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RESPONSES OBSERVED IN INDIVIDUAL ARTERIOLES AND VENULES OF RAT SKELETAL MUSCLE DURING SYSTEMIC HYPOXIA

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SUMMARY

1. In rats anaesthetized with Saffan, responses induced in individual arterial and venous vessels of the spinotrapezius muscle by systemic hypoxia (breathing 12 or 6% O_2 for 3 min) were directly observed by *in vivo* microscopy.

2. Both 12 and 6% O_2 induced gradual tachycardia and a fall in arterial pressure. Concommitantly, in each section of the vascular tree, some vessels showed a gradual increase in diameter, others, a gradual decrease.

3. During 12% O_2, mean diameter changes were graded from mean increases of $\sim$ 2% in main arteries (resting diameter 40–90 μm) to $\sim$ 20% in terminal arterioles (7–13 μm), and ranged from mean increases of 5–8% in collecting and secondary venules (9–18 μm, 18–30 μm), to a decrease of $\sim$ 2% in main veins (65–130 μm).

4. During 6% O_2, constrictor responses were more common in arterial vessels. Thus, mean changes amounted to diameter decreases of < 5% in main arteries and secondary arterioles (13–18 μm), and increases of $\sim$ 5% in primary arterioles (22–50 μm) and terminal arterioles. By contrast, diameter increases predominated in venous vessels being graded from $\sim$ 20% in collecting venules to $\sim$ 2% in main veins.

5. In seventeen rats, 6% O_2 was administered for eight 3 min periods separated by 30 min control periods. The changes evoked in arterial pressure and heart rate were consistent throughout. Diameter changes evoked in individual arterial and venous vessels were consistent in the first two hypoxic periods. However, diameter changes in the third and successive periods were significantly different from those recorded in the first period: increases in diameter became more common and pronounced.

6. These changes in vessel diameter, especially their variability, are considered in relation to recordings made previously of changes in gross blood flow and vascular conductance of limb muscle during systemic hypoxia.

INTRODUCTION

Recently we reported that in the rat, systemic hypoxia induced a gradual fall in arterial pressure, the magnitude of which was graded with the level of hypoxia. The fall in arterial pressure could be attributed to peripheral vasodilatation to which a major contributor was an increase in the vascular conductance of hindlimb muscles (Marshall & Metcalfe, 1988*a*, *b*). However, we also reported that some individuals

showed a decrease in the vascular conductance of limb muscle at one or more levels of hypoxia. This suggests that there are competing constrictor and dilator factors acting upon the vasculature of muscle during hypoxia, the latter tending to predominate (Marshall & Metcalfe, 1988*b*). These factors must be exerting their influence upon the various sections of the microcirculation of muscle. Thus, we have begun to analyse the responses induced by systemic hypoxia in individual vessels of muscle microcirculation. The muscle we have chosen for this purpose is the spinotrapezius muscle of the rat. We have already analysed the responses induced in the microcirculation of this muscle by changes in sympathetic nerve activity, by catecholamines and by locally released vasodilator metabolites (Marshall, 1982; Marshall & Tandon, 1984; Hébert & Marshall, 1986, 1988) and these are factors that we expected to contribute to the responses induced by systemic hypoxia (see Marshall & Metcalfe,1988*b*; Mian, Marshall & Kumar, 1990). As will become apparent below (see also Mian & Marshall, 1991), the responses induced in spinotrapezius microcirculation by systemic hypoxia seem to be qualitatively, but not necessarily quantitatively, comparable to those induced in limb muscle.

In initial experiments using *in vivo* microscopy, we found that systemic hypoxia produced both dilator and constrictor responses of the arterioles of the spinotrapezius, these occurring predominantly in small and large arterioles respectively (Booth & Marshall, 1987). In the present study we have made direct observations on the consecutive sections of the venous tree as well as further observations on the arterial tree, at two different levels of hypoxia. Moreover, as a preliminary to experiments in which we wished to observe the microvascular responses before and after application of pharmacological antagonists (see Mian & Marshall, 1991), we have assessed the reproducibility of diameter changes induced in individual vessels by systemic hypoxia. Some of our findings have been reported to The British Microcirculation Society and to The Physiological Society (Marshall & Mian, 1989, 1990).

