in chronic hypoxia, when the acute hypoxic challenge is more moderate and the level of anaesthesia less severe? Our previous studies have already demonstrated that in control animals reared in air, acute hypoxia produces much greater dilatation in skeletal muscle than in mesenteric circulation whether the comparison is made under light or deep anaesthesia (see Mian & Marshall, 1991*a*,*b*,*c*, 1995; cf. Langdown & Marshall, 1995). Given the role adenosine normally plays in acute hypoxia in skeletal muscle, we also proposed that during chronic hypoxia, the skeletal muscle microcirculation would be under a tonic influence of adenosine that would be removed on return to air-breathing.

To test these hypotheses we carried out studies on chronically hypoxic (CH) rats kept in a hypoxic chamber at 12% O_2 for 3–5 weeks and on weight-matched controls (N rats) that were maintained in air. Both groups were lightly anaesthetized with Saffan (Pitman–Moore, Uxbridge, Middlesex, UK), which preserves central nervous system influences over the cardiovascular system to a greater extent than more conventional anaesthetics (Marshall, 1994). Responses evoked in individual arterioles and venules of the spinotrapezius muscle were then directly compared when the inspirate of the CH rats was switched from 12 to 8% O_2, or to air and when that of the N rats was switched from air to 12 or 8% O_2. In both groups, the dilator responses evoked by acute hypoxia were also compared with those evoked by supramaximal concentrations of adenosine. All responses were re-tested after topical application of 8-SPT. Some of the results have been reported in brief (Mian & Marshall, 1993).

METHODS

Experiments were performed on eighteen male Wistar rats that had been housed in standard cages within a hypoxic chamber at 12% O_2 for 3–5 weeks from 6 to 7 weeks of age (CH) and on fourteen male Wistar rats that were housed in comparable cages, but breathed air (N). The hypoxic chamber has recently been described in detail (Thomas & Marshall, 1995). At the time of the acute experiments both CH and N rats weighed 250–300 g.

Anaesthesia was induced with 2–3% halothane in oxygen and maintained by continuous infusion of Saffan (7–12 mg kg^{-1}, I.V.) as described previously (Mian & Marshall, 1991*a*). During surgery, the anaesthetic level was adjusted so that the pinching of a paw evoked no withdrawal reflex. During the recording period, the anaesthetic level was reduced so that pinching the paw evoked a modest withdrawal reflex and a short-lasting rise in arterial pressure (Mian & Marshall, 1991*a*).

Once anaesthesia had been established and a cannula had been placed in the trachea, CH rats routinely breathed 12% O_2 that was delivered from a gas bag via an air pump, while N rats breathed air either directly from the atmosphere, or via the air pump (see Mian & Marshall, 1995, for details). Cannulae were placed in the brachial artery to allow 130 μl samples to be taken for blood gas analysis and in the femoral artery, for recording arterial pressure. The spinotrapezius muscle was then prepared for transillumination and the rat was moved to the modified stage of a microscope so that the muscle, with its vascular and nerve supply intact, could be arranged over a transparent column set into the stage. During the preparation it was irrigated with modified Krebs solution, otherwise it was covered with Saran wrap except when briefly removed to allow application of drugs (see below). The microcirculation was displayed on a video monitor and recorded on videotape with a video recorder so that the vessel diameters could be measured off-line by applying callipers to the monitor screen. All details of the surgical preparation and recording equipment have been described previously (Mian & Marshall, 1991*a*, 1995; Langdown & Marshall, 1995).

Experimental protocol

In each experiment, a field of view was chosen which contained two to three arterial or venous vessels from different sections of the vascular tree; this field of view was then maintained throughout the experiment. As in our previous studies, main arteries (40–90 μm) were defined as the arterial vessels that supplied the spinotrapezius; primary and secondary arterioles (22–50 μm and 13–18 μm respectively) as the arterial vessels of the primary and secondary anastomosing networks and terminal arterioles (7–13 μm), as the terminal branching vessels that give rise to the capillaries. Correspondingly, collecting venules (9–18 μm) were defined as the venous vessels into which the capillaries converged; secondary and primary venules (18–30 μm and 40–60 μm) as the vessels of the primary and secondary anastomosing venous networks and main veins (65–130 μm), as the vessels that drained the muscle (Marshall, 1982).

