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EFFECTS OF SYSTEMIC HYPOXIA ON THE DISTRIBUTION OF CARDIAC OUTPUT IN THE RAT

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SUMMARY

1. Studies were made in unanaesthetized rats of cardiovascular responses induced during 3 min periods of systemic hypoxia (inspirate 8 or 6% O_2). Arterial pressure and heart rate were recorded continuously; cardiac index and regional blood flows were measured in normoxia and at the 2nd min of hypoxia by injection of radiolabelled microspheres. Comparisons are made with changes recorded in Saffan-anaesthetized rats during 8% O_2 using microspheres and in previous studies using electromagnetic transducers on renal, mesenteric and femoral arteries (Marshall & Metcalfe, 1988*a*).

2. In unanaesthetized rats, the initial 1–1·5 min of hypoxia evoked behavioural arousal associated with a short-lasting rise in arterial pressure and heart rate. This agrees with our previous proposal that hypoxia activates the brain stem defence areas by stimulating peripheral chemoreceptors.

3. In unanaesthetized rats, these changes were superimposed upon a gradual fall in arterial pressure and tachycardia, the responses being greater during 6 than 8% O_2 (cf. Saffan-anaesthetized rats). Further, in all rats at the 2nd min of hypoxia, cardiac index and vascular conductance of most body tissues was increased. It is concluded that the fall in arterial pressure is due to peripheral vasodilatation.

4. In the unanaesthetized rats, the tendency for vascular conductance in kidney, intestine and gastrocnemius muscle to increase (more during 6 than 8% O_2) allowed increases in blood flow in the last two regions. These changes accord with those recorded under Saffan anaesthesia.

5. In both unanaesthetized and anaesthetized rats, hypoxia induced pronounced increases in vascular conductance of diaphragm, adrenal gland, cerebral hemispheres, cerebellum and brain stem, the resultant increases in blood flow being larger in the unanaesthetized rats.

6. It is proposed that in unanaesthetized, as in anaesthetized, rats the regional dilator responses predominantly reflect the local dilator effects of tissue hypoxia. Possible dilator factors are considered.

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INTRODUCTION

In recent studies we have analysed the cardiovascular responses produced by systemic hypoxia in spontaneously breathing rats, anaesthetized with Saffan (Glaxovet), using electromagnetic transducers to continuously record regional blood flows (Marshall & Metcalfe, 1988*a*, 1989*a*). We showed that graded levels of hypoxia produced by administering 15, 12, 8 or 6% O_2 in N_2 induced gradual tachycardia, a fall in arterial pressure and vasodilatation in renal, mesenteric and muscle vasculature. At the end of the 2nd min of a 3 min period of hypoxia, the magnitudes of these changes were graded with the level of hypoxia. In addition, the first 1–1·5 min of hypoxia often induced the characteristic pattern of the alerting stage of the defence response, viz. tachycardia, a rise in arterial pressure, and renal and mesenteric vasoconstriction with muscle vasodilatation. This pattern was superimposed upon the gradual changes already described. We deduced it was attributable to activation of the defence areas by hypoxic stimulation of the peripheral chemoreceptors (Marshall & Metcalfe, 1988*a*).

It is generally accepted that anaesthetics depress or distort central neural control of the cardiovascular system. However, it has been argued that Saffan offers a significant advantage over the commonly used anaesthetics like chloralose and barbiturates because it allows expression of hypothalamic, limbic and cortical influences over the cardiovascular system (Timms, 1981, 1982). Indeed, anaesthetics like chloralose, barbiturates, and urethane prevent the autonomic components of the alerting/defence response from being elicited by stimulation of peripheral chemoreceptors, or by well-recognized afferent inputs to the brain stem defence areas that can evoke the full autonomic and behavioural pattern of the defence response in the conscious animal (Abrahams, Hilton & Zbrozyna, 1960; Timms, 1981, 1982). Thus, it was particularly important for us to establish how well the cardiovascular responses evoked by systemic hypoxia under Saffan anaesthesia compared with those evoked in the unanaesthetized animal.

To this end we have carried out studies on unanaesthetized rats in which we made continuous recordings of changes in arterial pressure and heart rate induced by hypoxia and measured regional blood flows with the aid of radiolabelled microspheres (Laughlin, Armstrong, White & Rouk, 1982). The microsphere technique has the disadvantage that only a single instantaneous measurement of blood flow is obtained upon each injection of microspheres. However, it offers the advantage that blood flow can be measured in regions that could not be recorded by using electromagnetic transducers because of inaccessibility of, or low volume flow in, the supplying artery. In addition, we also carried out experiments in which microspheres were used in anaesthetized rats, so that direct comparisons could be made between the anaesthetized and unanaesthetized rats and between results obtained using microspheres and electromagnetic transducers. A brief report of some of these results has been made to the Physiological Society (Marshall & Metcalfe, 1988*b*).

METHODS

All experiments were performed on Sprague–Dawley rats; the body weights of the animals for which results are reported were 337·0 ± 6·4 g (mean ± S.E.M., $n = 8$) and 348·0 ± 9·1 g ($n = 7$) for those exposed to 8 and 6% O_2 respectively in the absence of anaesthesia and 430·0 ± 6·9 g ($n = 5$) for those exposed to 8% O_2 under Saffan anaesthesia.

Implantation of cannulae

The procedures we followed to allow the microsphere technique to be used in unrestrained, unanaesthetized rats were very similar to those described in detail by Laughlin *et al.* (1982). Thus, under anaesthesia (Immobilon, 0·2 ml I.P.), a cannula was implanted in the left renal artery to allow reference arterial samples to be taken and in the ascending aorta via the right carotid artery to allow radiolabelled microspheres to be injected and arterial pressure to be recorded.

After shaving the abdomen, and ventral and dorsal surfaces of the neck, these areas were swabbed with 0·5% chlorhexidine solution (Hibitane, ICI). The rat was then placed dorsal surface down on a sterile operating surface. All surgery was performed aseptically. Briefly, the skin of the abdomen and the body wall was opened with 1·5–2·0 mm mid-ventral incisions. The abdominal contents were carefully displaced to the right, covered with gauze soaked in physiological saline and, with the aid of a retractor, the left kidney was exposed. The left renal vein was separated from the left renal artery, ligated and divided to allow access to the renal artery. The latter was cannulated with a 250 mm length of 0·94 mm (outer diameter) sterile polyethylene tubing filled with 0·9% heparinized saline. This was advanced 2–3 mm until its tip lay just distal to the descending aorta, before being tied into place. The renal capsule was then opened, the left ureter ligated and divided, and the left kidney removed. The arterial cannula was secured to the psoas muscle with a suture, passed through the left side of the body wall and led between the skin and body musculature to be exteriorized at the nape of the neck. The body wall and skin were closed with silk sutures.

