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ANALYSIS OF THE CARDIOVASCULAR CHANGES INDUCED IN THE RAT BY GRADED LEVELS OF SYSTEMIC HYPOXIA

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SUMMARY

1. In rats anaesthetized with Saffan, we have further analysed the respiratory, cardiac and regional vascular responses induced by 3 min periods of graded hypoxia (breathing 15, 12, 8 or 6% O_2 in N_2).

2. Frequently, hypoxia evoked an episode, lasting 1–1·5 min, of tachycardia, renal and mesenteric vasoconstriction and skeletal muscle vasodilatation. The tachycardia and muscle vasodilatation persisted after vagotomy indicating they were not initiated by pulmonary stretch receptors secondary to hyperventilation. We propose that such episodes represented the cardiovascular components of the alerting–defence response initiated by activation of the brain stem defence areas by peripheral chemoreceptors.

3. Each of these episodes was superimposed upon gradual hyperventilation, tachycardia, fall in arterial pressure and vasodilatation in renal, mesenteric and muscle circulation the magnitudes of which at 2 min were generally graded with the level of hypoxia. In the 3rd minute, respiration and heart rate tended to wane below control levels.

4. Vagotomy had little effect on the heart rate changes and only slightly reduced the peripheral vasodilatation allowing the conclusion that the gradual tachycardia and peripheral vasodilatation was not a reflex initiated by pulmonary stretch receptors.

5. Guanethidine given after vagotomy abolished the tachycardia indicating it was sympathetically mediated; possible initiating factors are discussed. But the secondary bradycardia persisted indicating it reflected the direct effect of hypoxia on cardiac pacemaker tissue.

6. The peripheral vasodilatation persisted after guanethidine or phentolamine indicating it was mainly attributable to the local vasodilator effects of tissue hypoxia.

7. It is proposed that the components of the alerting response are an integral part of the response to systemic hypoxia. Further, that in the rat this response is superimposed upon, but may be overcome by the direct effects of hypoxia on peripheral vasculature, heart and central nervous system.

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INTRODUCTION

Recent experiments on cats and rats anaesthetized with the steroid agent Althesin/Saffan (Glaxo/Glaxovet), which does not depress afferent activation of the brain stem defence areas to the same extent as more commonly used anaesthetics like chloralose and barbiturates, have enabled us to show that peripheral chemoreceptors can act as an excitatory input to the defence areas. Thus, in cats and rats lightly anesthetized with Althesin/Saffan, selective stimulation of carotid chemoreceptors can evoke the autonomic components of the alerting stage of the defence response (visceral alerting response), which includes tachycardia and renal and mesenteric vasoconstriction, but vasodilatation in skeletal muscle (Hilton & Marshall, 1982; Marshall, 1987). The evidence to date (see Hilton & Marshall, 1982; Marshall, 1986, 1987) suggests that the chemoreceptor-evoked visceral alerting response is superimposed upon that response which is generally recognized as the primary cardiovascular response to carotid chemoreceptor stimulation, namely bradycardia and generalized vasoconstriction (see Daly, 1984). The obvious possibility raised by this work is that activation of the defence areas by peripheral chemoreceptors may be an integral part of the response to systemic hypoxia, which has not yet been recognized.

However, peripheral chemoreceptor stimulation is not the only determinant of the cardiovascular response to systemic hypoxia. Previous studies on the rabbit and dog have already revealed that the response observed depends upon the interaction between many different factors which include those secondary to hyperventilation and those resulting from the influence of hypoxia upon the central nervous system and from a direct action upon the heart and peripheral vasculature (e.g. Uther, Hunyor, Shaw & Korner, 1970; Koehler, McDonald & Krasney, 1980). Moreover, the indications are that the relative importance of these factors varies at different levels of hypoxia in a given species and varies between species. Thus, if chemoreceptor stimulation by systemic hypoxia can activate the defence areas to produce the visceral alerting response, it is impossible to predict how this may interact with the other effects of systemic hypoxia upon the cardiovascular system.

The purpose of the present experiments was therefore to elucidate these issues. The species chosen for study was the rat, whose cardiovascular response to systemic hypoxia has not previously been analysed. Therefore, as well as investigating whether systemic hypoxia can evoke the cardiovascular components of the visceral alerting response, we have analysed the role of the vagi and of the sympathetic nervous system in determining the response to hypoxia in this species. Some of these findings have been presented to the Physiological Society (Marshall & Metcalfe, 1987).

METHODS

Experiments were performed on twenty-five male Sprague rats ($356 \pm 113{\cdot}3$ mg body weight) using techniques and equipment as described recently (Marshall & Metcalfe, 1988*a*). Briefly, anaesthesia was induced with O_2 and N_2O (60%:40%) and halothane and was maintained with a continuous infusion of Saffan (Glaxovet) given via a cannula placed in the right jugular vein, at 13·6–9·5 mg total steroids kg^{-1} h^{-1} during surgery and at 7–4·5 mg kg^{-1} h^{-1} during the experimental

period. A stainless-steel T-shaped cannula placed in the trachea allowed monitoring of tidal volume and respiratory frequency via a flow-head and electrospirometer. Air or hypoxic gas mixtures (see below) were blown across the end of the flow-head at about 1·2 l min^{-1}, by means of an air pump. A cotton ligature was looped around each vagal trunk to facilitate sectioning of them later in the experiment. Arterial pressure was monitored via a cannula placed in the left femoral artery and heart rate was derived from the pressure recording via a rate-meter. In each experiment, blood flows were recorded from the right femoral artery, with paw excluded by a tight ligature placed around the ankle, and from the left renal artery, by means of cuff-type electromagnetic transducers and meters which were calibrated *in vitro*. In some experiments a cuff-type transducer was also placed on the cranial mesenteric artery via an incision in the left flank and the abdomen was then re-sealed. Mesenteric blood flow was only recorded in animals in which it was possible to expose a sufficient length of the mesenteric artery without damaging the nerve plexus that surrounds it; this limited us to six out of the total of twenty-five animals. For each artery, a zero-flow signal was regularly obtained during the experimental period by occluding the artery distal to the transducer with a pair of small PVC-covered forceps. Vascular conductance was computed on-line for each artery by a custom-built electronic divider. All variables were displayed on an eight-channel pen recorder.

The rat was allowed to equilibrate for 1–2 h at the experimental level of anaesthesia (see above), such that a strong pinch of the paw evoked withdrawal of the paw and a rise in arterial pressure, but there were no spontaneous movements. Then, continuous recordings of the respiratory and cardiovascular variables were made during air breathing and during 3 min periods of graded levels of hypoxia (breathing 15, 12, 8 or 6% O_2 in N_2). Arterial blood samples for measurement of blood gases and pH were taken during air breathing and at the end of the 2nd minute of hypoxic periods from a cannula placed in the right brachial artery. In experiments in which transducers were placed on both the renal and the mesenteric artery, two experimental runs were carried out under each experimental condition, one in which femoral and renal flows were recorded and the other in which femoral and mesenteric flows were recorded. The order of recording renal and mesenteric flow was randomized within and between experiments and the order of administration of the gas mixtures within each run was also randomized.