METHODS

Experiments were performed on male Sprague–Dawley or Wistar rats of 250–350 g body weight. They were anaesthetized with a continuous intravenous infusion of Saffan as described previously (Marshall & Metcalfe, 1988*a*). Arterial blood pressure was recorded from a cannula placed in the femoral arterial via a pressure transducer (Bell & Howell); heart rate was derived from the pressure recording by means of a rate meter (Devices). In some experiments a cannula was placed in the brachial artery to allow blood samples to be removed for measurement of arterial blood gases (see *Experimental protocol*). The trachea was cannulated with a stainless-steel T-shaped cannula, the main shaft of which was closed with a screw that could be removed to allow removal of mucus. In some experiments respiratory air flow was recorded by connecting the side arm of the tracheal cannula to a flow head (Mercury F100L) and thence to a pressure transducer (Grass Instruments PT5). Throughout the experimental period, air or one of the hypoxic gas mixtures was blown across the end of the side arm of the tracheal cannula (or flow head) by means of a reciprocator air pump, at a rate of $\sim 1{\cdot}2\ l\ min^{-1}$. The gas mixtures, 12 or 6% O_2 in N_2, were made up in PVC gas bags on the day of the experiment, with the aid of a mass spectrometer (see Marshall & Metcalfe, 1988*b*).

The left spinotrapezius muscle was prepared for *in vivo* microscopy as described previously (Marshall, 1982). It was arranged ventral surface uppermost over a Perspex column set into the modified stage of a Leitz Laborlux microscope and viewed with 10× eyepieces and long working distance objectives. During the preparation the muscle was irrigated with modified Krebs solution (Marshall, 1982). Once it was arranged on the column, Saran wrap (Dow Corning, USA) was

draped over it and the adjacent back muscles, to reduce the diffusion of O_2 and to prevent the muscle from drying out. The O_2 permeability of Saran wrap is only 1·5 ml 100 in^{-2} 24 h^{-1} (Sullivan & Johnson, 1981*a*). The microscope was fitted with a television camera which was connected to a television monitor and video recorder as described previously (Hébert & Marshall, 1986). Thus, the vasculature could be viewed on-line using the microscope and monitor and off-line by playing back videotape recordings. The final magnifications on the monitor were approximately 500, 1000 and 1500 with the objectives of 10×, 32× and 52× magnification respectively. On-line, changes in vessel diameter could be recorded semi-continuously by means of a video measurement system as described by Sullivan & Johnson (1981*a*). Briefly, this consists of electronic 'dividers' connected to a linear potentiometer. The circuit generates two parallel lines in the x-axis of the monitor, whose distance apart and position on the y-axis can be adjusted by means of three control knobs. The operator maintains the lines over the inner surfaces of the vessel wall and the recording circuit generates a DC voltage which is proportional to the distance apart of the lines. This signal was displayed, together with arterial pressure, heart rate and respiration, on a pen recorder (Devices M19), so allowing the time courses of change in vessel diameter and arterial pressure to be compared. In addition, in all experiments vessel diameters were measured from the video recordings by applying a pair of dividers and ruler to the monitor whilst the video recorder was in the freeze-frame mode. All video recordings were played back at least twice and measurements verified. This allowed the measurements made on-line to be checked and also allowed the diameters of other vessels in the field of view to be measured. A digital clock showing minutes and seconds displayed on the monitor throughout, facilitating accurate timing of measurements.

Experimental protocol

After completion of all surgery, the animal was allowed to equilibrate at the experimental level of anaesthesia for 30–45 min during which time an area of microcirculation was selected for observation. The magnification obtained on the video monitor limited the field of view to an average of two or three different, but not necessarily adjoining, vessels. This same field was maintained throughout the experiment. The diameter of each vessel was recorded on at least three occasions before the onset of the hypoxic stimulus, including measurements at 5 and 3 min and immediately prior to the stimulus. The onset of the stimulus (the beginning of delivery of 12 or 6% O_2 in N_2 to the animal) was designated time 0. *In vitro* experiments with the aid of the mass spectrometer showed that there was a latency of 5 s before the hypoxic mixture reached the tracheal cannula. Hypoxia was applied for 3 min. In addition to the continuous measurements of vessel diameter made using the video measurement system (see above), the diameter of all vessels was measured directly on the video monitor (see above) at 1, 2, 3, 5, 7, 9 and 10 min, or until it had returned to within 5% of its control. The maximum change in diameter from control at 1, 2 or 3 min was used for statistical analysis of the response evoked by hypoxia (see Results).

In some experiments, an arterial sample (140 μl) was removed from the brachial artery in normoxia and at the end of the 2nd min of hypoxia for measurement of arterial O_2 pressure (P_{a,O_2}), arterial CO_2 pressure (P_{a,CO_2}) and arterial pH which was the same time at which we measured arterial pressure and heart rate for analysis. Our previous studies have shown that at the end of the 2nd min, the respiratory responses, as well as the change in arterial pressure and heart rate, has reached a maximum (Marshall & Metcalfe, 1988*b*, 1989). Thus, we can be reasonably certain that at this time also the blood gas values have reached a steady state. The sample was analysed using a blood micro-system (Radiometer BMS Mk2) and digital acid–base analyser (Radiometer, PH M72 Mk2). Unfortunately, we found it difficult to keep the brachial cannula patent whilst the rat was arranged under the microscope for trans-illumination of the spinotrapezius. We did not wish to take blood from the femoral artery cannula as this disturbed the recording of arterial pressure and did not wish to cannulate the other femoral artery or a carotid artery as this would have removed a large mass of tissue from the general circulation, or disrupted the supply to the carotid body chemoreceptors respectively. Thus, we have only limited data on blood gases.