The right carotid artery was approached via a small 5–7 mm mid-ventral incision over the trachea and cannulated with a 15–20 mm length of tubing (as above). The cannula was advanced about 25 mm so that its tip would lie in the ascending aorta, just distal to the aortic valve, as estimated from cadavers. The position of the tip was verified at the end of each experiment. (As noted by Laughlin *et al.* (1982), we found that rats in which the catheter tip was inadvertently placed too close to, or even passed through the aortic valve, so that is probably interfered with normal valve functioning, were not inclined to exercise or play freely when they had recovered from anaesthesia; neither did they tolerate hypoxia. These animals were excluded from the study.) The carotid cannula was brought to the exterior via the same incision that was used for the renal artery cannula. This incision and that in the ventral surface of the neck were closed with sutures. The two cannulae were cleared with sterile, heparinized saline and each was plugged with the tip of a steel pin. They were marked so that they could be distinguished later, taped together with Sellotape and coiled together. A length of 2·5 cm wide Sellotape was used to enclose the coil in a flat pack at the nape of the neck.

Once surgery had been completed, the anaesthesia antidote was given (0·2 ml, Revivon I.P.) and antibiotic was given subcutaneously (0·05 ml Engomycin). The cannulae were flushed at approximately 12–18 h intervals and refilled with heparinized saline. All rats were allowed to recover for at least 2 days before the experimental session. Only those that appeared to be behaving normally were used, i.e. they ran around, played, groomed, fed, drank and looked healthy, as judged by eyes and hair.

Experimental procedure

The chamber used for the experiments was a wooden rectangular box (27 × 17 × 18 cm). One of the long sides had a Perspex 'window' and the lid was made of Perspex. The two short ends of the chamber opened into cones which were connected together via elephant tubing. A small fan in one of the cones allowed air and gas to be circulated through the chamber and mixed. A feeder tube, which was attached to a N_2 cylinder (BOC), was connected to the elephant tubing via a flow valve. When the chamber was to be made hypoxic, the N_2 cylinder was turned on and the flow rate adjusted so as to bring the percentage of O_2 in the chamber down to the desired level and maintain

it there. The composition of gas in the chamber was continuously monitored by a mass spectrometer, via PVC tubing which was inserted through a port in the Perspex lid, so that it hung down into the chamber.

The rat was placed in the chamber; the two cannulae were uncoiled and passed between the lid and the rim of the chamber via groves in the lid. The carotid artery cannula was connected via a double T-piece connector and a length of saline-filled tubing to a pressure transducer (Bell & Howell); one arm of the connector was used for injection of microspheres. The renal artery cannula was connected to a 1 ml syringe mounted on a withdrawal pump (Harvard). The cannulae were kept as short as possible to reduce dead space, but they were sufficiently long that the rat had complete freedom of movement within the chamber. Arterial pressure and heart rate, which was derived from the pressure signal via a rate meter (Devices), were recorded on a pen recorder (Electromed), together with the percentage of O_2 of the chamber, monitored via the mass spectrometer.

The rat was allowed approximately 30 min to settle before the experiment began. The three measurements of blood flows were made using three different infusions of 15 μm diameter microspheres labelled with Co^{57}, Sc^{46} or Sn^{113} (i) during normoxia (ii) at the end of the 2nd min of a 3 min period of hypoxia (8 or 6% O_2), and (iii) during normoxia, 10 min after the end of the period of hypoxia. The microsphere suspension consisted of 400000–500000 spheres ml^{-1} in 0·9% saline. They were thoroughly mixed on a vortex for at least 10 min prior to infusion, to ensure a uniform distribution (as determined by microscopic observation). In initial experiments, we used microspheres suspended in 10% Dextran. But, in these rats, infusion of the microspheres was frequently followed by severe hypotension and behavioural disturbances which we eventually attributed to the influence of the Dextran. These rats were excluded from the study. These effects probably reflected an anaphylactic responses to Dextran: others have noted hypotension in rats in response to Dextran (Stanek, Coleman, Smith & Murphy, 1985) and Armstrong & Laughlin (1983) reported swelling of the face and paws.

Each microsphere infusion was performed as follows: (1) withdrawal of the reference sample was begun from the renal artery at a rate of 0·542 ml min^{-1}; (2) 10 s later, 0·3 ml of microsphere suspension was injected into the ascending aorta via the carotid artery cannula and flushed in with 1 ml saline; this took about 15 s; (3) withdrawal was continued for a further 25 s after flushing, the final 140 μl being collected into a heparinized capillary tube and used to measure P_{a,O_2} and P_{a,CO_2} as described previously (Marshall & Metcalfe, 1988*b*). The total volume of blood withdrawn was 0·45 ml: in five animals in which haematocrit was measured in normoxia, at the end of hypoxia and after the final period of hypoxia, there was no significant change over the course of the experiment. The total number of microspheres given during each infusion was 130000–160000. By calculation, this should have been sufficient to ensure that the tissue sample with the lowest blood flow received at least 200 microspheres, which is the minimum required to provide 95% confidence and 10% precision for rats (Katz, Blantz, Rector & Seldin, 1971).

The blood sample withdrawn during the above procedure was placed in a 1·5 ml capped tube, the sample syringe was then flushed three separate times with 1 ml saline and these volumes were placed in three separate tubes; the radioactive counts from the four tubes were summed to give the total blood count. At the end of the experiment, the rat was killed with an overdose of Saffan. The following tissues were rapidly removed from all rats; the lungs, small intestine, the right kidney, right and left gastrocnemius muscles, spleen, adrenal glands, right and left cerebral hemispheres, cerebellum and brain stem. In addition, the diaphragm was removed from the group of rats that was exposed to 6% O_2. Each tissue sample was placed in a pre-weighed capped tube and weighed. Gamma emission from each of the tissue samples and from the blood samples was then counted using a Packard Auto Gamma 5650 counter. Tissue blood flows and cardiac index [output (100 g body weight)$^{-1}$] were calculated from the counts per minute and wet weights, using the equations:

$$\text{Blood flow} = \frac{[\text{counts (g tissue)}^{-1}] \times \text{reference blood flow (withdrawal rate)}}{\text{counts in reference sample}},$$

$$\text{Cardiac index} = \frac{\text{total counts injected} \times \text{reference blood flow}}{\text{counts in reference sample}},$$

according to standard procedures (Heymann, Payne, Hoffman & Rudolph, 1977). For the technique to be valid, one of the criteria is that the microspheres should all be trapped in the first

circulation through the tissues (see Discussion). Thus, animals in which more than 2% of the total counts occurred in the lungs in the control normoxic period were excluded from the study. The measured activity was corrected for cross-over radiation from Sc into Sn, from Sc into Co and from Sn into Co. Having obtained estimates of regional blood flows, these values were divided by mean arterial pressure at the time of measurement, to give regional vascular conductances.

Experiments under anaesthesia

Rats were anaesthetized with a continuous infusion of Saffan (7–12 mg kg^{-1} h^{-1}) and prepared for recording cardiovascular and respiratory variables as described previously (Marshall & Metcalfe, 1989*b*, *c*). Arterial pressure was recorded from the right femoral artery; heart rate was derived from the pressure recording. Respiration was recorded via a flow head attached to the trachea and an electrospirometer. Electromagnetic flow transducers were placed on the left renal artery and left femoral artery, with the left ankle ligated to exclude paw blood flow. Vascular conductances were computed on-line as blood flow divided by arterial pressure. The flow probes were calibrated by *in vitro* perfusion through freshly excised arteries. A cannula placed in the left brachial artery allowed a withdrawal sample to be taken, the final 140 μl of which was used for measurement of blood gases. In addition, as in the experiments described above, a cannula was inserted into the ascending aorta via the left carotid artery, for infusion of microspheres.