Results are expressed as mean ± S.E.M. All statistical analyses were carried out using Student's paired *t* test.

RESULTS

The basic pattern of response evoked by systemic hypoxia comprised hyperventilation, a fall in arterial blood pressure (ABP) and an increase in heart rate (HR) and in the vascular conductance of femoral, mesenteric and renal beds (FVC, MVC, RVC respectively). Each of these variables changed gradually and at the end of the 2nd minute of hypoxia the magnitude of the change in each variable was generally graded with the level of hypoxia as can been seen from Fig. 1. As can also be seen from Fig. 1, the frequency of augmented breaths (defined as an additional inspiratory effort at the peak of a normal inspiration) increased in a graded fashion with increasing levels of hypoxia, each of these being associated with transient peripheral vasodilatation and a fall in ABP. The mechanisms underlying these responses were analysed in our previous study (Marshall & Metcalfe, 1988*a*). In the present study we have concentrated upon the more gradual changes; to quantify them, a smooth line was drawn by eye through the recording of each variable and measurements were made at the end of the 2nd minute of each period of hypoxia. Figure 2 shows the averaged data ± S.E.M.s take from nineteen animals of which all were subsequently vagotimized and twelve were then given phentolamine.

Considering the gradual changes in more detail, the increase in respiratory minute volume ($\dot{V}_e$) shown in Fig. 2 was predominantly due to an increase in respiratory

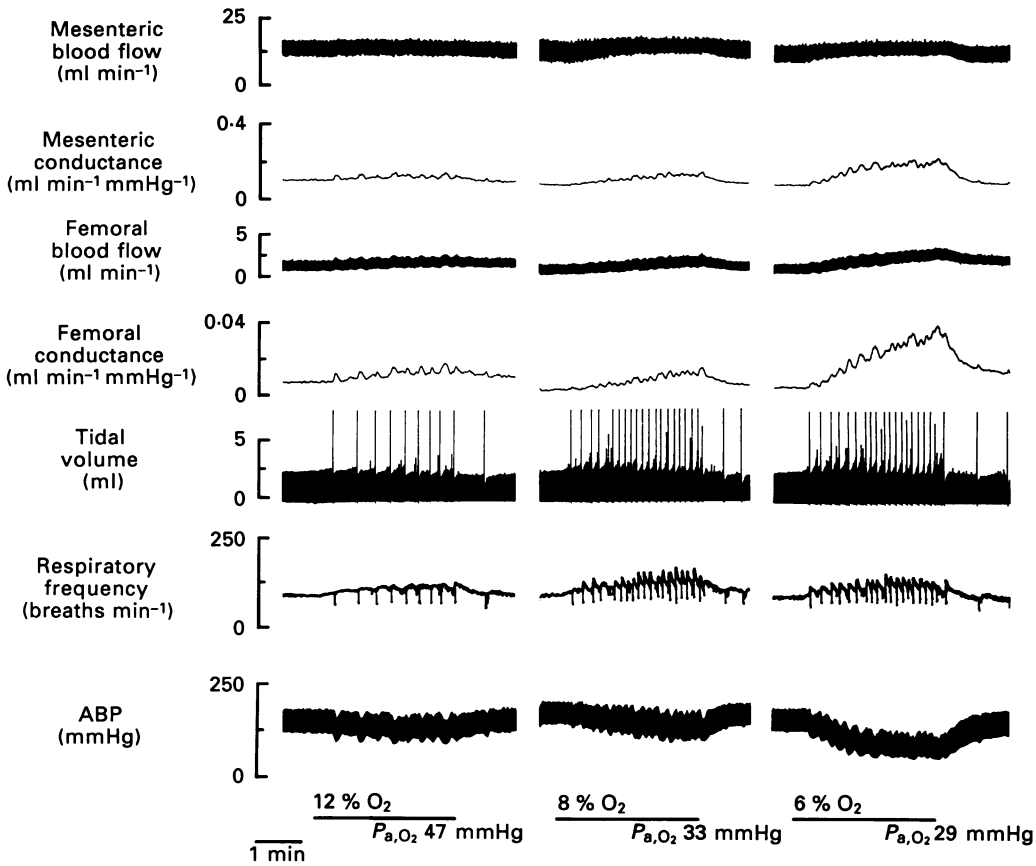

Fig. 1. Cardiovascular and respiratory responses induced by graded levels of systemic hypoxia. Traces from above down: blood flow in mesenteric artery, mesenteric vascular conductance, blood flow in femoral artery, femoral vascular conductance, respiratory tidal volume and frequency, and arterial pressure. Bar beneath each panel indicates 3 min period of breathing hypoxic mixture (12, 8, 6% O_2 in N_2). The P_{a,O_2} values are those measured at end of 2nd minute of hypoxia.

Fig. 2. Effects of graded levels of hypoxia upon respiration, arterial blood gases and cardiovascular variables. Graphs from above down show respiratory minute volume ($\dot{V}_e$) and P_{a,O_2} (closed symbols), P_{a,CO_2} (open symbols); arterial blood pressure ABP and heart rate (HR); femoral vascular conductance (FVC) and flow; renal vascular conductance (RVC) and flow; mesenteric vascular conductance (MVC) and flow. For each graph, abscissa is percentage O_2 in inspirate, ordinate is percentage change from baseline levels, with the exception of P_{a,O_2}, P_{a,CO_2} shown as absolute values, and HR shown as change in beats per minute. Each point represents mean of measurements made at end of 2nd minute of hypoxia: ●, ○, intact state; ■, □, after vagotomy; ▲, △, after phentolamine. S.E.M. indicated by bar except when encompassed within symbol. ***, **, * indicate significant difference between intact and post-vagotomy values, $P < 0{\cdot}001$, $< 0{\cdot}05$, $< 0{\cdot}1$ respectively. The number of pairs of values tested in each case (n) was 17, 18 or 19, except in the case of HR, when $n = 11$ at 8 and 6% O_2, and MVC and mesenteric flow when $n = 6$ throughout. †††; ††, † indicate significant difference between post-vagotomy and post-phentolamine values $P < 0{\cdot}001$, $< 0{\cdot}05$, $< 0{\cdot}1$ respectively. $n = 10$, 11 or 12 in each case.