In experiments on seventeen rats, a 6% O_2, hypoxic mixture was administered 8 times, separated by intervals of 30 min, in order to assess the reproducibility of the responses in single vessels. Measurements were made exactly as described above.

Accuracy of measurements. The measurements of vessel diameter made on-line using the video measurement system were within 4–8% of those made directly from video recordings played back on the monitor. The discrepancies were largely attributable to movement artifacts caused by

respiration. The estimated error of measurements made directly from the monitor was 2–3% of the control diameter.

Statistical analyses. All results are given as a means ± S.E.M. Changes in vessel diameter are expressed as a percentage of the control diameter, the latter being a mean of the measurements made before the onset of the hypoxia stimulus (see above). Comparisons within and between groups were made using Student's paired and unpaired *t* test respectively; $P < 0.05$ being taken to indicate a significant difference.

RESULTS

Comparison of the effects of 12 and 6% O_2

Arterial pressure and heart rate

Hypoxia produced an increase in respiration and generally a gradual fall in arterial pressure and increase in heart rate (see Fig. 1). In response to a given level of inspired O_2, the evoked fall in arterial pressure tended to be smaller when the animal was lightly anaesthetized, as judged by corneal and paw withdrawal reflexes, while the evoked tachycardia tended to be greater (cf. Marshall & Metcalfe, 1988*b*). Moreover, under light anaesthesia, hypoxia sometimes evoked a short-lasting increase in arterial pressure and heart rate. Such episodes usually occurred within the first 1–1·5 min of hypoxia and were accompanied by pupillary dilation, exophthalmus, whisker twitching and sometimes piloerection. They presumably represented activation of the brain stem defence areas by peripheral chemoreceptor stimulation (see Marshall, 1987; Marshall & Metcalfe, 1988*b*). As such episodes were unpredictable, and not consistent from one hypoxic stimulus to the next, we concentrated on the gradual changes in arterial pressure and heart rate. These usually reached their maximum at the 2nd min of hypoxia, so measurements for statistical analyses were made at this time (cf. Marshall & Metcalfe, 1988*b*). In twenty-seven rats during 12% O_2 mean arterial pressure fell on average by 11·9 ± 3·4% from the control value of 118 ± 23 mmHg, while heart rate increased from 417 ± 10 to 423 ± 15 beats min^{-1}. In a further eighty-four rates during 6% O_2 mean arterial pressure fell by 19·0 ± 4·0% from the control value of 135 ± 18 mmHg, while heart rate increased from 432 ± 4 to 447 ± 5·1 beats min^{-1}. The differences between the changes evoked by 12 and 6% O_2 did not reach significance. In experiments in which arterial blood gases were analysed, P_{a,O_2}, P_{a,CO_2} and arterial pH changed from 80·5 ± 6·5 mmHg, 37·0 ± 2·1 mmHg and 7·36 ± 0·04 respectively (n = 22 where n is the number of rats) during air breathing, to 45·0 ± 6·9 mmHg, 26·3 ± 2·4 mmHg and 7·43 ± 0·02 (n = 9) respectively by the end of the 2nd min of 12% O_2 and to 28·9 ± 3·7 mmHg, 22·9 ± 3·7 mmHg and 7·47 ± 0·04 (n = 13) respectively by the end of the 2nd min of 6% O_2.

Vessel diameter

The vessels of the spinotrapezius muscle were classified according to their positions and diameters as described previously (Marshall, 1982). Briefly, the arteries that supply the muscle and their immediate branches are designated main arteries (40–90 μm, internal diameter i.d.). Arterial vessels of 22–50 μm (i.d.) and 13–18 μm (i.d.) are termed primary arterioles and secondary arterioles, while the branches that give rise to capillaries are designated terminal arterioles (7–13 μm i.d.). On the

venous side, the vessels that drain capillaries are termed collecting venules (9–18 μm i.d.). These converge into secondary and primary venules (18–30 μm and 40–60 μm i.d. respectively) and thence into main veins (65–130 μm i.d.) which drain the muscle.