Table 1. Values of blood gases, arterial pH (pH_a) and mean arterial pressure (ABP) recorded in N and CH rats during air-breathing, 12 and 8% O_2 before and after topical application of 8-SPT to the spinotrapezius

	P_{a,O_2} (mmHg)	P_{a,CO_2} (mmHg)	pH_a	ABP (mmHg)
		CH rats		
Before 8-SPT				
Air	89·13 ± 2·94	47·42 ± 2·96	7·266 ± 0·034	151·0 ± 4·8‡
12% O_2	52·40 ± 5·98*†	41·60 ± 2·71*	7·308 ± 0·027*	128·0 ± 3·5*
8% O_2	38·32 ± 2·32*	39·25 ± 7·66*	7·315 ± 0·035*	98·3 ± 8·8*
After 8-SPT				
Air	87·42 ± 9·90	46·50 ± 3·33	7·271 ± 0·045	147·5 ± 2·6‡
12% O_2	54·97 ± 1·69*†	41·52 ± 2·74*	7·317 ± 0·022*	130·5 ± 6·5*
8% O_2	40·34 ± 2·59*	37·82 ± 3·18*	7·315 ± 0·043	97·5 ± 8·5*
		N rats		
Before 8-SPT				
Air	83·77 ± 2·59	44·75 ± 3·10	7·260 ± 0·025	125·8 ± 2·01
12% O_2	43·76 ± 2·05*	33·07 ± 1·23*	7·381 ± 0·016*	94·5 ± 4·2*
8% O_2	40·93 ± 2·07*	36·13 ± 3·47*	7·359 ± 0·038*	82·5 ± 4·48*
After 8-SPT				
Air	81·52 ± 1·88	45·95 ± 2·10	7·288 ± 0·034	124·1 ± 3·0
12% O_2	43·02 ± 1·80*	34·45 ± 1·00*	7·390 ± 0·016*	102·0 ± 4·4*
8% O_2	38·92 ± 2·13*	37·38 ± 4·42	7·339 ± 0·041*	79·0 ± 4·5*

*Significant difference within CH or N rats, from values recorded during air-breathing. †Difference between values recorded in CH rats breathing 12% O_2 and N rats breathing air. ‡Difference between values recorded in CH rats breathing air and N rats breathing air. In each case significance was recognized at $P < 0·05$.

Following an equilibrium period of at least 30 min during which the field of view was selected, the inspirate of the CH rats was switched from 12% O_2 to air for 3 min and then back to 12% O_2. Following a period of at least 10 min to allow all variables to return to their original control values, the inspirate was changed from 12 to 8% O_2 for 3 min and then back to 12% O_2. Following a further stabilization period of 10 min, the Saran wrap was removed to allow adenosine (Sigma, 10^{-3} M in Krebs solution) to be topically applied to the muscle; the Saran wrap was immediately replaced. Ten minutes later, the Saran wrap was temporarily removed again to allow 8-SPT (Sigma, 10^{-3} M in Krebs solution) to be topically applied to the muscle. After a further 10 min, the whole protocol just described was repeated. Measurements of vessel diameter were made under control conditions (12% O_2) and at the end of the 1st, 2nd and 3rd minute of breathing air or 8% O_2, the largest change in diameter from control being taken for analysis (Mian & Marshall, 1991*a*); the peak of the response to adenosine was used for analysis. Measurements of arterial pressure were taken immediately before and at the end of the 2nd minute of breathing air or 8% O_2. Samples for blood gas analysis were taken during 12% O_2 and in the 3rd minute of breathing air or 8% O_2. Arterial blood samples were also taken for measurement of haematocrit.

The N rats underwent an exactly comparable protocol except that the inspirate was switched from air to 12% O_2 for 3 min and from air to 8% O_2 for 3 min.

Statistical analyses

All results are expressed as means ± S.E.M. Comparison of responses in individual vessels before and after 8-SPT within CH or N rats were made by Student's paired t test, where n is number of vessels. Comparisons between CH and N rats for mean arterial pressure and blood gases were made by Student's unpaired t test, where n is number of animals. $P < 0·05$ was considered significant.

RESULTS

Chronically hypoxic (CH) rats

The values of mean arterial pressure and blood gases recorded in CH and N rats under their control conditions (12% O_2 and air respectively) and when the inspirate was changed, before and after 8-SPT, are shown in Table 1. The haematocrit of the CH rats was 55 ± 0·9% while that of the N rats was 41·9 ± 0·5%.

Responses evoked by air

The change from 12% O_2 to air-breathing induced an increase in arterial O_2 pressure (P_{a,O_2}) from its low level of ~52 mmHg to a level comparable with that of N rats breathing air (Table 1). Concomitantly, there was a rise in P_{a,CO_2} and a fall in arterial pH (pH_a) due to hypoventilation. Simultaneously, arterial pressure rose to well above the level recorded in N rats breathing air (Table 1). In the spinotrapezius muscle, all arterial and venous vessels in each section of the vascular tree showed a decrease in diameter (Fig. 1).

Responses evoked by 8% O_2

Not surprisingly the change from 12 to 8% O_2 produced a further fall in P_{a,O_2}, while P_{a,CO_2} fell and arterial pH rose due to hyperventilation. Concomitantly arterial pressure fell (Table 1). The majority of the arterial and venous vessels showed an increase in diameter, but some showed a decrease (Fig. 1). There was no consistency within preparations in that vessels that constricted commonly occurred within the same field of view as those that dilated; this was also the case in the present and previous studies on N rats (see Mian & Marshall, 1991*a*).