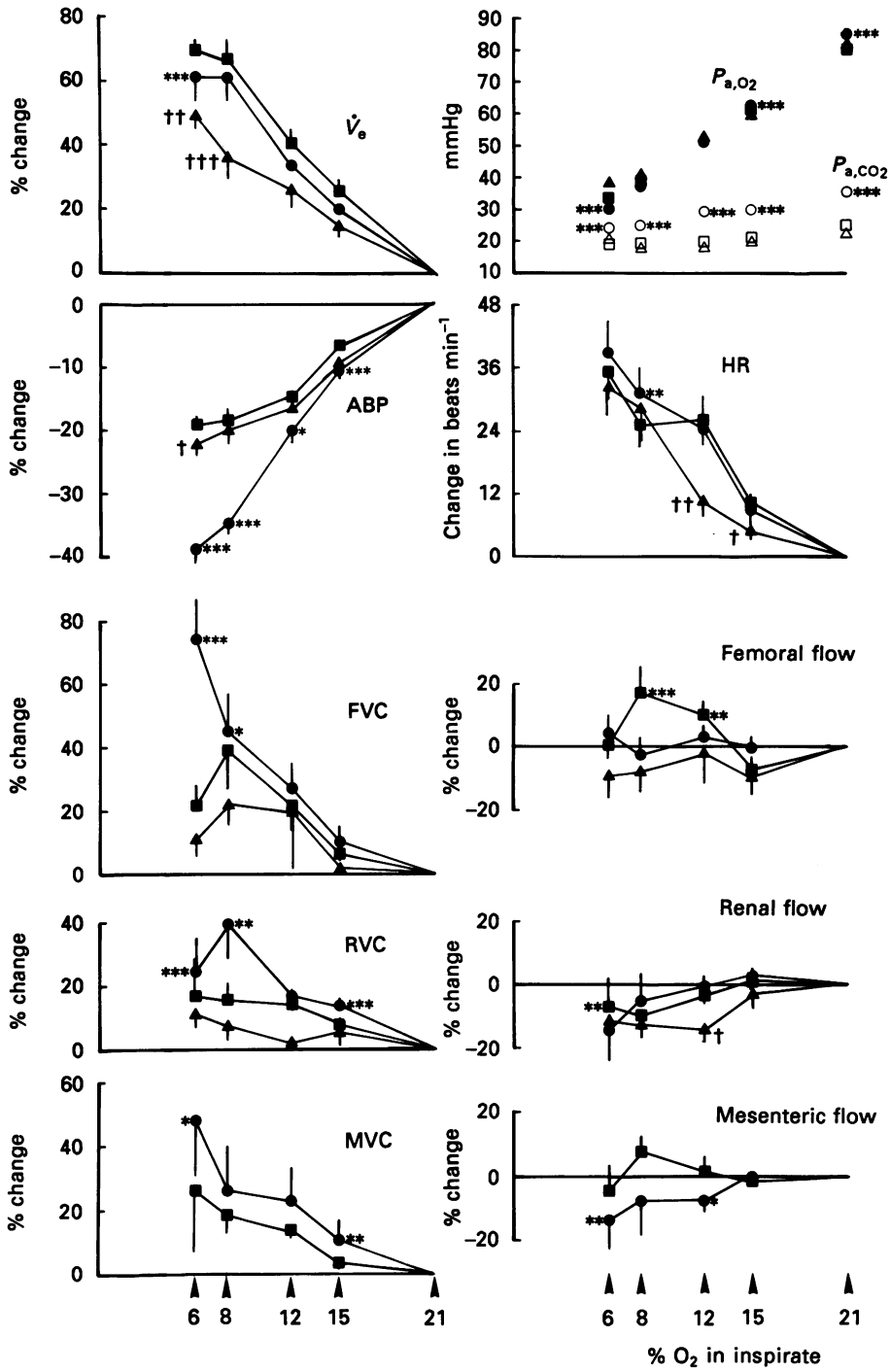

Fig. 2. For legend see facing page.

frequency (F_r), for example in response to 15% O_2, the 21% increase in $\dot{V}_e$ comprised an 18% increase in F_r and a 2% increase in tidal volume (V_t), while in response to 6% O_2, increase in $\dot{V}_e$ comprised a 37% increase in F_r and an 18% increase in V_t. Meanwhile arterial O_2 and CO_2 pressure (P_{a,O_2} and P_{a,CO_2}) fell substantially and to extents which were graded with the O_2 percentage in the inspired air; arterial pH (pH_a) increased in a graded fashion from 7·4 during air breathing to 7·52 with 6% O_2. The levels of P_{a,O_2} P_{a,CO_2} and pH_a attained in different animals in response to a given hypoxic mixture were remarkably similar as indicated by the low variance of the data: the S.E.M. bars of each point in Fig. 2 are actually encompassed within the area of the symbols.

During air breathing, mean ABP was $144 \pm 4{\cdot}7$ mmHg and reached $89 \pm 28{\cdot}6$ mmHg in response to 6% O_2, while average HR was $441 \pm 8{\cdot}01$ beats min^{-1} and reached $463{\cdot}8 \pm 51{\cdot}0$ beats min^{-1} in response to 6% O_2; again there was good comparability between animals in the change observed in each of these variables at each level of hypoxia. By contrast, there was far more variability in the changes recorded in the regional vascular beds. Thus, while RVC and MVC generally increased, indicating vasodilatation, one or two animals showing a decrease in one or both of these conductances at each level of hypoxia; these averaged $10{\cdot}8 \pm 6{\cdot}3$% ($n = 7$) and $10{\cdot}2 \pm 4{\cdot}3$% ($n = 6$) from control levels for renal and mesenteric vasculature respectively. It may also be noted that the mean increase in RVC induced by 6% O_2 was actually less than that induced by 8% O_2, the difference between the changes with 8 and 6% O_2 being significant at the $P < 0{\cdot}01$ level. Similarly, for the femoral vascular bed, although the predominating response at all levels of hypoxia was an increase in FVC, these increases being larger than in renal and mesenteric beds, six out of the total of nineteen animals showed a decrease in FVC in response to 15% O_2 (amounting to $13{\cdot}2 \pm 8{\cdot}0$% from control levels), four showed a decrease in response to 12% O_2 (of $10 \pm 2{\cdot}7$%) and two animals showed decreases in response to 8 and 6% O_2 of 40 and 24% respectively. Renal blood flow (RBF) either remained more or less constant or tended to fall (Fig. 2), particularly in response to 6% O_2 when the fall was significantly greater than that induced by 8% O_2 ($P < 0{\cdot}01$). Mesenteric blood flow (MBF) generally fell in a graded fashion with increasing severities of hypoxia, while the change in femoral blood flow (FBF) varied considerably between animals, some showing marked increases, others marked decreases, so that on average blood flow remained virtually constant.