An hypoxic mixture (8% O_2 in N_2) was administered for 3 min via the respiratory flow head as described previously (Marshall & Metcalfe, 1988*b*, *c*). Regional blood flows were measured in normoxia, at the end of the 2nd min of hypoxia and 10 min after hypoxia as described above, microspheres being injected via the carotid cannula, and the reference sample being taken from the brachial artery. The animal was killed with an overdose of Saffan and the tissues removed and counted as described above.

All data are expressed as mean ± S.E.M. Statistical comparisons within groups were made using Student's paired *t* test, and comparisons between groups using Student's unpaired *t* test. $P < 0.05$ was considered significant.

RESULTS

Changes in behaviour

The time taken to lower the percentage of O_2 in the experimental chamber to 8 or 6% was 22–23 and 28–29 s respectively. Within the first 1 min after the N_2 inflow began, all rats showed obvious signs of behavioural arousal. Some individuals ran to the corner of the chamber as if looking for a means of escape. Frequently they stood upright on hindlimbs, sniffed vigorously with whiskers twitching rapidly and looked around. Others sat with weight on the hindquarters, front limbs in the air and looked around the chamber. Often the tail was raised as a stiff vertical rod. When it was possible to clearly observe the eyes, exophthalmus was apparent and the pupils were dilated. This behaviour died away during the following 0·5–1 min so that within 2 min of the percentage of O_2 in the chamber reaching the desired level, the rat was usually sitting still. Measurements of regional blood flow were made at this time, or at the earliest possible time thereafter when the rat was not moving.

In many rats there was a second and sometimes a third period of arousal during the final 45 s of hypoxia, but these episodes were shorter and less dramatic. Other rats showed no further signs of arousal; rather they sat on the floor of the chamber with front paws and head lowered, and exophthalmus and pupillary dilatation was still apparent. This latter behaviour seemed more common during exposure to 6% O_2, but otherwise there were no obvious differences between the behaviour that occurred during exposure to 8 and 6% O_2.

The percentage of O_2 in the chamber could be raised to the atmospheric level within 3 s by opening the tubing connected to the chamber to the air. Within 30 s

to 1 min, behaviour returned to normal. The rats sat on their hindquarters and began grooming, explored the floor of the chamber or reached up to grasp the arterial cannula that ran upwards to the rim of the chamber. The final measurement of regional blood flows was taken 10 min later or at the earliest time thereafter when the rat was not moving around the chamber.

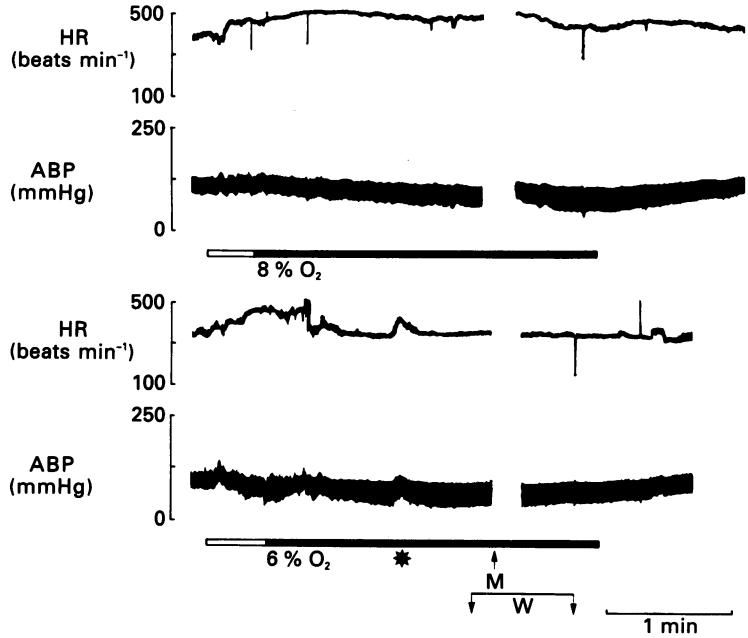

Fig. 1. Original traces showing changes in arterial pressure and heart rate evoked in two different unanaesthetized rats by exposure to 8 and 6% O_2 (above and below respectively). In each case, top trace shows heart rate (HR; beats min^{-1}); bottom trace shows arterial pressure (ABP; mmHg). The beginning of the bar beneath each set of traces indicates the time from which N_2 was added to the experimental chamber; the length of the filled part of the bar indicates the time over which the chamber was maintained at 8 or 6% O_2. W and associated arrows indicate start and finish of withdrawal from renal artery. M indicates injection of microspheres followed by washing through ABP cannula; hence break in ABP trace. Note short-lasting increase in HR during 6% O_2 (✱) which was associated with behavioural arousal.

Changes in arterial pressure and cardiac index

The average levels of mean arterial pressure in normoxia were $103{\cdot}7 \pm 6{\cdot}4$ and $124{\cdot}2 \pm 15{\cdot}2$ mmHg in the rats that were subsequently exposed to 8 and 6% O_2 respectively: these values were not significantly different. During the initial period of arousal at the onset of hypoxia and during any subsequent episodes of arousal thereafter, arterial pressure tended to rise, albeit in a rather transient, or erratic manner (Fig. 1). However, the underlying trend was that arterial pressure gradually fell during hypoxia to $90{\cdot}0 \pm 7{\cdot}7$ and $98{\cdot}0 \pm 16{\cdot}0$ mmHg at the end of the 2nd min exposure to 8 and 6% O_2 respectively. These changes represented a significant fall of $13{\cdot}75 \pm 3{\cdot}2$ and $22{\cdot}7 \pm 3{\cdot}4$% respectively from control levels. At the end of the