Responses evoked by adenosine (10^{-3} M)

As can be seen from Fig. 1, all arterial and venous vessels dilated in response to adenosine. Comparison of the responses evoked by 8% O_2 and by adenosine shows that, of the arterioles that dilated in response to 8% O_2, the magnitude of this dilatation was as large as that induced by adenosine. Furthermore, the arterial and venous vessels that constricted in response to 8% O_2 showed just as great a dilatation in response to adenosine as those vessels that dilated in response to 8% O_2 (Fig. 1).

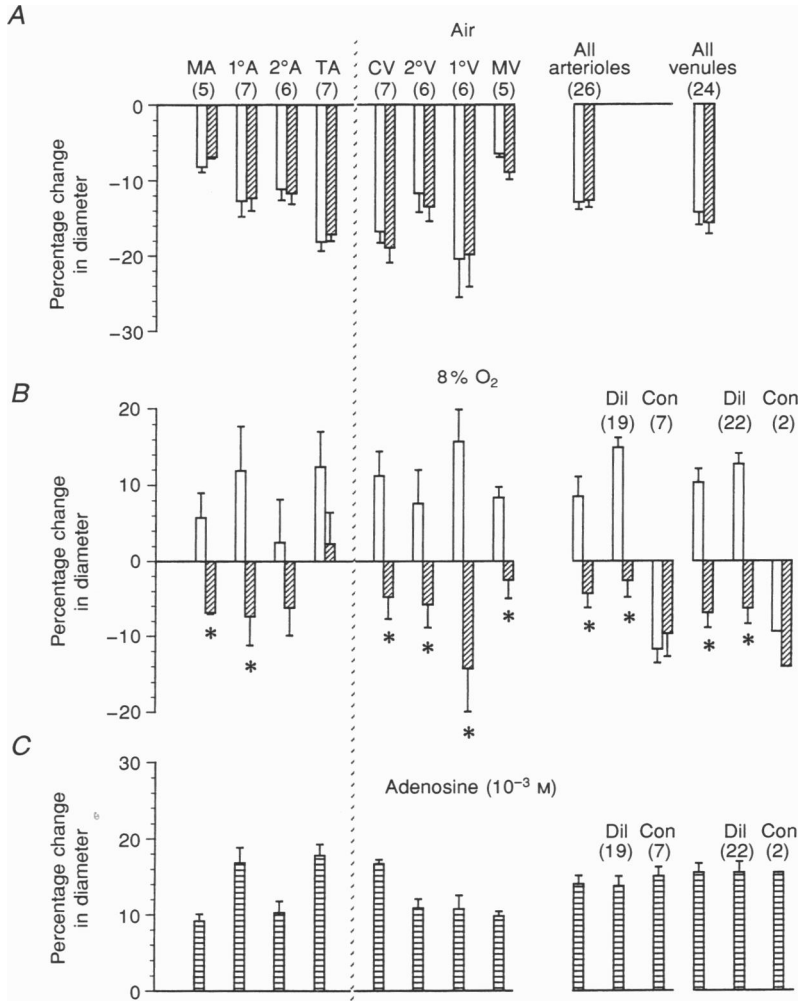

Figure 1. Responses evoked in arterial and venous vessels of muscle microcirculation of CH rats breathing 12% O_2 by the change to breathing air or 8% O_2 for 3 min and by topically applied adenosine

Responses are shown as percentage change in diameter (± S.E.M.). In A, the change to breathing air, and *B*, the change to 8% O_2 for 3 min, the columns indicate responses before (□) and after (▨) 8-SPT. *C*, responses evoked by adenosine. Abbreviations on the left-hand side of *A*, *B* and *C* indicate vessel types: MA, main arteries; 1°A, primary arterioles; 2°A, secondary arterioles; TA, terminal arterioles; CV, collecting venules; 2°V, secondary venules; 1°V, primary venules; MV, main veins. On the right-hand side in *A*, columns represent means of responses in all arterioles and venules. On the right-hand side in *B* and *C*, columns represent means of responses in all arterioles and venules and means of responses of arterioles and of venules that dilated (Dil) or constricted (Con) in response to 8% O_2. Numbers above columns indicate number of vessels. * Significant difference between responses before and after 8-SPT, $P < 0{\cdot}05$.

Effects of 8-SPT

Ten minutes after applying 8-SPT to the spinotrapezius muscle, all arterioles and venous vessels were at their original control diameter. Topical application of 8-SPT had no effect on the changes in arterial blood gases or arterial pressure, nor on the changes in vessel diameter induced by air-breathing (Table 1, Fig. 1).