In addition to the changes so far described, the first 1–1·5 min of hypoxia often evoked a short-lasting episode of further hyperventilation, tachycardia and increase in FVC but with a decrease in MVC and RVC so that ABP rose above its gradual downward trend (Fig. 3*A*). This was accompanied by pupillary dilatation and movement of the vibrissae. Such episodes most commonly occurred in response to 8 and 6% O_2, but they could also be evoked by 15 and 12% O_2; there was no obvious correlation between the magnitudes of the individual components of the response and the level of hypoxia. Each episode lasted 1–1·5 min, at the end of which FVC had apparently returned to the level of the underlying gradual increase in conductance. Following this, we often chose to terminate the period of hypoxia as in Fig. 3. However, if hypoxia was allowed to continue for our standard 3 min, a second episode rarely occurred. These episodes seemed most likely to occur at lighter levels

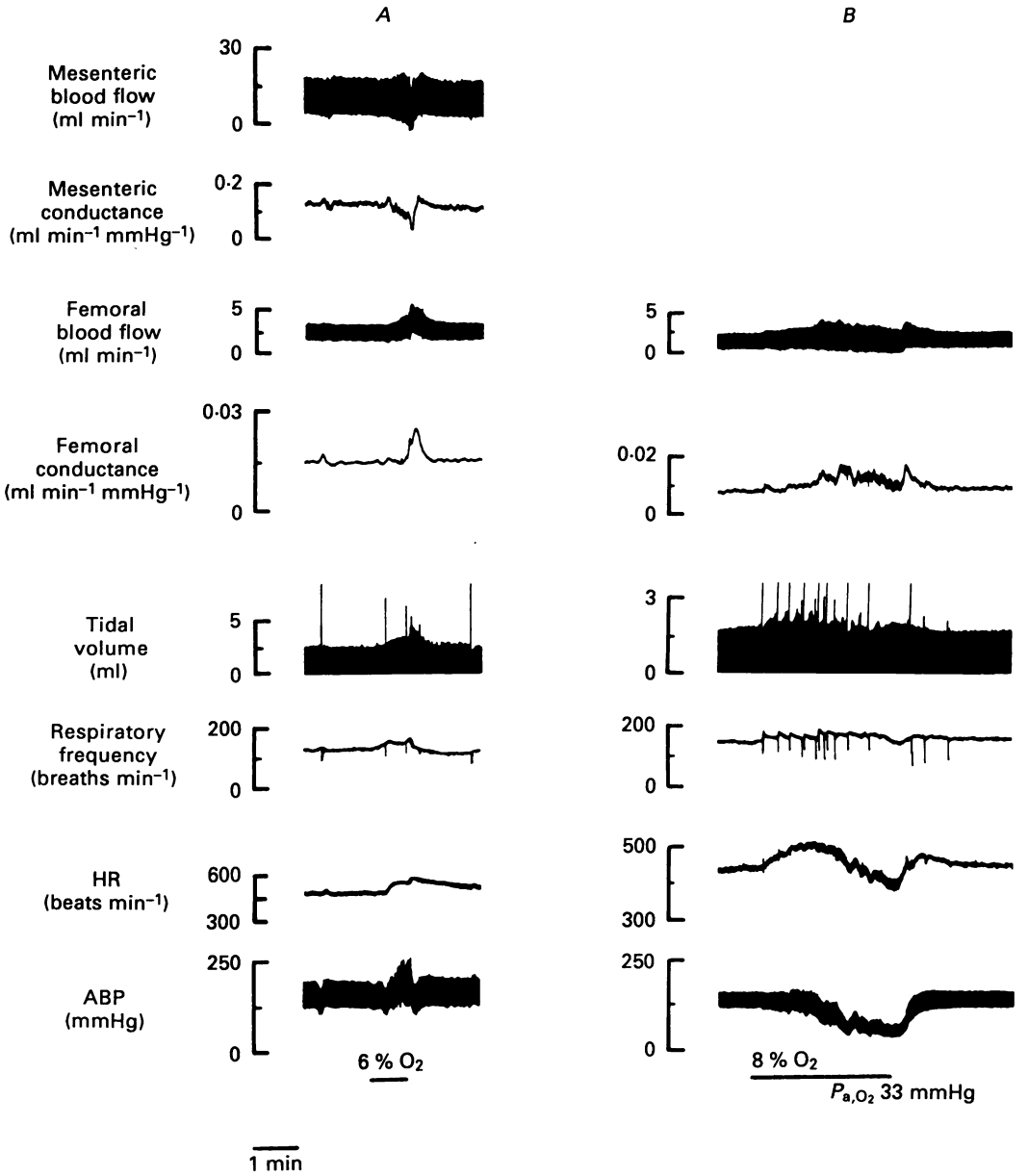

Fig. 3. *A*, example of an episode of rapid mesenteric vasoconstriction and muscle vasodilatation, with tachycardia and a rise in arterial pressure, evoked at the onset of administration of 6% O_2. Traces as in Fig. 1, but with addition of heart rate (HR). *B*, example of waning of gradual hyperventilation and tachycardia towards the end of a 3 min period of breathing 8% O_2. Traces as in *A* with the exception of mesenteric blood flow and conductance. For further explanation see text.

of Saffan anaesthesia; they could be terminated early by bolus injection of Saffan (1–1·5 mg kg^{-1} I.V.) and could be prevented by increasing the rate of infusion of Saffan within the range given in the Methods section.

During the final 1 min of the 3 min test periods of hypoxia the gradual hyperventilation and tachycardia upon which the above episodes were superimposed, often waned towards, or even to below control levels, by up to 20% and 45 beats min^{-1} for $\dot{V}_e$ and HR respectively (see Fig. 3*B*). This tendency was most pronounced during exposure to 8 and 6% O_2, but could be accentuated at all levels of hypoxia, by increasing the rate of infusion of Saffan, within the range given in the Methods section.

Effect of vagotomy

Vagotomy produced the expected increase in baseline V_t and reduction in F_r (see Fig. 4). The effect of this was that during air breathing, P_{a,O_2} was significantly increased with respect to pre-vagotomy values, P_{a,CO_2} significantly decreased (Fig. 2) and pH_a significantly increased to 7·5. In three animals, the baseline level of HR was decreased by vagotomy, but average HR increased significantly from $440 \pm 6{\cdot}9$ to $465 \pm 5{\cdot}6$ beats min^{-1}. There was no significant change in mean ABP (mean $144 \pm 4{\cdot}7$ mmHg before, $139 \pm 4{\cdot}1$ mmHg after vagotomy). Augmented breaths were abolished by vagotomy, at least temporarily, but sometimes re-appeared 1–2 h later and then each one was associated with transient cardiovascular changes as before vagotomy (Fig. 4, cf. Marshall & Metcalfe, 1988*a*).

Qualitatively, the changes induced by hypoxia were comparable to those induced with vagi intact, but there were quantitative differences. Thus, with each hypoxic mixture the increase in V_e at the end of the 2nd minute of hypoxia tended to be greater than with the vagi intact (see Fig. 2). Moreover, the increase in V_t played a larger part in the hyperventilation, particularly at the more severe levels of hypoxia. For example, in response to 15% O_2 the 26% increase in $\dot{V}_e$ comprised a 21% increase in F_r and a 5% increase in V_t while the 70% increase in $\dot{V}_e$ induced by 6% O_2 reflected a 29% increase in F_r and a 41% increase in V_t. Although the P_{a,O_2} attained with the 15% O_2 mixture was significantly lower than with vagi intact, P_{a,O_2} was better maintained at the more severe levels of hypoxia, the difference between the pre- and post-vagotomy changes being significant with 6% O_2. P_{a,CO_2} fell with each hypoxic mixture, but the decrease in absolute terms from the air-breathing value was less than with vagi intact, amounting to a mean of 12 mmHg in response to 6% O_2 with vagi intact and only 6 mmHg after vagotomy.