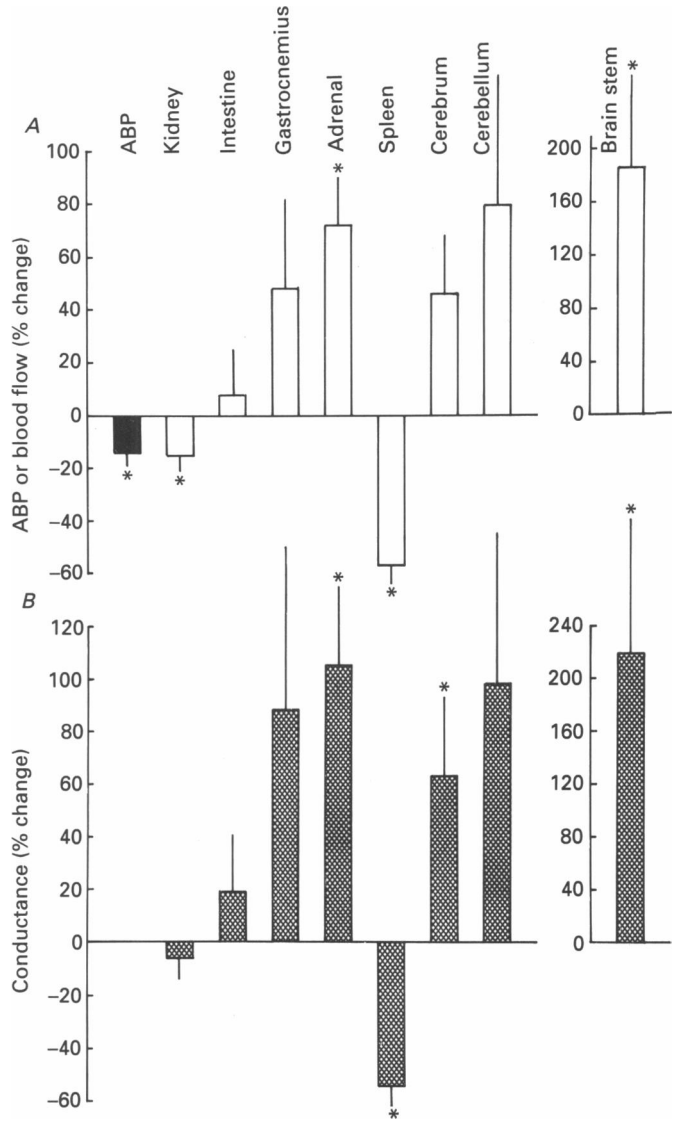

Fig. 2. Changes in arterial pressure, regional blood flows and vascular conductances induced in unanaesthetized rats by the end of the 2nd min of exposure to 8% O_2. *A*, changes in arterial pressure (ABP, filled columns), tissue blood flows (open columns). *B*, changes in regional conductances (cross-hatched columns). All values are expressed as mean percentage change (±S.E.M.) from control (normoxic) values ($n = 8$). Asterisks indicate significant change from control values ($P < 0.05$). Note scales for brain stem are half those for other tissues.

period of hypoxia arterial pressure gradually returned to the control level (Fig. 1) and at the 10th min after hypoxia it was not significantly different from the original control level.

Heart rate was very labile in normoxia. It varied between 325 and 500 beats min^{-1} tending to rise whenever the animal moved, even if the movement just involved clean-

ing the face with the forepaws. For this reason it seems inappropriate to give a value for 'resting' or 'control' heart rate. However, at the onset of hypoxia, heart rate consistently rose as can be seen from Fig. 1. It remained high whilst the animal was aroused and when the animal quietened down it fell somewhat, to a level that was generally higher than that recorded just prior to hypoxia. When the rat showed a further episode of arousal during hypoxia this was associated with a short-lasting increase in heart rate (see Fig. 1).

During normoxia, cardiac index, as estimated from the microsphere injections, was $404{\cdot}0 \pm 43{\cdot}7$ ml min^{-1} (100 g body wt)$^{-1}$ in the rats exposed to 8% O_2 and $475{\cdot}0 \pm 48{\cdot}5$ ml min^{-1} (100 g body wt)$^{-1}$ in those exposed to 6% O_2. From these levels cardiac index rose by $22{\cdot}9 \pm 19{\cdot}1$% by the 2nd min of 8% O_2 and by $25{\cdot}9 \pm 17{\cdot}6$% by the 2nd min of 6%; these changes were not significantly different.

Changes in blood gases

In normoxia, the mean levels of P_{a,O_2} were $81{\cdot}7 \pm 7{\cdot}9$ and $78{\cdot}9 \pm 7{\cdot}0$ mmHg in the groups of animals that were exposed to 8 and 6% O_2 respectively, while the mean levels of P_{a,CO_2} were $35{\cdot}3 \pm 4{\cdot}5$ and $34{\cdot}9 \pm 2{\cdot}8$ mmHg respectively. There were no significant differences between the two groups. By the end of the 2nd min of exposure to 8 and 6% O_2, P_{a,O_2} had decreased significantly to $29{\cdot}5 \pm 3{\cdot}9$ and $23{\cdot}7 \pm 4{\cdot}2$ mmHg respectively, these values being significantly different. Concomitantly, P_{a,CO_2} decreased significantly to $22{\cdot}9 \pm 3{\cdot}5$ and $19{\cdot}0 \pm 4{\cdot}4$ mmHg in 6 and 8% O_2 respectively, these values not being significantly different.

Regional blood flows

The control values of blood flows to various organs, as measured using the radiolabelled microspheres, are shown in Table 1 in columns (i) and (ii) for those subsequently exposed to 8 and 6% O_2 respectively. There were no significant differences between them except for the small intestine (Table 1); there was no obvious reason for this disparity. Of particular importance was that in each group of animals there was no significant difference between the control blood flows recorded in the right and left gastrocnemius muscles, nor between those recorded in the right and left cerebral hemispheres (see Discussion).

The percentage changes in the regional blood flow and vascular conductances that occurred by the end of the 2nd min of exposure to 8 and 6% O_2 are shown in Figs 2 and 3 respectively. As there was no significant difference between the changes recorded in the right and left gastrocnemius, nor in the right and left cerebral hemispheres during either 8 or 6% O_2, the mean changes for the two sides are presented in each case. As can be seen, renal blood flow showed a small, but significant decrease in response to 8% O_2, which could be accounted for by the fall in arterial pressure, since renal vascular conductance hardly changed (Fig. 2). By contrast, at 6% O_2 renal vascular conductance increased significantly, allowing renal blood flow to remain more or less constant despite the larger fall in mean arterial pressure (Fig. 3). Apart from this, the general trend was for blood flow and vascular conductances to increase during hypoxia in each of the vascular beds and for the increases to be larger during 6% O_2 (cf. Figs 2 and 3). However, as is clear from the S.E.M. bars, there was considerable variation in the changes recorded particularly in response to 8% O_2.

In the small intestine and gastrocnemius muscle this variability represented qualitative as well as quantitative differences between animals. Thus, during 8% O_2, intestinal blood flow increased in three animals, but showed no change or a decrease in the remainder, while intestinal vascular conductance increased in five out of eight