Similarly, 8-SPT had no effect on the changes in arterial blood gases or arterial pressure induced by 8% O_2 (Table 1). However, there were marked changes in the responses induced in the microcirculation. Thus, the mean increases in diameter in the main arteries, primary arterioles, collecting venules, secondary and primary venules and main veins were significantly reduced, resulting in mean decreases in diameter; similar trends were apparent in the secondary and terminal arterioles (Fig. 1). When the vessels were grouped according to whether they showed dilatation or constriction in response to 8% O_2 before 8-SPT, it became clear that 8-SPT preferentially affected the dilator responses (Fig. 1).

When the effects of adenosine were re-tested at the end of the experiment (see protocol), there were no detectable changes in diameter in any section of the arterial or venous trees showing that 8-SPT produced an effective blockade of the adenosine receptors that lasted during the second exposure to air and to 8% O_2.

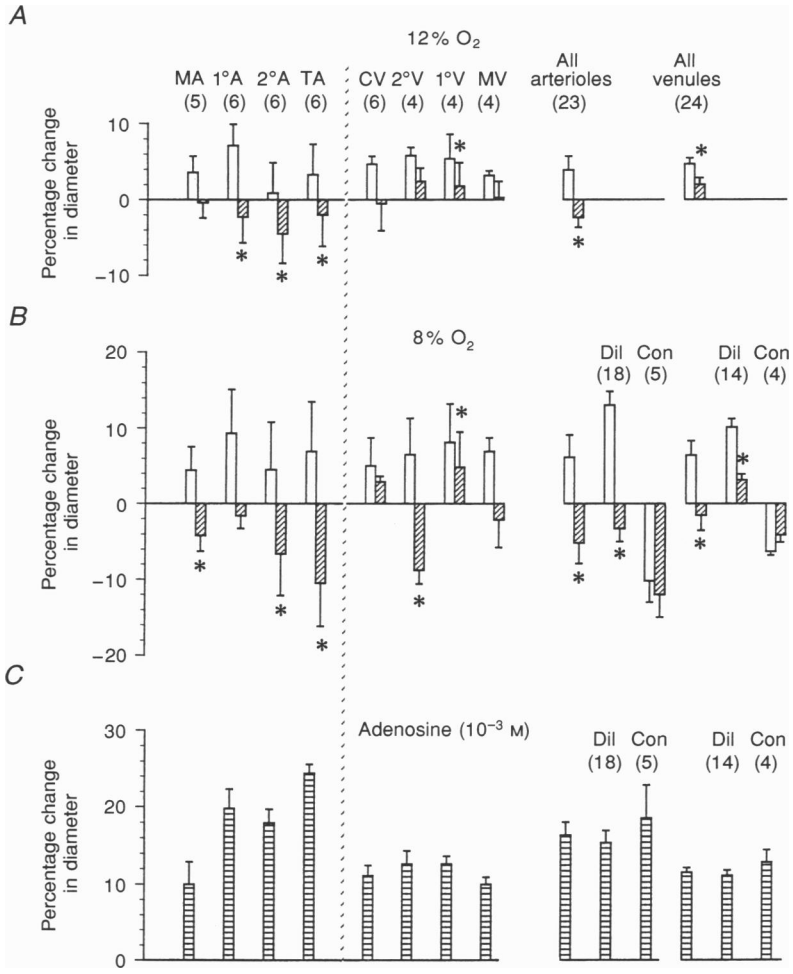

Figure 2. Responses evoked in arterial and venous vessels of muscle microcirculation of N rats breathing air by the change to breathing 12 or 8% O_2 and by topically applied adenosine

Responses are shown as percentage change in diameter (± S.E.M.) In *A*, the change to breathing air, and *B*, the change to 8% O_2, the columns indicate responses before (□) and after (▨) 8-SPT. *C*, responses evoked by adenosine. Abbreviations above the columns in *A*, *B* and *C* indicate vessel types as in Fig. 1. On the right-hand side in *A*, columns represent means of responses in all arterioles and all venules. On the right-hand side in *B* and *C*, columns represent means of responses in all arterioles and venules and means of responses of arterioles and venules that dilated (Dil) or constricted (Con) in response to 8% O_2. Numbers above columns indicate number of vessels. * Significant difference between responses evoked before and after 8-SPT, $P < 0.05$.