Hypoxia (12 and 6% O_2) induced changes in diameter in all sections of the arterial and venous trees (Fig. 2). The recordings made with the video measurement system

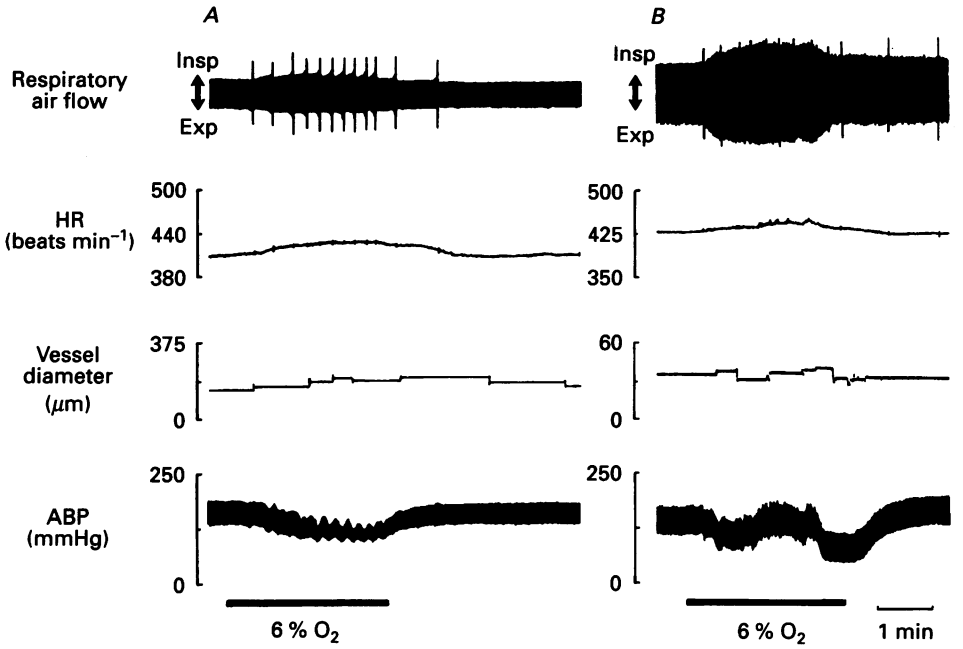

Fig. 1. Examples of responses evoked in two different animals by systemic hypoxia. Traces from above downwards are respiratory airflow (inspiration (Insp) up, expiration (Exp) down), heart rat (HR, beats min^{-1}), vessel diameter (μm) of secondary arteriole (*A*) and primary arteriole (*B*) as recorded by video measurement system, and arterial pressure (ABP). Horizontal bars below traces indicate 3 min period of breathing 6% O_2. Note in *A* gradual increase in vessel diameter and gradual fall in ABP, in *B* more complex changes in vessel diameter and ABP which were associated with pupillary dilatation, exophthalmus (see text).

showed that all vessels responded within 20 s of the onset of the hypoxic stimulus. They generally showed a gradual change reaching their maximum response by the end of the 2 min of hypoxia (see Fig. 1*A*). Most vessels returned to their baseline diameters within 5–8 min of the onset of hypoxia. Thus, these changes in diameter seemed to occur simultaneously with the gradual changes in arterial pressure (Fig. 1*A*).

By contrast, approximately 30% of the primary and secondary arterioles showed more complicated changes in diameter; these comprised a more rapid increase or a decrease that reached a peak within the first 1–1·5 min of hypoxia followed by a decrease, or increase respectively, towards or beyond control diameter (Fig. 1*B*). Such responses seemed to occur most commonly when the rat was lightly anaesthetized and were often associated with a short-lasting increase in arterial

pressure and heart rate as described above. These responses were difficult to separate objectively from the gradual responses. Thus, for the purposes of analysis we have taken the maximum change in diameter recorded in each vessel at 1, 2 or 3 min after the onset of hypoxia (see Methods). It should be noted that for the great majority of