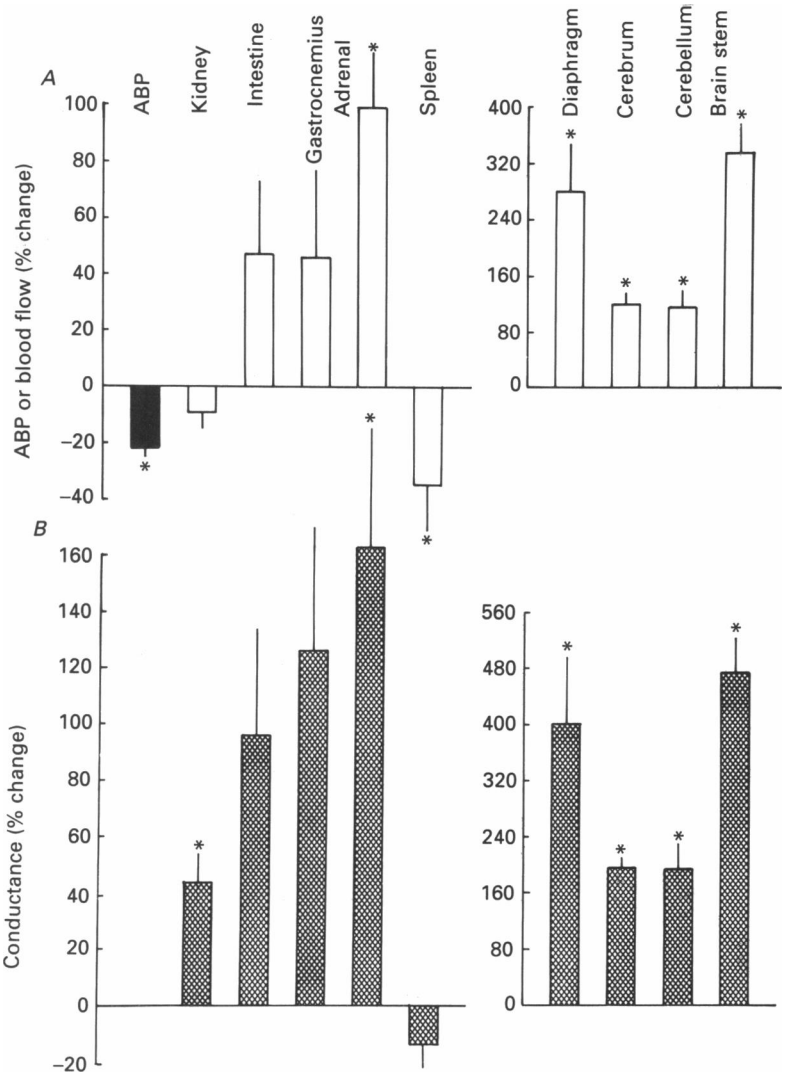

Fig. 3. Changes in arterial pressure, regional blood flows and vascular conductances induced in anaesthetized rats by the end of the 2nd min of exposure to 6% ($n = 7$). Abbreviations, shading and ordinates as in Fig. 2, but note different scales as indicated.

animals. During 6% O_2, intestinal blood flow and vascular conductance decreased in only one animal and increased or remained constant in the remainder. On the other hand, during 8% O_2, gastrocnemius blood flow and vascular conductance increased in only three out of eight animals and decreased in the remainder, while at 6% O_2,

both gastrocnemius blood flow and vascular conductance increased in five animals and decreased in the other two.

The direction of change was far more consistent in the other tissues (see Figs 2 and 3). In the brain regions during 8% O_2, blood flow and vascular conductance to the cerebral hemispheres and cerebellum increased in six animals; in the remaining two

TABLE 1. Tissue blood flows (ml min^{-1} 100 g^{-1}) during initial period of normoxia in unanaesthetized rats subsequently exposed to 8 and 6% O_2 (columns (i) and (ii) respectively) and in anaesthetized rats (column (iii))

	(i)	(ii)	(iii)
Kidney	5·47 ± 0·39	4·52 ± 0·21	6·44 ± 0·34
Small intestine	1·00 ± 0·14	2·29 ± 0·21†	2·48 ± 0·49
Right gastrocnemius	0·21 ± 0·05	0·23 ± 0·04	—
Left gastrocnemius	0·19 ± 0·03	0·22 ± 0·06	0·024 ± 0·0045*
Diaphragm	—	0·61 ± 0·09	0·87 ± 0·39
Adrenals	2·01 ± 0·23	2·32 ± 0·30	3·23 ± 1·19
Spleen	2·25 ± 0·21	1·53 ± 0·25	—
Right cerebum	0·94 ± 0·14	0·97 ± 0·07	0·49 ± 0·05
Left cerebum	1·03 ± 0·13	0·93 ± 0·07	0·73 ± 0·06
Cerebellum	0·72 ± 0·10	0·80 ± 0·09	1·029 ± 0·17
Brain stem	0·39 ± 0·04	0·46 ± 0·03	0·74 ± 0·19

Values are means ± S.E. of the mean.

* Indicates significant difference between (iii) and (i) and between (iii) and (ii) ($P < 0·05$ in each case). † indicates significant difference between (i) and (ii) ($P < 0·05$).

cerebral blood flow fell with the arterial pressure. However, in the brain stem during 8% O_2 and in the cerebral hemispheres, cerebellum and brain stem during 6% O_2, blood flow increased in all animals, i.e. vascular conductances increased consistently and to extents that more than compensated for the fall in arterial pressure. Similarly, blood flow and vascular conductances in the adrenal glands increased both during 8 and 6% O_2. Diaphragm blood flow and vascular conductance, which was only recorded in the rats exposed to 6% O_2, showed substantial increases in all animals (Fig. 3). By contrast, blood flow and vascular conductance of the spleen decreased significantly both during 8 and 6% O_2 (Figs 2 and 3).

The regional blood flows measured 10 min after the end of the hypoxic period were not significantly different for any of the tissues, from the values recorded in the initial normoxic period.

Experiments under anaesthesia

In the rats under Saffan anaesthesia, the average level of mean arterial pressure in normoxia was 153·3 ± 7·0 mmHg, which was significantly higher than the levels recorded in the unanaesthetized rats (see above). Cardiac index during normoxia was 298·0 ± 18·9 ml min^{-1} (100 g body wt)$^{-1}$; this was not significantly lower than the values recorded for the unanaesthetized rats.

The regional blood flows recorded in normoxia are shown in Table 1, column (iii). In these animals we measured flow to the left kidney both using microspheres and an electromagnetic transducer (see below), whereas in the unanaesthetized rats we

recorded blood flow to the right kidney. The blood flows recorded using microspheres in the left kidney in the anaesthetized rats and in the right kidney in the unanaesthetized rats were not significantly different.

The blood flow in the *right* gastrocnemius of the anaesthetized rats was significantly less than that measured in the *left* gastrocnemius muscle because the blood flow to the right hindlimb was impaired by the cannula in the right femoral artery. Thus, we have excluded data from the right gastrocnemius muscle. The blood flow to the left gastrocnemius of the anaesthetized rats was significantly less than that of the unanaesthetized rats (Table 1).

Further, in the anaesthetized rats, the blood flow to the *right* cerebral hemisphere was significantly lower than that to the *left* hemisphere (Table 1, column (iii)). This presumably reflected the presence of the cannula in the right carotid artery (see Discussion). Thus, we have not presented any further data from the right hemisphere.

Apart from these discrepancies, the control blood flows for the small intestine, cerebellum, brain stem, diaphragm and adrenal glands were not significantly different from those recorded in the unanaesthetized animals, although cerebral blood flow tended to be lower and cerebellar and brain stem blood flow tended to be higher in the anaesthetized than in the unanaesthetized animals (Table 1).

During 8% O_2, mean arterial pressure fell to $101{\cdot}0 \pm 11{\cdot}7$ mmHg which represents a mean change of $34{\cdot}4 \pm 6{\cdot}7$% from the control levels. Mean cardiac index increased by $19{\cdot}95 \pm 8{\cdot}14$%. Meanwhile, P_{a,O_2} and P_{a,CO_2} fell from $78{\cdot}0 \pm 1{\cdot}88$ and $37{\cdot}7 \pm 1{\cdot}19$ mmHg respectively, values which were not significantly different from those recorded in the unanaesthetized animals (see above), to $27{\cdot}24 \pm 1{\cdot}06$ and $25{\cdot}14 \pm 0{\cdot}84$ mmHg respectively, which were not significantly different from the values recorded in the unanaesthetized rats during 8% O_2.