Table 2. Control diameters of arterial and venous vessels from each section of the vascular tree in CH rats breathing 12% O_2 and in N rats breathing air

	CH (μm)	n	N (μm)	n
Main arteries	58·85 ± 4·40	5	56·16 ± 4·14	5
Primary arterioles	31·46 ± 3·68	7	35·70 ± 7·39	6
Secondary arterioles	16·50 ± 0·47	7	16·10 ± 0·70	6
Terminal arterioles	10·85 ± 0·75	7	12·86 ± 0·34	6
Collecting venules	14·80 ± 0·73	7	14·50 ± 0·96	6
Secondary venules	23·87 ± 1·45	6	26·73 ± 0·94	4
Primary venules	48·86 ± 2·63	6	40·68 ± 3·72	5
Main veins	71·74 ± 10·50	5	84·58 ± 10·65	4

Values are given as means ± S.E.M.; n, number of vessels.

Control (N) rats

As expected, the P_{a,O_2} of the N rats under their control conditions (breathing air) was higher than that of the CH rats under their control conditions (breathing 12% O_2) (Table 1). However, mean arterial pressure was similar in N and CH rats under their respective control conditions. As indicated in Methods, the vessels chosen for study were placed in categories on the basis of their anatomical position and internal diameter. There were no significant differences between the control internal diameters of the arterial or venous vessels in N and CH rats when N rats were breathing air and CH rats were breathing 12% O_2 (Table 2).

Responses evoked by 12 and 8% O_2

As shown in Table 1, P_{a,O_2} fell during 12 and 8% O_2, the level attained in 8% O_2 being slightly lower than that attained in 12% O_2. P_{a,CO_2} fell and arterial pH rose from control values at both levels of hypoxia.

As in CH rats, hypoxia produced a fall in arterial pressure, the levels attained being lower in 8% than in 12% O_2 (Table 1). Breathing 12 or 8% O_2 also produced increases in diameter in some arterial and venous vessels of each section of the vascular tree and decreases in diameter in the remainder (Fig. 2), these qualitative differences being observed within individual preparations as in CH (see above). The majority of the vessels showed dilatation in response to both levels of hypoxia (Fig. 2, subgroup data for 12% O_2 not shown). Comparison of Figs 1 and 2 shows that the dilator and the constrictor responses evoked by 8% O_2 in the arterial and venous vessels of N rats were similar in magnitude to those evoked by 8% O_2 in CH rats. Furthermore, it is clear from Figs 1 and 2 that the changes in diameter that occurred in N rats when they were switched from air to 8% O_2 were much smaller than the differences between the diameters recorded in air and those recorded during 8% O_2 in CH rats. Thus, if the changes in diameter evoked in CH rats by 8% O_2 are expressed as a percentage of the diameters reached in air, then 8% O_2 evoked mean increases in diameter of all arterioles of ~25%; of arterioles that dilated, ~31%; of all venous vessels, ~28%; and of venous vessels that dilated, ~30%. However, in N rats the changes in diameter recorded in these same categories of vessels were ~6, 13, 6 and 10%, respectively.

Responses evoked by adenosine

As in CH rats, adenosine evoked dilator responses in all sections of the arterial and venous trees (Fig. 2). The magnitudes of these dilator responses were not significantly different from those evoked in N rats. In agreement with the results obtained in CH rats, the magnitudes of the dilator responses that were evoked in arterial and venous vessels by 8% O_2 were comparable with the dilator responses evoked in those same vessels by adenosine (Fig. 2). Notably, the dilator responses evoked by adenosine in those arterial and venous vessels that were constricted by 8% O_2 were just as large as those evoked by adenosine in the vessels that were dilated by 8% O_2 (Fig. 2). It is clear that the dilator responses evoked by adenosine in the arterial and venous vessels of N rats were similar to those induced in CH rats (compare Figs 1 and 2).

Effects of 8-SPT

As in CH rats, 10 min after application of 8-SPT to the spinotrapezius all vessels were at their original control diameter. 8-SPT had no effect on the control levels of blood gases and arterial pressure, nor on the values attained during 12 and 8% O_2 (Table 1).

However, as in CH rats, 8-SPT did affect the responses induced in the microcirculation by hypoxia. Thus, 8-SPT tended to reduce mean increases in diameter evoked by 12 and 8% O_2, or convert mean increases in diameter to mean decreases in each section of the arterial and venous trees. The effects of 8-SPT reached significance in the primary, secondary and terminal arterioles and primary venules in 12% O_2, in the main arteries, secondary and terminal arterioles and secondary and primary venules in 8% O_2 and in all arterioles and all venules when considered as a group during both 12 and 8% O_2. As in CH rats, when the vessels were grouped according to whether they dilated or constricted in response to 8% O_2, then 8-SPT preferentially affected the vessels that dilated (Fig. 2).

Comparison of Figs 1 and 2 reveals that 8-SPT was just as effective in reducing the dilator responses evoked by 8% O_2 in N and CH rats.

As in CH rats, 8-SPT was fully effective in blocking the dilator responses evoked by adenosine when re-tested at the end of the experiment.