The increases in HR evoked by 15 and 12% O_2 were sometimes larger, sometimes smaller than those induced before vagotomy, the differences between them being statistically insignificant. Statistical tests carried out on the HR changes induced by 8 and 6% O_2 indicated that they were significantly smaller than those induced with vagi intact. However this effect was probably not real, for the baseline levels of HR were higher after vagotomy than before (see above), and the rate-meter we used could not follow HR values higher than 500 beats min^{-1}. Thus, animals in which the HR change was larger after vagotomy than before were more likely to be excluded from our sample; in fact eight were excluded after vagotomy both at 8 and 6% O_2. By contrast, the fall in mean ABP induced by hypoxia was obviously smaller after

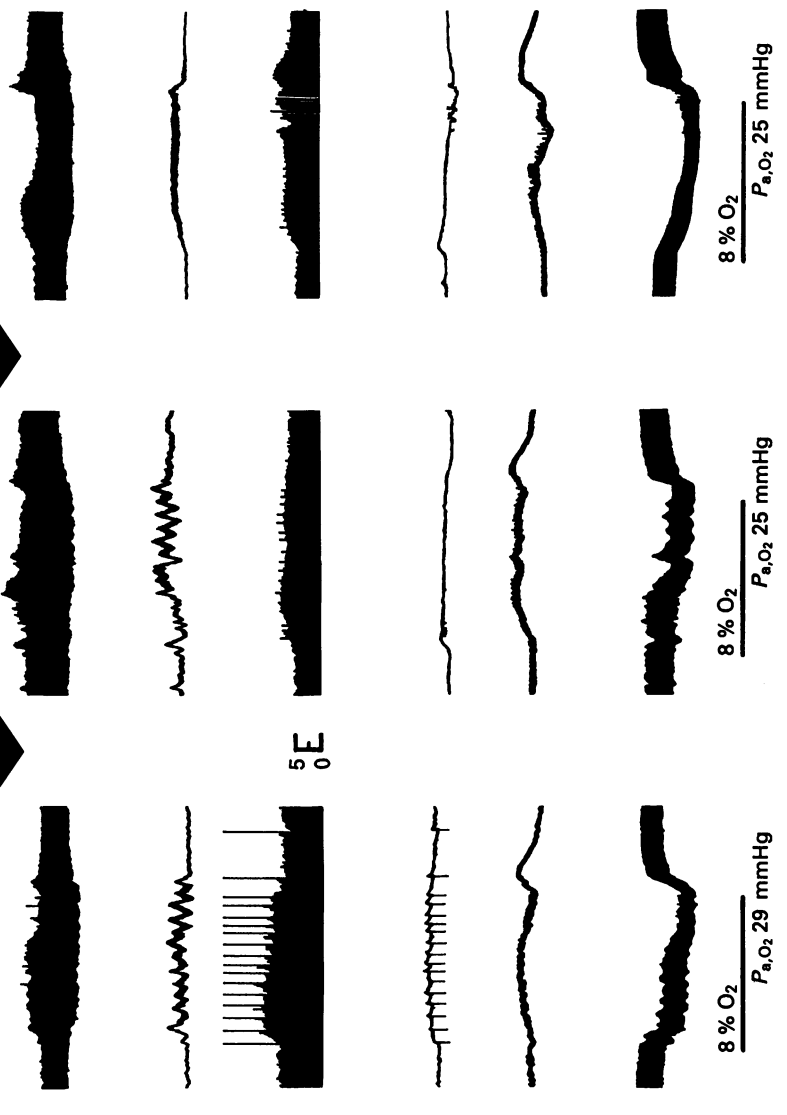

Fig. 4. Effect of vagotomy and subsequent sympathetic blockade with guanethidine upon cardiovascular and respiratory responses induced by 8 % O_2. Traces as in Fig. 3*B*.

vagotomy the difference being most marked at the most severe levels of hypoxia (Fig. 2), and arterial pulse pressure was generally better maintained as can be seen from Fig. 4. The increases in RVC, MVC and FVC also tended to be smaller after vagotomy (see Fig. 2). But it is clear from the S.E.M. bars in Fig. 2 that the variability of the changes in each of the regional vascular conductances was just as great as with the vagi intact, for example a total of twelve vasoconstrictor responses were recorded in the femoral vascular bed in response to the full range of hypoxic mixtures before vagotomy and eight after vagotomy. MBF and FBF were significantly better maintained than with vagi intact, indeed FBF showed an increase relative to control levels in response to 12 and 8% O_2. On average RBF tended to fall, but only by $\sim 10\%$ from control levels.

Vagotomy had no obvious effect on the short-lasting episodes of further hyperventilation, tachycardia, muscle vasodilatation, renal and mesenteric vasoconstriction and rise in ABP, that occurred at lighter levels of anaesthesia as described above (Fig. 5). Moreover, the gradual hyperventilation and tachycardia still tended to wane during the final 3 min of hypoxia, particularly at deeper levels of anaesthesia, just as occurred with vagi intact (Fig. 4).

Effect of guanethidine

Six vagotomized animals were given guanethidine in an attempt to elucidate the role of the sympathetic nervous system. As can be seen from Fig. 4, guanethidine produced the expected falls in the baseline levels of ABP and HR. Thereafter, the hypoxia-induced tachycardia was virtually abolished and HR always fell to below control levels during the 3rd minute of hypoxia, by up to 200 beats min^{-1}. Meanwhile the increases in the regional vascular conductances persisted, so that mean ABP usually fell to even lower values than before administration of the drug. During each period of hypoxia the hyperventilation during the first minute was just as great as in the absence of guanethidine, but the waning of this response was more pronounced to the extent that in some animals apnoea occurred and artificial ventilation was required.

Effect of phentolamine

A further twelve vagotomized animals were given phentolamine to block α-receptor-mediated sympathetic influences on the vasculature whilst preserving sympathetic influences on the heart. Phentolamine (10 mg kg^{-1} I.V.) abolished pressor responses evoked by bolus injection of phenylephrine (10 μg kg^{-1} I.V.) and reduced baseline ABP significantly to $97{\cdot}5 \pm 3{\cdot}6$ mmHg. The baseline level of HR was increased significantly from the post-vagotomy level, to $472 \pm 9{\cdot}2$ beats min^{-1}, but there were no significant changes in baseline levels of R_f, V_t, P_{a,O_2}, P_{a,CO_2} or pH_a. Qualitatively, the respiratory and cardiovascular changes induced by hypoxia after phentolamine plus vagotomy were similar to those seen after vagotomy alone as indicated by the changes measured at the end of the 2nd minute of hypoxia (Fig. 2). Quantitatively, the increases in $\dot{V}_e$ were smaller after phentolamine, but the falls in P_{a,O_2} and P_{a,CO_2} were similar, except in response to 6% O_2 when the P_{a,O_2} after phentolamine was significantly higher than after vagotomy alone.