The changes in regional blood flows and vascular conductances during 8% O_2 were essentially similar to those seen in the unanaesthetized rats (cf. Figs 4 and 2). Thus, renal blood flow decreased significantly, with the fall in systemic arterial pressure, as there was little change in renal vascular conductance. On average, the vascular conductances of the small intestine and cerebral hemisphere increased, though not significantly, but there were significant increases in the vascular conductances of the cerebellum, brain stem and adrenal. In each case the mean increases as percentage changes from the control levels were similar to those seen in the unanaesthetized animals. However, the percentage increases in blood flow in the brain regions were apparently rather smaller than in the unanaesthetized animals exposed to 8% O_2 (cf. Figs 4 and 2). By contrast, the percentage changes in both vascular conductance and blood flow to the gastrocnemius muscle were substantially greater than the unanaesthetized animals (cf. Figs 4 and 2). This was in part because only one of the anaesthetized rats did not show an increase in gastrocnemius blood flow and conductance. However, considering only those animals in which gastrocnemius blood flow and conductance did increase, these were apparently larger as percentage changes from control in the anaesthetized animals (mean 224 *vs.* 114% for flow and 435 *vs.* 294% for conductance, anaesthetized *vs.* unanaesthetized). Diaphragm blood flow and vascular conductance, which were not recorded in the unanaesthetized animals exposed to 8% O_2, were substantially increased, but to rather lesser extents than in the unanaesthetized animals exposed to 6% O_2 (cf. Figs 4 and 3).

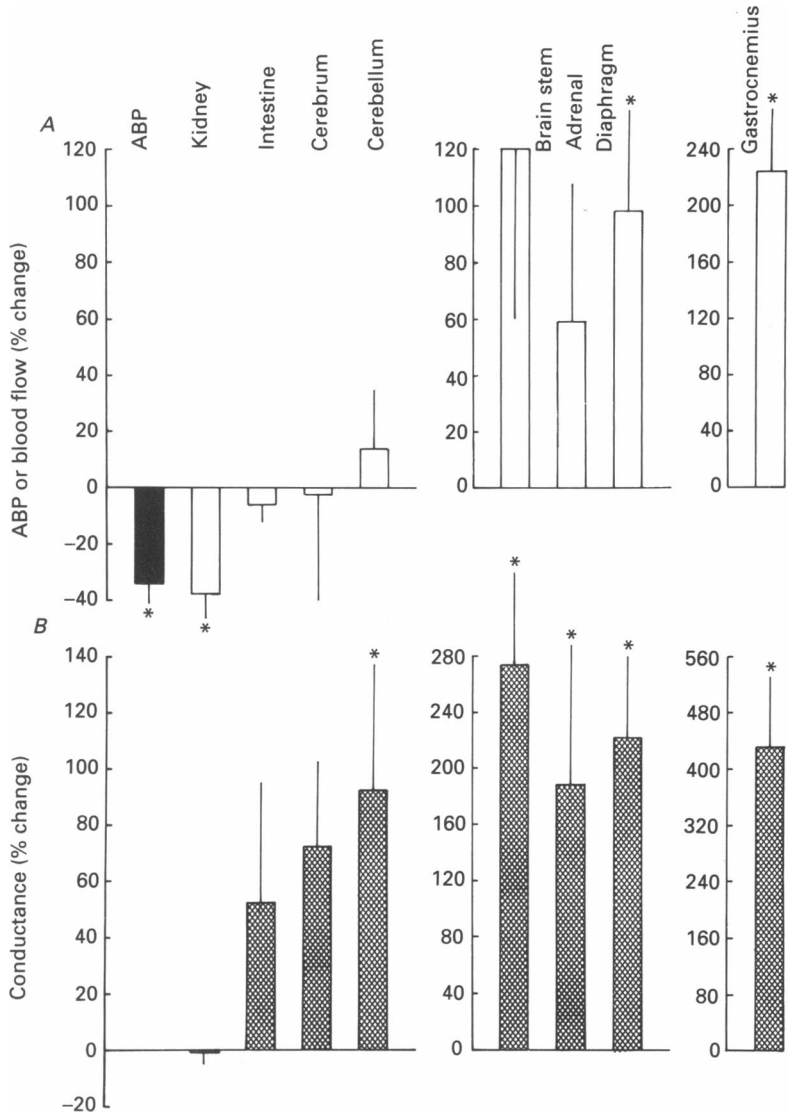

Fig. 4. Changes in arterial pressure, regional blood flows and vascular conductances induced in Saffan-anaesthetized rats by the end of the 2nd min of exposure to 8% O_2 ($n = 5$). Abbreviations, shading and ordinates as in Fig. 2, but note different scales as indicated.

As in the anaesthetized animals the blood flow rates recorded in normoxia at the 10th min after hypoxia were not significantly different from those recorded in the initial period of normoxia.

For the kidney, direct comparisons were possible between the blood flows measured with microspheres and those recorded with the electromagnetic transducer on the renal artery. Considering all the blood flow measurements made with microspheres, both during normoxia and hypoxia, and the measurements made at

the same time with the transducers, there was no significant difference between the two sets of values using the paired t test. The correlation coefficient was 0·9524, this being highly significantly different from zero ($P < 0·0001$).

Comparisons were also possible between the changes induced by hypoxia in the left gastrocnemius muscle as measured with the microspheres and in left hindlimb muscles that were served by the left femoral artery. Whilst blood flow to the gastrocnemius muscle increased significantly by 224 ± 55 % during hypoxia, blood flow to the hindlimb muscles was only 117 ± 9 % of control which was not a significant increase. Moreover, while the vascular conductance of the gastrocnemius increased by 435 ± 105 %, the vascular conductance of whole hindlimb in hypoxia was 195 ± 38 % of control which did not represent a significant increase.

DISCUSSION

Behaviour and associated cardiovascular changes

In the present experiments on anaesthetized rats, systemic hypoxia produced a similar overall pattern of cardiovascular changes as in rats under Saffan (Marshall & Metcalfe, 1988*a*, *c*, 1989*a*), in that there was tachycardia and a gradual fall in arterial pressure. Moreover, hypoxia frequently evoked behavioural arousal with features comparable to those seen upon stimulation of the defence areas (Yardley & Hilton, 1986), each episode of arousal being accompanied by further tachycardia and a short-lasting rise in arterial pressure. This accords with previous evidence that hypoxia evokes behavioural arousal in dogs, rabbit and man (Uther, Hunyor, Shaw & Korner, 1970; Koehler, MacDonald & Krasney, 1980; Rowell & Blackmon, 1986). It also accords with our evidence that, under Saffan anaesthesia, hypoxia can evoke the cardiovascular components of the alerting stage of the defence response and with our proposal that activation of the defence areas by peripheral chemoreceptors is an integral part of the response to systemic hypoxia (Marshall & Metcalfe, 1988*a*, 1989*b*). That periods of arousal were less common and less pronounced during the last minute of the 3 min period of hypoxia accords with the idea that activation of the defence areas may be gradually overcome by the central depressant effects of hypoxia (Marshall & Metcalfe, 1988*a*, 1989*b*).