DISCUSSION

The results obtained in the control (N) rats of the present study are fully consistent with those of our previous studies. Thus, under light Saffan anaesthesia, administration of 12 and 8% O_2 for 3 min evoked a fall in arterial pressure and hyperventilation associated with respiratory alkalosis (Marshall & Metcalfe, 1988). In the spinotrapezius muscle, there was a net increase in the diameters of arterial and venous vessels, though some individual arterioles and venules showed a decrease in diameter (Mian & Marshall, 1991*a*,*b*,*c*). Our previous results indicated that these diameter changes represent active dilator and constrictor responses in both arterioles and venules: notably, venous vessels within skeletal muscle do not show passive changes in diameter as a consequence of changes in intravascular pressure caused by active arteriolar responses (see Marshall, 1982; Mian & Marshall, 1991*a*,*b*,*c*). Comparisons of the magnitudes of the responses observed in the present study with those seen in our previous studies indicate that the arteriolar and venous responses evoked by systemic hypoxia are graded with the level of hypoxia. For example, the mean diameter changes evoked in the arterioles amounted to increases in diameter of 4 and 6% during 12 and 8% O_2, respectively, in the present study, and of 16% during 6% O_2 in our previous study (Mian & Marshall, 1991*c*). Our previous studies in which P_{a,CO_2} was controlled indicated that the responses observed in skeletal muscle during systemic hypoxia are attributable to the consequences of the fall in P_{a,O_2} rather than to the fall in P_{a,CO_2} that accompanies the evoked hyperventilation; the fall in P_{a,CO_2} made a small contribution to the muscle vasodilatation only during 6% O_2 and not during more moderate hypoxia (Marshall & Metcalfe, 1989).

In our previous study (Mian & Marshall, 1991*c*), the blocking action of the adenosine receptor antagonist 8-phenyltheophylline (8-PT) waned over the period of the experiment. In the present study, we had no such problems with 8-SPT; its blocking action on the dilator responses evoked by a supramaximal dose of adenosine was fully effective at the end of the experiment. The effects of 8-SPT on the hypoxia-induced responses indicated, in agreement with our previous studies (Mian & Marshall, 1991*c*), that a large part of the dilatation of both arterioles and venules was mediated by adenosine in that the mean dilator responses were reversed to mean constrictor responses. With these effects blocked, the remaining dilator influences on the arterioles and venules (Marshall, 1995) were presumably overcome by vasoconstrictor influences, such as those of α-adrenoreceptor stimulation by nerve-released noradrenaline and circulating catecholamines and by the actions of circulating vasopressin and angiotensin (Mian & Marshall, 1991*b*; Cohen, Mian & Marshall, 1992; Marshall, Lloyd & Mian, 1993).

In CH rats breathing 12% O_2, P_{a,O_2} was lower, but arterial pressure was the same as that of N rats breathing air. This contrasts with our observations on deeply anaesthetized CH rats breathing 10% O_2, which showed that arterial pressure was lower than in N rats breathing air (Mian & Marshall, 1995). Thus, it seems that after 3–5 weeks of chronic exposure to 12% O_2, and when examined under light anaesthesia, any tonic dilator influence of hypoxia upon total peripheral resistance was effectively counterbalanced by other factors.

The arterial and venous vessels we chose to study from each section of the spinotrapezius were of comparable control diameters in CH rats breathing 12% O_2 and N rats breathing air. This is in agreement with the observation we made on mesenteric vasculature (Mian & Marshall, 1995), but is of no value in indicating whether there was a tonic dilator influence on mesenteric, or muscle microcirculation in the CH rats: we may have preferentially selected vessels of similar diameters. However, the diameters of vessels from each section of muscle circulation in CH rats breathing 12% O_2 were probably smaller than in N rats breathing 12% O_2, given that the latter were acutely dilated during 12% O_2. Moreover, supramaximal concentrations of adenosine produced dilator responses of similar magnitude in the two groups of animals. This suggests that the resting tone in vessels of similar size was, in fact, comparable in CH rats breathing 12% O_2 and N rats breathing air. Furthermore, within 10 min of applying 8-SPT to the spinotrapezius, the diameters of vessels in CH rats breathing 12% O_2 were the same as their original control diameters. We made the same observation in N rats breathing air in the present and in our previous study (Mian & Marshall, 1991). In the latter study, observations made during the 10 min after application of the adenosine antagonist showed a transient vasoconstriction in all vessels, indicating a small tonic dilator influence of adenosine. We omitted measurements over this time in the present study because it is difficult to restore focus quickly on the vessels after removing and replacing the Saran wrap (see Methods). Nevertheless, we can conclude that any tonic dilator influence of adenosine upon the muscle microcirculation of CH rats breathing 12% O_2 was small and unlikely to have been any greater than in N rats breathing air. This is again in agreement with the conclusion we drew for mesenteric circulation of CH rats (Mian & Marshall, 1995), but the conclusion is, perhaps, more surprising for muscle vasculature, given that adenosine plays a much greater role in the dilatation induced by acute hypoxia in muscle than in the mesentery (see introduction). Indeed, it seems that within 3–5 weeks of chronic exposure to 12% O_2, the increase in haematocrit

had allowed arterial O_2 content to increase to the extent that the O_2 supply to the spinotrapezius of CH rats breathing 12% O_2 was comparable to that of N rats breathing air and the hypoxic stimulus for adenosine release had become similarly small. This view is supported by our recent measurements of O_2 delivery to hindlimb muscles of CH and N rats (Davies, Thomas & Marshall, 1994).