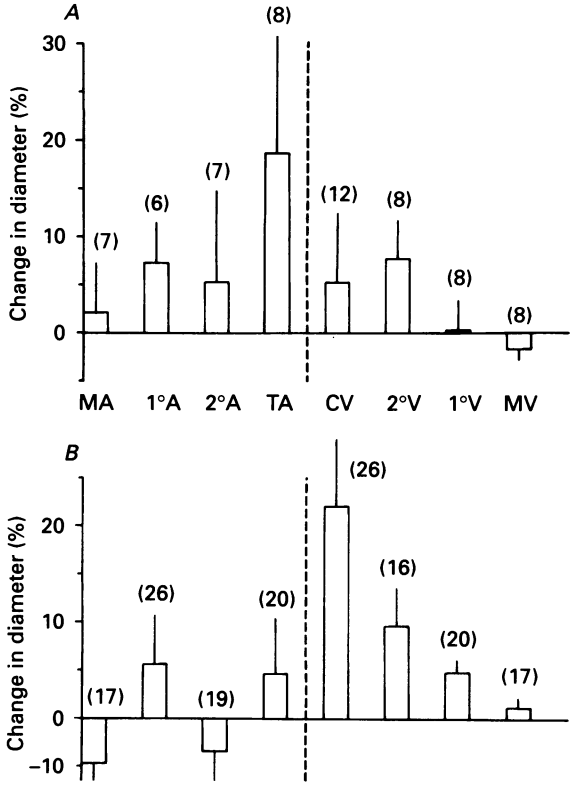

Fig. 2. Responses evoked in individual sections of arterial and venous trees by two different levels of hypoxia. *A*, 12% O_2; *B*, 6% O_2. Columns show mean change (±S.E.M.) in diameter in main arteries (MA), primary arterioles (1°A) secondary arterioles (2°A), terminal arterioles (TA), collecting venules (CV), secondary venules (2°V), primary venules (1°V) and main veins (MV). Numbers in parentheses indicate number of vessels.

vessels this maximum was that attained at the end of the 2nd min of hypoxia; the exceptions were those vessels for whom the rapid change in diameter in the first 1–1·5 min was greater than the diameter recorded at the end of the 2nd min. For analysis we used only those responses evoked during the 1st or 2nd exposure to hypoxia, since the responses evoked by a given level of hypoxia in individual vessels became more variable in subsequent exposures (see below).

Considering these maximum changes, during 12% O_2, there was a mean increase in diameter in all sections of the arterial tree, the largest mean increase being in the terminal arterioles (Fig. 2). The large S.E.M. bars reflect the fact that some arterial vessels of each type showed a decrease in diameter, this occurring in > 50% of main arterioles, but only 25% of terminal arterioles. Considering all arterial vessels

together, the mean magnitude of the decreases in diameter was $-10{\cdot}6 \pm 1{\cdot}6\%$ from control diameter ($n = 13$), while the mean magnitude of increases in diameter was $+22{\cdot}9 \pm 5{\cdot}1\%$ ($n = 15$).

Similarly, the predominating change in the venous side of the circulation was an increase in diameter, the largest mean increase occurring in the collecting and secondary venules. Decreases in diameter also occurred in all sections of the venous tree, these having their strongest influence in the main veins, which showed a net decrease in diameter (Fig. 2). The mean magnitudes of the decreases and increases in diameter were $-11{\cdot}1 \pm 2{\cdot}1\%$ ($n = 17$) and $+16{\cdot}8 \pm 3{\cdot}2$ ($n = 17$) from control diameter respectively. Approximately 12–14% of each vessel type showed no measurable change in diameter during 12% O_2.

During 6% O_2, considerably more vessels showed a measurable change in diameter than during 12% O_2, i.e. 94% of all arterial vessels and 100% of venous vessels. Amongst the arterial vessels, the direction of change was just as variable as during 12% O_2, the resultant mean change being a decrease in the diameter of main arteries and secondary arterioles, but a mean increase in the diameter of secondary and terminal arterioles (Fig. 2). The mean magnitudes of the decreases and increases in diameter were $-16{\cdot}0 \pm 7{\cdot}8\%$ ($n = 45$) and $+18{\cdot}2 \pm 3{\cdot}1\%$ ($n = 37$) from control respectively. By contrast, there was more uniformity on the venous side; the great majority of all venous vessels showed increases in diameter, the mean increases being greatest in collecting venules and smallest in main veins (Fig. 2). Considering all venous vessels together, the mean magnitudes of the decreases and increases in diameter were $-13{\cdot}3 \pm 1{\cdot}9\%$ ($n = 20$) and $+21{\cdot}1 \pm 3{\cdot}6\%$ ($n = 59$) from control respectively.

Analysis of responses recorded in individual spinotrapezius preparations during a single period of hypoxia revealed no obvious correlation between the direction of diameter changes seen in arterial vessels that branched from one another, nor in venous vessels that branched from one another. For example, whilst a primary arteriole showed an increase in diameter, of the two secondary arterioles that branched from it, one showed an increase and the other a decrease in diameter. Further, a terminal arteriole that showed an increase in diameter could be a branch either of a secondary arteriole that showed an increase, or of one that showed a decrease in diameter. Although we do not have enough data on blood gas values to test whether there was a correlation between the changes in vessel diameter and systemic P_{a,O_2}, P_{a,CO_2} or pH, these observations indicate that such a correlation is extremely unlikely.

We wondered whether the magnitude or direction of the change in vessel diameter observed during hypoxia might be correlated with the magnitude of the concomitant fall in arterial pressure. However, this was not the case for any type of arteriole nor venule, nor for the grouped populations of arterioles or of venules (Pearson correlation coefficients $< 0{\cdot}5$ in each case).