To establish finally whether hypoxia can elicit the full pattern of the alerting response in the conscious animal, we still need to show whether the characteristic pattern of renal and splanchnic vasoconstriction, but not muscle vasodilatation, is associated with the initial stage of arousal, i.e. before the animal shows any locomotor response. This could not be attempted using radiolabelled microspheres since they allow only an instantaneous measurement of regional blood flows and the exact timing of arousal was unpredictable.

Blood gases

In normoxia, P_{a,O_2} and P_{a,CO_2} recorded in the anaesthetized rats were comparable to those recorded in rats under Saffan anaesthesia in the present and previous studies (Marshall & Metcalfe, 1988*a*, 1989*a*). Further, the levels to which P_{a,O_2} and P_{a,CO_2} fell by the end of the 2nd min of 8 or 6 % O_2 were comparable in the unanaesthetized and anaesthetized rats (Marshall & Metcalfe, 1988*a*, 1989*a*). This indicates that the

resting ventilation and the ventilatory responses evoked by the two levels of hypoxia were comparable in the two conditions. The similar levels of P_{a,O_2} are of particular importance, for we proposed that in the anaesthetized rat the changes in arterial pressure and in the regional vascular conductances are predominately due to the local dilator effects of tissue hypoxia (Marshall & Metcalfe, 1988*a*).

Arterial pressure and cardiac output

In normoxia, mean arterial pressure was rather lower in the unanaesthetized rats than in the Saffan-anaesthetized rats. Further, the fall in arterial pressure induced by hypoxia was smaller in the unanaesthetized rats, whether considered as a percentage of control or in absolute terms (cf. Marshall & Metcalfe, 1988*a*, 1989*a*). However, if our estimates of cardiac index are acceptable (see below), our results indicate that cardiac output tends to increase in hypoxia both in the presence and absence of anaesthesia. Thus, in both groups of animals, the fall in systemic arterial pressure can be attributed to a fall in total peripheral resistance as we deduced for anaesthetized rats (Marshall & Metcalfe, 1988*a*) and as reported by Walker (1986) for unanaesthetized rats. In fact, calculations from the measurements of arterial pressure and cardiac output indicate that in normoxia, total peripheral resistance was higher in the Saffan-anaesthetized rats (0·5 mmHg min^{-1} kg^{-1}) than in either groups of unanaesthetized rats (0·25 and 0·26 mmHg min^{-1} kg^{-1} in those subsequently exposed to 8 and 6% O_2 respectively) and that the fall in total peripheral resistance during hypoxia was larger in the anaesthetized rats (44% from control during 8% O_2, compared with 20 and 38% in the unanaesthetized rats during 8 and 6% O_2 respectively). Thus, these observations indicate that Saffan anaesthesia tends to raise the level of sympathetic vasoconstrictor tone in normoxia, but that in the face of competition from vasodilator influences in hypoxia, sympathetic vasoconstrictor activity is better able to maintain pressure in the absence of anaesthesia.

Validity of the microsphere measurements

For the microsphere technique to give valid measurements of cardiac output and regional blood flows, a number of conditions must be met (Katz *et al.* 1971; Heyman *et al.* 1977; Laughlin *et al.* 1982). These include the facts that the microspheres must be uniformly distributed in the suspension medium prior to infusion and well mixed with blood upon injection such that they are distributed in proportion to the distribution of cardiac output. On the other hand, it is obviously important that the injection of the microspheres should not disturb the normal functioning of the cardiovascular system. These points were all taken into consideration in the design of our experiments, for which we relied heavily on the modifications introduced by Laughlin *et al.* (1982) for experiments on unanaesthetized, unrestrained rats. However, the most important test of whether the technique has been used appropriately is provided by the results obtained.

In the unanaesthetized rats, the blood flows we estimated from the microsphere results were fully comparable for the right and left gastrocnemius and for the right and left cerebral hemispheres, both in normoxia and hypoxia, indicating that the microspheres were well mixed in the blood stream. The fact that in the anaesthetized rats the flow estimated for the right cerebral hemisphere was significantly lower than

that for the right, probably indicates that with a cannula on the right carotid artery, the Circle of Willis was less efficient in distributing blood equally to the right and left sides of the forebrain after 1–2 h under anaesthesia , than after 2 days in the absence of anaesthesia. The flows to the right and left gastrocnemius muscles cannot be compared in the anaesthetized rats because of the cannula in the right femoral artery. However, as the procedures adopted were comparable in the unanaesthetized and anaesthetized rats it is reasonable to assume that in the anaesthetized rats also the mixing and distribution were acceptable. The observation that both in unanaesthetized and in anaesthetized rats, the estimated blood flows for each tissue and the levels of arterial pressure were comparable in the final and initial periods of normoxia indicates that infusion of microspheres *per se* did not affect the functioning of the cardiovascular system. Further the very close correlation in the anaesthetized rats between the estimate of renal blood flow from the microscopes and from the electromagnetic transducers indicates that the estimates made from the microspheres were reliable quantitatively, as well as qualitatively. This view is supported by the close similarity of our estimates of cardiac output in the unanaesthetized rats and those of Walker (1986) who used thermodilution (412 ml min^{-1} kg^{-1}) and between our estimates in the anaesthetized rats and those of Takacs, Kallay & Vaida (1962) who used indicator dilution (307 ml min^{-1} kg^{-1}).

Regional blood flows

Assuming that our results are reliable, comparisons can be made between the anaesthetized and unanaesthetized rats for those tissues studied previously using electromagnetic transducers, viz. renal, mesenteric and hindlimb muscle (Marshall & Metcalfe, 1988*a*, 1989*a*).

Comparison of the renal vascular changes is not straightforward because renal vasculature shows pronounced myogenic responses to changes in arterial perfusion pressure and the hypoxia-induced fall in arterial pressure was much smaller in the absence of anaesthesia. However, the renal responses were not dissimilar under the two conditions: renal blood flow tended to fall in response to 8% O_2 in the anaesthetized and unanaesthetized rats of the present study and in the anaesthetized rats of our previous studies (Marshall & Metcalfe, 1988*a*) because any change in renal vasculature conductance was not sufficient to counteract the fall in arterial pressure. In response to 6% O_2, there was substantial renal dilatation both in the absence and presence of anaesthesia, but in the unanaesthetized rats renal blood flow was better maintained (cf. Marshall & Metcalfe, 1988*a*).

Comparison is easier between the vascular changes estimated from the small intestine using microspheres and from the flow probe placed on the mesenteric artery which supplies the small intestine. Again, blood flow was better maintained during hypoxia in the unanaesthetized rats. But, in these animals, as in anaesthetized rats (Marshall & Metcalfe, 1988*a*), whereas on average mesenteric vascular conductance increased during hypoxia and to a greater extent during 6% than 8% O_2, there were individual rats that showed a fall in mesenteric conductance. Presumably, in unanaesthetized, as in anaesthetized, rats (Marshall & Metcalfe, 1988*a*), there are competing constrictor and dilator influences upon the mesenteric circulation during hypoxia, the latter tending to predominate.