This view is also consistent with the present finding that 8-SPT did not affect the arteriolar and venous constrictor responses induced in the spinotrapezius of CH rats when they were switched from breathing 12% O_2 to air: these responses could not be attributed to a decrease in adenosine concentration. Again, we made similar observations and drew a similar conclusion for the mesenteric circulation of CH rats (Mian & Marshall, 1995). As the vessels of the mesentery have negligible tissue parenchyma around them and the rats were deeply anaesthetized, we proposed that the mesenteric vasoconstrictor responses must have been due to the direct action of the oxygen on the vascular smooth muscle or to a change in the release of some substance(s) from the blood vessel wall. We proposed an increase in the release of oxygen-derived free radicals since they are known to inactivate endothelium-derived relaxing factor (Rubanyi & Vanhoutte, 1986).

In skeletal muscle of lightly anaesthetized animals there are other possible explanations. A decrease in the tonic discharge from the peripheral chemoreceptors of CH rats on exposure to air, as evidenced by the increase in P_{a,CO_2}, would have been expected to lead to a small decrease in sympathetic vasoconstrictor activity and muscle vasodilatation (Marshall, 1987). This would have attenuated, rather than contributed to, the rise in arterial pressure and vasoconstriction in muscle microcirculation that we actually saw. However, in CH rats breathing 12% O_2, constrictor responses induced by noradrenaline in mesenteric and muscle microcirculation (Mian & Marshall, 1994) and increases in total peripheral resistance (Doyle & Walker, 1991) evoked by phenylephrine, angiotensin and vasopressin were greatly attenuated compared with those induced in N rats breathing air and when CH rats breathed air, the changes in total peripheral resistance induced by these agonists were greatly increased (Doyle & Walker, 1991). This raises the possibility that the vasoconstrictor responses observed in the present study when CH rats breathed air reflected an increase in the tonic vasoconstrictor influence of sympathetic noradrenergic activity on the arterioles which are innervated (see Marshall, 1982) and of circulating catecholamines, vasopressin and angiotensin on arteriolar and venous vessels. These factors are known to exert a tonic vasoconstrictor influence on muscle in N rats under light Saffan anaesthesia (Hebert & Marshall, 1988; Louwerse & Marshall, 1992; Marshall, Lloyd & Mian, 1993).

Furthermore, even in normoxic animals, hyperoxia produces vasoconstriction in skeletal muscle by a local action, but not so readily in mesentery. For example, in microcirculatory studies, an increase in superfusion P_{O_2} from ~0 to > 60 mmHg (Lang & Johnson, 1988), or even to equilibration with air (Langdown & Marshall, 1995) had no effect on arterioles of the mesentery. However, an increase in superfusion P_{O_2} from 5 to 35 mmHg, or from ~0 to 66 mmHg produced ~18 and ~11% decreases in the diameter of arterioles of hamster cremaster and cat sartorius muscle, respectively (Sullivan & Johnson, 1981; Gorczynski & Duling, 1989); this is similar to the mean 13% decrease in diameter of spinotrapezius arterioles of CH rats when P_{a,O_2} increased from 52 to 89 mmHg. Furthermore, in N rats in which the vasoconstrictor influences of the sympathetic nervous system had been pharmacologically blocked, addition of O_2 to the inspired air produced a rise in arterial pressure and vasoconstriction in limb muscle (Marshall, 1987). Such local constrictor responses have been attributed to a decrease in the release of a vasodilator substance, or to an increase in the release of a vasoconstrictor substance from the skeletal muscle fibres (Sullivan & Johnson, 1981). The substance(s) involved have not been fully investigated, but Jackson (1989) implicated the synthesis of vasoconstrictor leukotrienes in hyperoxia-induced arteriolar constriction in hamster cheek pouch. Whatever substance(s) were involved in the spinotrapezius muscle must also have been capable of constricting venous vessels, for they too showed a 14% decrease in diameter when CH rats breathed air. These venous responses were in accord with the known comparability of the responsiveness of muscle arterial and venous vessels to constrictor and dilator influences (see Marshall, 1982; Marshall & Tandon, 1985; Mian & Marshall, 1991*a*, *b*, *c*; Marshall, Lloyd & Mian, 1993).