Effect of repeated exposure to 6% O_2

In a group of seventeen rats, there were no significant differences between the changes in arterial pressure and heart rate evoked during eight exposures to 6% O_2 and there was no significant change in the baseline level of arterial pressure or heart

rate over the course of the experiment. The changes evoked by the first three exposures are shown in Fig. 3; on the 8th exposure, arterial pressure fell by $14{\cdot}0 \pm 4{\cdot}4\%$ and heart rate increased by $11{\cdot}0 \pm 7{\cdot}2$ beats min^{-1}.

However, whilst the changes in vessel diameter evoked the first two exposures to 6% O_2 were not significantly different for any vessel type, the changes recorded in

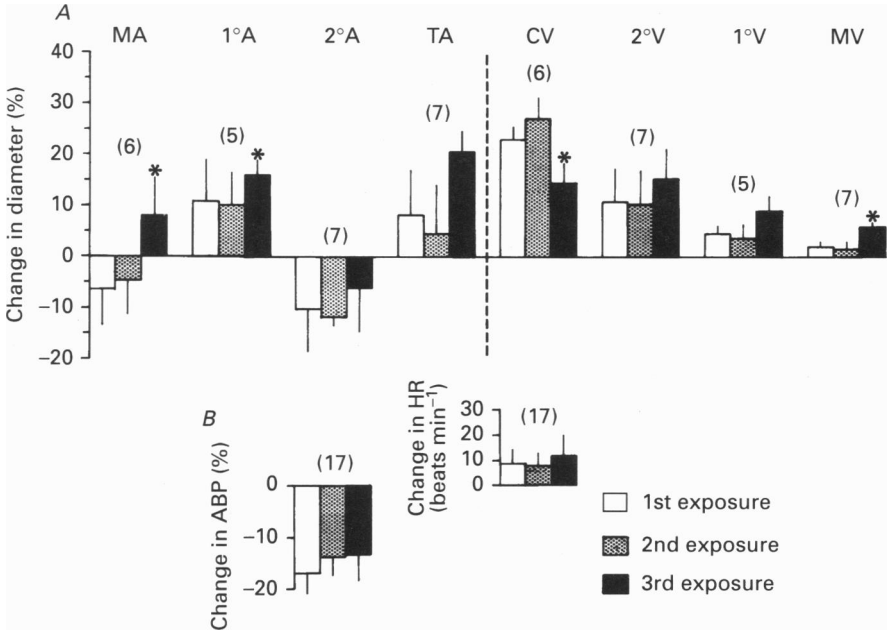

Fig. 3. Changes in vessel diameter, arterial pressure and heart rate evoked by repeated exposure to hypoxia. *A*, percentage changes in vessel diameter, abbreviations as in Fig. 2. *B*, percentage changes in arterial pressure (ABP) and changes in heart rate (HR, beats min^{-1}) recorded during changes in vessel diameter, the number of animals from which measurements were made being indicated in parentheses. Open, shaded and filled columns indicate 1st, 2nd, 3rd exposure to 6% O_2 respectively. Asterisks indicate significant differences from control response ($P < 0{\cdot}05$).

the 3rd and subsequent exposures to 6% O_2 were different from those recorded in the first exposure (Fig. 3). In all types of arterial and venous vessels, except collecting venules, the mean changes in diameter tended towards a larger increase in the 3rd exposure (Fig. 3). This tendency was maintained in subsequent exposures but the variability of the changes seen in each vessel type, even in collecting venules, also increased. Thus during the 8th exposure the mean percentage changes in diameter were $+11{\cdot}5 \pm 17{\cdot}0$*, $+30{\cdot}0 \pm 30{\cdot}5$*, $+25{\cdot}5 \pm 31{\cdot}5$*, $+32{\cdot}0 \pm 35{\cdot}4$* in main arteries, primary arterioles, secondary arterioles and terminal arterioles respectively, and $+35{\cdot}0 \pm 26{\cdot}3$, $+13{\cdot}0 \pm 13{\cdot}2$, $+11{\cdot}3 \pm 7{\cdot}2$* and $+8{\cdot}9 \pm 11{\cdot}2$* in collecting venules, secondary venules, primary venules and main veins respectively (* indicating $P < 0{\cdot}05$ for the difference between the change recorded in the 1st and 8th exposure).

DISCUSSION

Changes in arterial pressure and heart rate

The systemic cardiovascular changes induced by systemic hypoxia in the present study were comparable to those reported previously (Marshall & Metcalfe, 1988*b*, 1989) in that they included a gradual tachycardia and fall in arterial pressure. The short-lasting increases in arterial pressure and heart rate that occurred particularly at the onset of hypoxia, could be attributed to activation of the brain stem defence areas (see Results; Marshall, 1987; Marshall & Metcalfe, 1988*b*).