Only one animal was excluded from the post-phentolamine sample because HR

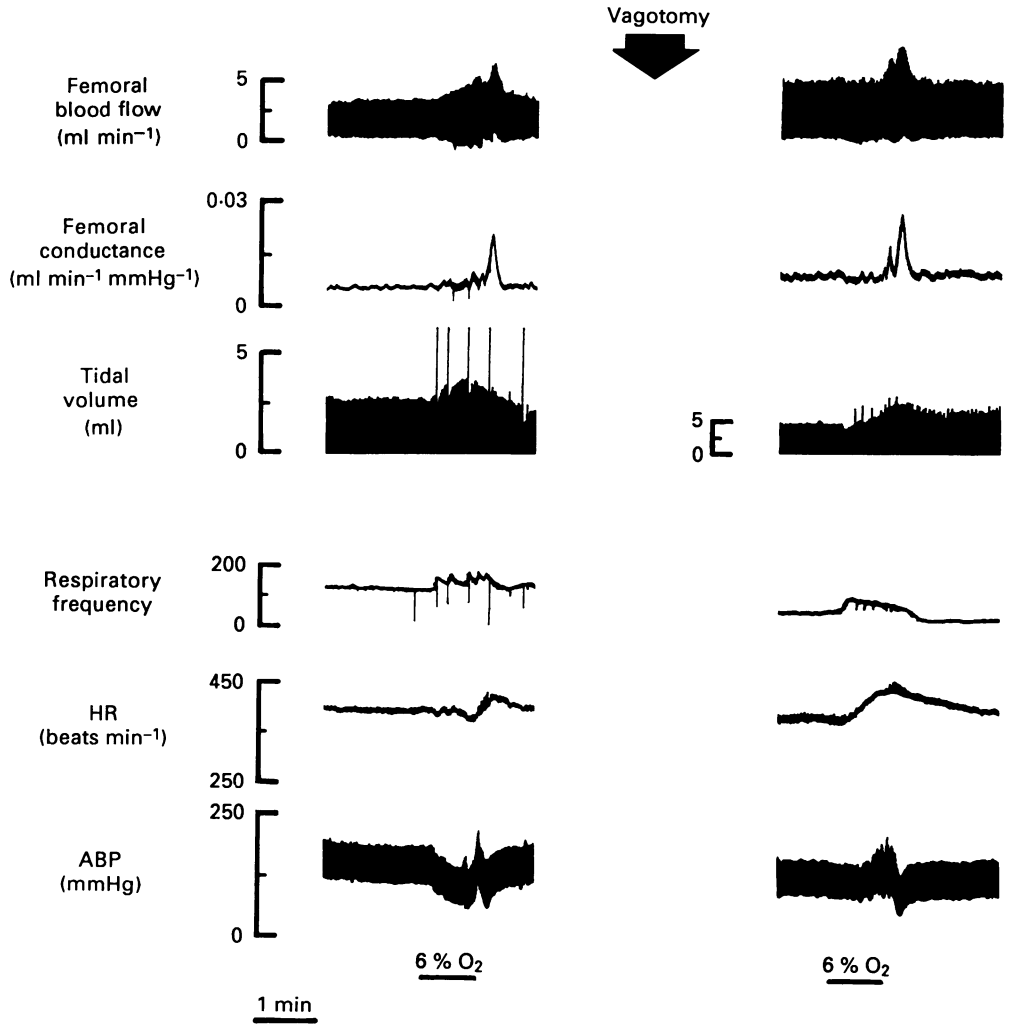

Fig. 5. Effect of vagotomy upon a response pattern, comparable to that seen in Fig. 3*A*, evoked by 6% O_2. Note: the tachycardia and vasodilatation in muscle persist indicating they are not reflex responses mediated by pulmonary vagal afferents secondary to the hyperventilation. Traces as in Fig. 3*B*.

exceeded 500 beats min^{-1} and therefore could not be recorded (see above). Thus the finding that the changes in HR induced by 15 and 12% O_2 were smaller than after vagotomy, while the changes induced by 8 and 6% O_2 were similar to those evoked after vagotomy alone, can be accepted as real. If the changes induced in ABP are expressed in absolute terms, then the change induced by each hypoxic mixture was smaller after phentolamine than before. However, if expressed as a percentage of the baseline level, then the changes induced after phentolamine were somewhat greater than after vagotomy alone (Fig. 2), the difference reaching significance with 6% O_2: the absolute level of mean ABP reached in response to 6% O_2 was 78·9±6·5 mmHg.

We have insufficient data to comment on the effect of phentolamine upon the changes in MBF and MVC. On average RVC and FVC were still markedly increased in response to each level of hypoxia (Fig. 2). The average increases were most pronounced in the femoral bed and 6% O_2 still induced a smaller change in FVC than did 8% O_2, as had occurred after vagotomy alone. A gradual decrease, rather than an increase in FVC and/or RVC, was still seen in some animals; the decreases averaged $14 \pm 4{\cdot}8$% ($n = 9$) from control levels for FVC and $5{\cdot}1 \pm 1{\cdot}3$% ($n = 11$) for RVC. Both FBF and RBF tended to fall at each level of hypoxia, the differences between pre- and post-phentolamine changes being most pronounced in the femoral bed (Fig. 2).

Short-lasting episodes of further hyperventilation and tachycardia accompanied by pupillary dilatation and movement of vibrissae were still superimposed upon these gradual changes during exposure to 8 and 6% O_2 as before phentolamine (see above) but any vascular changes associated with them were minimal. The tendency for the gradual tachycardia to wane during the final 1 min of hypoxia was not obviously different from that seen after vagotomy alone, but the tendency for the hyperventilation to wane was more pronounced; three out of the twelve animals showed apnoea in the final 1 min of hypoxia and in the rest, $\dot{V}_e$ always fell to the control level, or below it, during the final 1 min.

DISCUSSION

The present experiments were a continuation of our recent study on rats anaesthetized with Saffan which showed that systemic hypoxia evoked an increase in the frequency of augmented breaths, each one being associated with transient peripheral vasodilatation and sometimes bradycardia (Marshall & Metcalfe, 1988*a*); we provided evidence that all of these changes were a reflex initiated predominantly by pulmonary irritant receptors. In the present study we have attempted a more detailed analysis of the underlying, gradual cardiovascular changes of the waning of these changes after the 2nd minute of hypoxia and have paid particular attention to the short-lasting episodes of further tachycardia, vasodilatation in muscle, and the renal and mesenteric vasoconstriction that occurred during the first 1–2 min of hypoxia.