Similarly, the present estimates of blood flow and vascular conductance to the gastrocnemius muscle in the unanaesthetized rats indicated net dilatation in the muscle vasculature that was greater during 6% than 8% O_2, with some individuals showing vasoconstriction at each level of hypoxia. This concurs with the present observations on anaesthetized rats and with the present and previous observations on changes in femoral vascular conductance (Marshall & Metcalfe, 1988*a*, *c*).

But, it is clear that in normoxia the blood flow to the gastrocnemius muscle of the unanaesthetized rats was about ten times higher than that of the anaesthetized rats, reflecting a higher vascular conductance in the former (0·017 *vs.* 0·0002 ml min^{-1} $mmHg^{-1}$). The limb muscles of the unanaesthetized animals may have been showing a degree of functional hyperaemia caused by small movements made just before the time of measurement. However, the higher tonic level of sympathetic activity proposed above to account for the higher total peripheral resistance in the anaesthetized rats, may have been particularly directed towards skeletal muscle. That the increases in gastrocnemius conductance induced by hypoxia were smaller in the unanaesthetized rats would then accord with the suggestion that sympathetic vasoconstrictor activity was better able to maintain arterial pressure in the absence of anaesthesia (see above).

Further, the magnitude of the changes in gastrocnemius conductance recorded in unanaesthetized rats of the present study was much greater than the changes in femoral vascular conductance recorded previously in anaesthetized rats (Marshall & Metcalfe, 1988*a*, 1989*a*). This was also obvious in the anaesthetized rats of the present study when direct comparison was possible between the changes in gastrocnemius and femoral conductance. This is of interest because calculations from the data of Armstrong & Laughlin (1983) indicate that fast glycolytic fibres make up ~ 62% of the gastrocnemius muscle of the rat, but only about 50% by weight of the hindlimb muscles that are served by the femoral artery. Since glycolytic fibres would be expected to be most easily compromised by hypoxia and to switch to anaerobic metabolism, these observations concur with the idea that the vasodilatation that occurs in limb muscles during hypoxia is mediated by metabolites released as a consequence of local tissue hypoxia. The vasodilator factor could be any of those proposed for functional hyperaemia, including inorganic phosphate K^+ (Hilton, Hudlicka & Marshall, 1978) and adenosine (Berne, Knabb, Ely & Rubio, 1983). We have obtained evidence that K^+ may be involved (Kumar, Marshall & Mian, 1990). Given that adenosine is released by skeletal muscle under hypoxic conditions, with or without muscle contraction (see Berne *et al.* 1983), the idea that adenosine plays a role is attractive. Accordingly, theophylline, which antagonizes the actions of adenosine, considerably reduced the vasodilatation induced in limb muscle by severe hypoxia (Marshall & Neylon, 1990).

Turning to other tissues, the blood flow and vascular conductance of the diaphragm increased substantially during hypoxia in both unanaesthetized and anaesthetized rats. This presumably reflected functional hyperaemia associated with the hyperventilation accentuated by the direct effect of hypoxia upon muscle metabolism. In the adrenal gland, also, there was an increase in blood flow and vascular conductance both in the absence and presence of anaesthesia. This may reflect dilatation associated with increased metabolic activity of the cortex and/or

medulla, or simply dilatation induced by the effect of hypoxia upon resting metabolism. There is evidence that plasma adrenaline increases during hypoxia (Rose, Althaus, Kaiser, Miller & Carey, 1983). Further, we recently provided evidence that during moderate hypoxia, the β_2-receptor-mediated effects of catecholamines promote the reuptake of K^+ skeletal muscle thereby limiting the vasodilatation induced by locally released K^+ (Kumar *et al.* 1990), while during severe hypoxia, catecholamines contribute to the muscle vasodilatation by a direct β_2-mediated influence on the arteriolar vessels (Marshall & Mian, 1990).

By contrast, the vascular conductance and blood flow of the spleen decreased during hypoxia, both in the absence and presence of anaesthesia. This may be explained by a reflex increase in the sympathetic activity to the spleen. As the haematocrit did not change during hypoxia (see Methods), it seems unlikely that the spleen mobilizes red cells into the general circulation in the rat as it does in the dog (Berns, Anderson & McDonald, 1979).

In the brain, blood flow to the cerebral hemispheres in normoxia tended to be higher in the unanaesthetized than in the anaesthetized rats. This is not surprising for neuronal activity in the cerebrum was presumably reduced by Saffan anaesthesia. On the other hand, the baseline levels of cerebellar and brain stem blood flow tended to be higher in the anaesthetized rats. This raises the possibility that total blood flow to the brain was similar in both groups of rats in accord with well-known autoregulation of brain blood flow and that under anaesthesia there was redistribution from the cerebrum towards cerebellum and brain stem.

Despite the differences in baselines, hypoxia induced considerable dilatation in all brain regions, both in the absence and presence of anaesthesia. The percentage changes in conductance to the three regions during 8% O_2 were similar in both groups of rats, although the consequent increases in blood flow were greater in the unanaesthetized rats. Moreover, in the latter the increases in vascular conductance and blood flow evoked by 6% O_2 were even larger than those induced by 8% O_2 and far greater than the changes induced in other tissues. This must be of relevance for brain function. From the haemoglobin–O_2 dissociation curve, the fall in P_{a,O_2} from 80 to 30 mmHg induced by 6% O_2 would be expected to cause a 50% reduction in arterial O_2 content. Thus, the 120% increase in cerebral and cerebellar blood flow that occurred during 6% O_2 would provide an O_2 supply to those areas comparable to that delivered during normoxia. Obviously the increase in brain stem blood flow of > 300% seen during 6% O_2 should have ensured that the O_2 delivery to that region was far greater than during normoxia. This may have been used to support the increase in neuronal activity involved in integrating the various changes in afferent and efferent activity that occur during hypoxia. Indeed, some by-product of neuronal activity presumably induced the vasodilatation in each of the brain areas. Adenosine is a strong candidate for this role: it is released from brain tissue of the rat within 4 s of exposure to 10% O_2 and it is a potent vasodilator of cerebral vessels (Winn, Rubio & Berne, 1981).

Thus, the present experiments have shown that the cardiovascular changes induced in the unanaesthetized rat by systemic hypoxia are very similar to those we have described fully in the Saffan-anaesthetized rat (Marshall & Metcalfe, 1988*a*, 1989*a*). They have provided further evidence that hypoxia evokes the cardiovascular

components of the alerting response and that these are superimposed upon a gradual tachycardia and fall in arterial pressure attributable to peripheral vasodilatation. In view of our evidence that in the anaesthetized rat, lung stretch receptor stimulation and hypocapnia secondary to hyperventilation make no significant contribution to the vasodilator responses induced in renal mesenteric and muscle circulation (Marshall & Metcalfe, 1988*c*, 1989*a*), it is reasonable to propose that this is also the case for the unanaesthetized rat. Rather, it seems that in the unanaesthetized, as in the anaesthetized, rat vasodilator metabolites released by tissue hypoxia can account for mesenteric and muscle vasodilatation and may augment the myogenic response of the kidney to the fall in systemic pressure. The present study has also shown that both produce pronounced vasodilatation in the adrenal gland and brain. The mechanisms underlying these responses and their functional significances remain to be determined.

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