The magnitude of the gradual fall in arterial pressure recorded during 12% O_2 (−12% from control) was similar to that recorded previously (−20 and −10% from control; Marshall & Metcalfe, 1988*b*, 1989). However, the fall recorded during 6% O_2 (−19% from control) was smaller than those recorded previously (−40 and −35%; Marshall & Metcalfe, 1988*b*, 1989). We previously showed that the fall in arterial pressure was graded with the P_{a,O_2} and was also dependent on the level of Saffan anaesthesia (see Results; Marshall & Metcalfe, 1988*b*). However, the levels of P_{a,O_2}, P_{a,CO_2} and pH recorded in the present study were comparable to those in rats prepared for recording regional blood flows, both during air breathing and when breathing 12 or 6% O_2 (cf. Marshall & Metcalfe, 1988*b*). We aimed to keep the same level of anaesthesia in the present, as in previous experiments. The only obvious differences between the present and previous studies is that, we previously used home-bred Sprague–Dawley rats, whereas the present experiments were performed predominantly on home-bred Wistars. Whatever the reason, if the fall in arterial pressure is predominantly attributable to a decrease in the vascular conductance of skeletal muscle as we have proposed (Marshall & Metcalfe, 1988*b*), then it might be expected that, in the present experiments, vasodilator influences upon skeletal muscle would be smaller and/or that vasoconstrictor influences would be greater.

The nature of the changes in vessel diameter

The relatively fast increases and decreases in the diameter of primary and secondary arterioles that occurred in the first 1–1·5 min of hypoxia in association with a short-lasting rise in arterial pressure (see above), were probably part of the alerting stage of the defence response. This response includes dilatation in skeletal muscle, which is sometimes preceded by constriction, whether it is initiated by peripheral chemoreceptor stimulation, or by stimulation in the central nervous system (Marshall, 1987; Yardley & Hilton, 1987). We could not analyse these responses because they did not occur consistently and were not easy to separate temporally from more gradual changes in diameter. That they were included in our statistical analyses probably contributed to the variability of the data.

Since the more gradual diameter changes that occurred in the majority of vessels were accompanied by a gradual fall in systemic arterial pressure, consideration must be given to whether they were a direct consequence of the arterial pressure change, or contributed to it. Muscle arterioles generally do not respond passively to falls in perfusion pressure (Sullivan & Johnson, 1981*b*). Moreover, it is unlikely that the *increases* in arteriolar diameter reflected a myogenic dilatation, for this would have been expected to be preceded by an initial passive decrease in diameter, or at least

by a period when diameter did not change (Johnson, 1980). As far as we could tell, the gradual increases in arteriolar diameter occurred simultaneously with the fall in arterial pressure. Further, there was no statistical correlation between the changes in diameter and arterial pressure, which supports the idea that the former were not consequences of the latter. Thus, we conclude that the decreases and increases in arteriolar diameter reflected active constrictor and dilator responses respectively that contributed to the change in vascular conductance of the spinotrapezius and in arterial pressure.

In previous studies, the venous vessels of the spinotrapezius showed no measurable changes in diameter during stimulation of the sympathetic supply to the muscle, baroreceptor stimulation and functional hyperaemia when we had good reason to suppose that the pressure within them changed substantially (Marshall, 1982; Marshall & Tandon, 1984; Hébert & Marshall, 1988). This is consistent with the direct evidence of House & Johnson (1986) that venules of the cat sartorius muscle (25–185 μm diameter) showed no change in diameter when arterial pressure was reduced in steps to 20 mmHg and venular pressure fell proportionately. Thus, we propose that the venous diameter changes observed during hypoxia represented their active constriction and dilatation, rather than passive collapse or distension respectively.

The effects of different levels of hypoxia

Although constrictor responses occurred in some arterioles during 12% O_2, dilator responses predominated in all sections of the arterial tree. This accords with the mean increase in the vascular conductance of limb muscle during 12% O_2 (Marshall & Metcalfe, 1988*b*, 1989). During 6% O_2, constrictor responses were more apparent in all arterial sections, which contrasts with the greater mean increase in conductance of limb muscle at 6 than 12% O_2 (Marshall & Metcalfe, 1988*b*, 1989). But, in the latter studies, some individual animals did show a decrease in limb conductance in response to each level of hypoxia and, during 6% O_2, these decreases were greater in magnitude than those recorded in response to 12% O_2. Indeed, this was our reason for suggesting that there is competition between constrictor and dilator influences upon skeletal muscle during hypoxia (Marshall & Metcalfe, 1988*b*). That the constrictor influences were stronger in the present study is consistent with the fact that the hypoxia-induced fall in arterial pressure was smaller than in our previous studies (see above). Moreover, if locally released metabolites make a major contribution to the dilatation seen in skeletal muscle during hypoxia (Marshall & Metcalfe, 1988*b*), then this dilator influence might well be weaker in spinotrapezius than in hindlimb muscle. For, fast glycolytic fibres, whose oxidative metabolism would be most easily compromised by hypoxia and so would be expected to release vasodilator metabolites, make up over 50% of the total muscle mass of rat hindlimb (Armstrong & Laughlin, 1983), but only 33% of the spinotrapezius muscle (Taylor & Calvey, 1977).