First, it should be noted that the levels of P_{a,O_2} and P_{a,CO_2} that we recorded during air breathing and during exposure to 8% O_2 were virtually identical with those recorded in unanaesthetized rats (Walker, 1986; Marshall, & Metcalfe, 1988*b*), indicating that baseline ventilation and the hyperventilation induced by hypoxia were comparable in the two situations. Further, the baseline levels of heart rate and the increases in heart rate evoked by 8% O_2 in the present study were comparable to those recorded in unanaesthetized rats (Walker, 1986; J. M. Marshall & J. D. Metcalfe, unpublished observations). The mean level of arterial pressure recorded during air breathing in the present study was higher than that recorded in unanaesthetized rats (Walker, 1986: 144 *vs.* 104 mmHg, cf. J. M. Marshall & J. D. Metcalfe, unpublished observations), which suggests that Saffan anaesthesia raises the background level of sympathetic activity. However, both Walker (1986) and ourselves (Marshall & Metcalfe, 1988*b*) found that in unanaesthetized rats hypoxia

induced a fall in arterial pressure, which was similar as a percentage of the control values to that recorded in the present study. Thus, in general terms at least, the changes that hypoxia induced in rats under Saffan are comparable to those evoked in rats in the absence of anaesthesia. It seems therefore that the arterial pressure change induced in the rat by hypoxia is directionally opposite from that generally recorded in larger species like cat, dog, rabbit and indeed man, all of whom tend to show a rise in arterial pressure (Black & Roddie, 1958; Uther *et al.* 1970; Koehler *et al.* 1980; Marshall & Metcalfe, 1987).

Evidence for activation of the brain stem defence areas

Each short-lasting episode of tachycardia, vasodilatation in skeletal muscle and vasoconstriction in kidney and mesenteric circulation that occurred in the first 1–2 min of hypoxia was accompanied by pupillary dilatation and movement of the vibrissae. This is comparable with the visceral alerting response evoked in the anaesthetized rat by electrical stimulation in the brain stem defence areas and which in the conscious animal is accompanied by behavioural signs of alerting or defensive behaviour (Yardley & Hilton, 1986). It is also comparable with the pattern of response that can be evoked in rats under Saffan anaesthesia by selective stimulation of the carotid chemoreceptors (Marshall, 1987). In full accord with the general characteristics of the visceral alerting response, the muscle vasodilatation was not a reflex initiated by lung stretch receptors secondary to hyperventilation for it persisted after vagotomy. Further, in agreement with the particular characteristics of the alerting response in the rat, the muscle vasodilatation was greatly attenuated or abolished by phentolamine indicating that it was mediated predominantly by inhibition of sympathetic noradrenergic activity (cf. Yardley & Hilton, 1987; Marshall, 1987). Thus, the present findings are consistent with the hypothesis put forward in the Introduction, that activation of the defence areas by peripheral chemoreceptors is an integral part of the response to systemic hypoxia. This is supported by the fact that in three separate experiments the episodes described above could not be evoked by hypoxia after bilateral section of the carotid sinus nerves (J. M. Marshall, unpublished observations). That the visceral alerting response was only evoked in the present study at light levels of anaesthesia is understandable for previous studies showed that when employed at high doses, Saffan, like other anaesthetics, can block afferent activation of the defence areas (Marshall, 1987). The present results are consistent with previous observations on conscious animals in as much as hypoxia has been shown to elicit behavioural alerting in the dog, rabbit and man (Uther *et al.* 1970; Koehler *et al.* 1980; Rowell & Blackmon, 1982) and in the dog this was abolished when the carotid sinus nerves were sectioned (Koehler *et al.* 1980). Unfortunately, in these previous studies effects of hypoxia upon the cardiovascular system were only followed discontinuously, or in the steady state, so that any short-lasting changes associated specifically with periods of behavioural alerting were not reported.

The gradual cardiovascular changes

Since heart rate and vascular conductance in renal, mesenteric and femoral bed increased simultaneously during hypoxia, this suggests that the gradual fall in

arterial pressure was predominantly due to a fall in total peripheral resistance, rather than to a fall in cardiac output. In fact Walker (1986) showed, by using the thermal dilution technique in unanaesthetized rats, that cardiac output increased by ~ 50% during exposure to 8% O_2, while calculated total peripheral resistance decreased significantly. Our recordings of regional blood flows showed that blood flow to the kidney remained more or less constant when the O_2 in the inspirate was reduced over the range 21–6%. Thus, it could be concluded that the renal vasculature simply showed autoregulation, reflecting a myogenic dilator response of the vascular smooth muscle to the fall in systemic arterial pressure. However, the increase in renal conductance evoked by 6% O_2 was significantly less than that evoked by 8% O_2, despite the greater fall in systemic arterial pressure during 6% O_2 and renal blood flow fell significantly more during 6% than 8% O_2. This, together with the fact that at each level of hypoxia there were individual animals that showed a decrease rather than an increase in renal vascular conductance, suggests that a constrictor influence affected the kidney, particularly at the most severe level of hypoxia and competed with the dilator influence that generally predominated during more moderate hypoxia. The fact that individual animals sometimes showed a decrease, rather than an increase, in mesenteric vascular conductance, similarly suggests that in the mesenteric vasculature, constrictor and vasodilator influences were in competition. In the femoral vasculature the mean increases in conductance were substantially larger than those recorded in renal and mesenteric vascular beds and femoral blood flow was well maintained. Given that skeletal muscle vasculature comprises over 25% of total peripheral resistance, it seems likely that vasodilatation in skeletal muscle made the largest contribution to the hypoxia-induced fall in total peripheral resistance and in arterial pressure. That some animals showed a substantial decrease rather than an increase in femoral vascular conductance again suggests competition between dilator and constrictor influences and this is supported by our recent direct observations on the microvasculature of rat skeletal muscle: while the predominating response of the arterioles to systemic hypoxia was vasodilatation (Booth & Marshall, 1987), constrictor responses were observed, particularly at more proximal arterioles.