Even if adenosine exerted no tonic dilator influence upon the muscle microcirculation of CH rats breathing 12% O_2, it did play a major role in the dilator responses evoked by an acute hypoxic challenge. This was as we had proposed from our experiments on mesentery and occurred even though the rats were lightly anaesthetized and under the potential vasoconstrictor influences of the sympathetic nervous system and hormones (see above). Thus, when CH rats were switched from breathing 12 to 8% O_2, there was a further fall in P_{a,O_2} to a level similar to that reached in N rats when they breathed 8% O_2 and mean dilator responses occurred throughout the arterial and venous trees of spinotrapezius muscle that were at least as great as those evoked in N rats when they were switched from air-breathing to 8% O_2. These responses were also much greater than the differences between the responses evoked in N rats by 12 and 8% O_2. Moreover, 8-SPT greatly reduced the mean increases in diameter in CH rats, or reversed them to mean decreases in diameter by preferentially affecting those arterioles and venous vessels that dilated, just as it did to the responses evoked in N rats by 8% O_2, the magnitude of these effects being comparable in the two groups of animals. Whether the effects of

breathing 8% O_2 are considered as a fall in P_{a,O_2} (a change from 52 to 38 mmHg in CH and from 84 to 41 mmHg in N rats), or as a fall in arterial O_2 content, it is clear there must have been a much smaller decrease in the oxygen transported by the supply artery of the spinotrapezius muscle of CH rats. Given that vasodilator responses to hypoxia within the microcirculation provide a means of increasing the blood flow, improving the homogeneity of the blood supply to the capillary network and therefore of helping to maintain the O_2 supply to the tissue cells (Harrison, Kessler & Knauf, 1990) the present results suggest that the CH rats had become more efficient at protecting the O_2 supply to the skeletal muscle fibres in the face of an acute systemic hypoxic challenge. They further suggest this can be largely attributed to the dilator influences of adenosine (see Marshall, 1995).

In CH rats, as in N rats, the arterioles and venous vessels that constricted in response to acute hypoxia were just as dilated by a supramaximal concentration of exogenous adenosine as those that dilated to acute hypoxia. This suggests that in CH as in N rats, the adenosine that dilated the arterial and venous vessels during acute hypoxia must have been released from within, or very close to, the blood vessel wall (see Mian & Marshall, 1991*c*, and introduction to this paper) and that this adenosine was more readily released in CH rats. Our experiments on mesenteric circulation in CH rats showed that adenosine, newly synthesized, or stored and released from the wall itself can contribute to the dilatation (see Mian & Marshall, 1995). But, in soleus muscle of rats, which is predominantly oxidative, 5′-nucleotidase was also closely associated with the skeletal muscle fibres that were adjacent to the blood vessel walls (Rubio *et al.* 1973). Now, adenosine is more readily released from oxidative than glycolytic fibres (Bockman & McKenzie, 1983) and recent studies on Sprague–Dawley rats showed that chronic hypoxia (10% O_2) for 5 weeks from 5 weeks of age, inhibited the normal transition of oxidative to more glycolytic fibres in soleus muscle so that at 10 weeks, the proportion of oxidative fibres was ~50% greater than in N rats (Ishihara, Itoh, Oishi, Itoh, Hirofujii & Hayashi, 1995). If this phenomenon also occurred in our CH Wistar rats which were exposed to 12% O_2 from the age of 6–7 weeks, then the spinotrapezius muscle may have had a greater preponderance of oxidative fibres; in the normoxic adult it has oxidative : glycolytic : mixed fibres in the ratio 1 : 1 : 1 (Mian & Marshall, 1991*c*). This may in turn have been associated with an increase in the 5′-nucleotidase activity in the immediate vicinity of the blood vessels and an increase in adenosine synthesis around vessels where P_{O_2} had become particularly low (see Mian & Marshall, 1991*c*).

In summary, the present study provided the first evidence that within 3–5 weeks of chronic exposure to 12% O_2, rats have acclimated to the extent that the arterial and venous vessels of skeletal muscle have a resting tone equivalent to that of normoxic rats breathing air. Any tonic dilator influence of adenosine is no greater than in normoxic rats; this may be explained by the increase in haematocrit that is induced by chronic hypoxia and a consequent normalization of O_2 supply to muscle. It further suggests that an acute change to air-breathing serves as a hyperoxic stimulus that produces constriction of both arterial and venous vessels in skeletal muscle and may be caused by an increased effect of a tonic neural, or hormonal influence, or by a locally released substance with vasoconstrictor action. Finally, it indicates that muscle microcirculation of chronically hypoxic rats is much more sensitive to the dilator influence of acute systemic hypoxia than that of normoxic rats and that this effect is largely mediated by adenosine.

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