By contrast, on the venous side of the circulation, dilator responses were more predominant during 6 than 12% O_2. Further, during 6% O_2, the magnitude of the dilatation was graded from the collecting venules to the main veins, the former showing the largest mean change. This pattern is reminiscent of that observed in the spinotrapezius following muscle contraction and which led us to conclude that the

venous vessels are dilated by metabolites released from skeletal muscle, the collecting venules being the most sensitive (Marshall & Tandon, 1984). It is tempting to suggest that locally released metabolites also contributed to the venous dilator responses observed during hypoxia.

The effects of repeated exposure to hypoxia

When the rat was made hypoxic on more than two occasions, the variance of the diameter changes of all types of arterial and venous vessels increased, largely because the dilator responses evoked in some vessels became greater. This was unexpected and not easy to explain as the change in vascular conductance of limb muscle evoked in an individual animal by a given level of hypoxia is consistent and the diameter changes evoked in individual vessels by sympathetic stimulation, catecholamines and muscle contraction, were reproducible (J. M. Marshall, unpublished observations).

These findings are very important in relation to the design of experimental protocols. For example, only if an antagonist is applied between the first and second period of hypoxia could we reasonably propose that any change in the response of a given vessel is attributable to the action of the antagonist (see Mian & Marshall, 1991).

The variability of the microvascular responses

Even if we consider only the first two exposures to hypoxia, there was considerable variability amongst the sections of the spinotrapezius microcirculation in the responses induced by hypoxia. Although the hypoxic mixtures the animals breathed were standard (12 or 6% O_2), there were differences between animals in the magnitude of the reflex hyperventilation induced by each mixture, to judge from the variance of the changes in P_{a,O_2} and P_{a,CO_2}. Differences in the changes in P_{a,O_2} and P_{a,CO_2} *per se* and in the associated reflex effects upon the cardiovascular system might explain some of the variability at the level of the microcirculation, but would not explain why two branches of the same vessel should respond differently during a given period of hypoxia. Rather, we propose that variability is characteristic of the behaviour of muscle microcirculation during systemic hypoxia. There are no studies with which ours may be directly compared, but there have been two studies on the rat cremaster muscle. Hutchins, Bond & Green (1974), who observed arterioles of less than 50 μm during administration of 18% O_2 for 1–2 min, and Morff, Harris, Weigman & Miller (1981), who observed arterioles and venules of over 100 μm diameter during administration of 10% O_2 for 1 h, showed a mean increase and a mean decrease in vessel diameter respectively and in both studies there was great variability about the mean values.

Such variability, at least amongst arterial vessels, could be inferred from the large variance of the changes in vascular conductance of the muscles of the whole hindlimb (Marshall & Metcalfe, 1988*b*, 1989), for changes in gross vascular conductance represent an average of the changes in vascular conductances in the many individual vessels of the microcirculation that are in series and parallel with one another. Further, in previous studies on the spinotrapezius muscle in which we observed the effects of more specific stimuli, for example, selective stimulation of sympathetic nerve supply, metabolites released by contracting muscle fibres and exogenous

catecholamines (Marshall, 1982; Marshall & Tandon, 1984; Hébert & Marshall, 1988), there were major differences between vessel types in their behaviour during a given stimulus and differences within vessel types in the magnitude of the response. During systemic hypoxia, the influences of sympathetic activity, of local metabolites and of circulating catecholamines upon muscle microcirculation, would be expected to change simultaneously (see Marshall & Metcalfe, 1988*b*, 1989; Mian *et al.* 1990). Thus, the response seen in any given vessel would be expected to reflect the relative importance of such influences; this may be dependent on density of the sympathetic innervation, or adrenergic receptors and on the O_2 consumption and metabolism of nearby muscle fibres.

In summary, the present study has shown that systemic hypoxia produces both increases and decreases in the diameter of individual arterioles of the spinotrapezius muscle, these responses occurring simultaneously with a fall in arterial pressure. The direction and magnitude of diameter change in any given arteriole was consistent during the first two repetitions of the hypoxic stimulus. Similarly, systemic hypoxia induced both increases and decreases in the diameter of individual venules. But, increases in diameter predominated, there being a gradient from the venules nearest to the capillary bed which showed the largest increases in diameter, to those furthest from the capillary bed which showed the smallest diameter changes. Further studies will be required to elucidate the factors that are responsible for these diameter changes and whether they vary from one section of the vascular tree to another; the following paper indicates the roles of α- and β-adrenoreceptor stimulation (Mian & Marshall, 1991).

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