The role of vagal afferents. The obvious possibility to be considered is whether the gradual tachycardia and peripheral vasodilatation induced by hypoxia was a reflex initiated by pulmonary stretch receptors secondary to the hyperventilation, for this reflex makes a major contribution to the cardiovascular response evoked in the dog by selective stimulation of carotid chemoreceptors and by systemic hypoxia (Koehler *et al.* 1980; Daly, 1984). In the present study, vagotomy increased the baseline level of tidal volume and decreased the baseline level of respiratory frequency as would be expected from interruption of pulmonary stretch receptor afferent fibres and consequent loss of the Breuer–Hering reflex. The average baseline level of heart rate was increased by vagotomy which may be explained by loss of tonic inhibitory influences upon heart rate from aortic baroreceptors and various cardiac receptors (cf. Manica & Donald, 1970), and possibly by loss of cardiac vagal tone. Indeed this result suggests that any tonic excitatory influence from pulmonary stretch receptors upon heart rate was weak. This is consistent with our recent experiments on the rat which showed that inhalation of SO_2, which produced a more or less selective blockade of pulmonary stretch receptor activity, had no effect on

resting heart rate (Marshall & Metcalfe, 1988*a*). The increases in heart rate induced by 15 and 12% O_2 were not affected by vagotomy and as explained in the Results section, the observation that the increases in heart rate induced by 8 and 6% O_2 were apparently less after vagotomy could be attributed to our inability to record heart rates higher than 500 beats min^{-1}. In fact, since blockade of pulmonary stretch receptors with SO_2 had no significant effect on the tachycardia induced by 8% O_2 and since experimentally induced hyperinflation had no effect on heart rate (Marshall & Metcalfe, 1988*a*) we reiterate our proposal that in the rat, tachycardia initiated by pulmonary stretch receptors with vagal afferents makes little or no contribution to the tachycardia induced by hypoxia.

As the tachycardia persisted after vagotomy it presumably reflected an increase in cardiac sympathetic activity, rather than a fall in vagal tone. The primary effect of carotid chemoreceptor stimulation upon heart rate is bradycardia in the rat as in other species (Marshall, 1987). A sympathetically mediated increase in heart rate in response to hypoxia could be explained by the excitatory influence of increased central inspiratory drive upon cardiac sympathetic activity, the ability of hypoxia of the central nervous system to increase sympathetic activity as discussed previously (Marshall, 1987), by reflex effects exerted by baroreceptor unloading resulting from the fall in systemic arterial pressure, and by a reflex induced by metaboreceptors in skeletal muscle stimulated by the fall in local O_2 tension (Thimm, Dicntsal & Meier zu Vesl, 1986).

Vagotomy certainly did significantly reduce the hypoxia-induced fall in arterial pressure at all levels of hypoxia and tended to reduce the mean increases in the regional vascular conductances, particularly those induced by the more severe levels of hypoxia. We have previously provided evidence that pulmonary stretch receptors make no significant contribution to the fall in arterial pressure and rise in regional vascular conductance induced by hypoxia (Marshall & Metcalfe, 1988*a*). Thus, we suggest that the pronounced effects of vagotomy in the present experiments can be explained by other mechanisms. Firstly, as the increase in tidal volume induced by hypoxia was greater after vagotomy, this may have increased venous return to the heart and by promoting an increase in stroke volume and cardiac output, may have allowed arterial pressure to be better maintained. This is supported by our observation that arterial pulse pressure was noticeably greater during hypoxia after the vagi had been cut. Secondly, the fact that after vagotomy the greater hyperventilation allowed P_{a,O_2} to be better maintained at the most severe level of hypoxia, while P_{a,CO_2} was lower at all levels of hypoxia, may be relevant, for the magnitude of vasodilatation in the intact animal was generally graded with the fall in the level of P_{a,O_2}, while the lower levels of P_{a,CO_2} would have been expected to exert a constrictor influence (Hargreaves & Marshall, 1986). Thirdly, interruption of the known tonic inhibitory influences upon sympathetic vasomotor tone which emanate from the cardio-pulmonary receptors (Mancia & McDonald, 1975) may have allowed the primary sympathetically mediated reflex vasoconstriction induced by hypoxic stimulation of the peripheral chemoreceptors (see Introduction) to have a greater effect upon the regional vascular beds. Lastly, removal of a tonic inhibitory influence from vagal afferents upon vasopressin secretion (Bishop, Thames & Schmidt, 1984) may have allowed this hormone to play a greater role in maintaining arterial

pressure. Vasopressin levels in plasma of both anaesthetized and unanaesthetized rats were substantially raised by levels of hypoxia comparable to those achieved in the present experiments (Forsling & Aziz, 1983) and our recent studies suggest that such raised levels of vasopressin tend to ameliorate hypoxia-induced vasodilatation, at least in muscle (Lloyd & Marshall, 1988).

Having accepted that arterial pressure was better maintained during hypoxia after vagotomy and given that the renal bed is noted for its ability to autoregulate, the smaller rise in mean renal conductance could be explained by a less-marked myogenic dilatation, this still allowing mean renal blood flow to remain within 10% of control levels. By contrast, even though the increases in femoral and mesenteric vascular conductance were smaller as percentages of their respective control levels after vagotomy, both vascular beds tended to receive a higher blood flow during hypoxia after vagotomy, and in response to 8% O_2 the levels of blood flow reached were substantially greater than during air breathing. This is consistent with the suggestion made above, that after vagotomy, hypoxia induced a greater increase in cardiac output.

Whatever the relative importance of the various factors discussed above in explaining the effects of vagotomy, we can certainly conclude that the tachycardia and peripheral vasodilatation induced by hypoxia in the intact animal were largely produced by factors other than the reflex effects of pulmonary stretch receptor stimulation.

The role of sympathetic activity. When background levels of and changes in sympathetic activity had been attenuated with phentolamine, hypoxia still induced comparable falls in arterial pressure to those induced after vagotomy alone, with substantial vasodilatation in skeletal muscle and kidney. These responses also persisted after guanethidine. Thus, it seems that the dilator responses were not neurally mediated. The increase in vascular conductance in skeletal muscle could reasonably be attributed to the local dilator effects of metabolites released as a consequence of tissue hypoxia. This accords with our recent direct observations that following local application of sympathetic antagonists to the spinotraprezius muscle, the dilatation induced in individual arterioles of the muscle by systemic hypoxia was accentuated and the constrictor responses seen in proximal arterioles were attenuated or converted to dilator responses (Booth & Marshall, 1987). Moreover, there is substantial evidence that muscle arterioles are exquisitely sensitive to the vasodilator effects of locally released metabolites (see Marshall & Tandon, 1984). The possibility that tissue metabolites contributed to the vasodilatation seen in the renal and in the mesenteric vasculature can also be considered, for perfusion of the kidney or mesenteric circulation with blood of P_{a,O_2} comparable to the lowest levels reached in the present experiments has been shown to produce significant vasodilatation in these vascular beds (Daugherty, Scott, Dabney & Haddy, 1967; Svanvik, Tyllstrom & Wallentu, 1968). The direct actions of a fall in P_{a,O_2} upon the vascular smooth muscle may also have played a part for hypoxia can induce substantial relaxation of femoral, renal and mesenteric arteries *in vitro* (Marriott & Marshall, 1988*a*, *b*).

Putting the evidence discussed above together, we suggest that the peripheral vasodilatation induced by hypoxia in the intact rat reflects a predominance of the local vasodilator effects of tissue hypoxia. The vasoconstrictor effect of the reflex

increase in sympathetic discharge initiated as a primary response to peripheral chemoreceptor stimulation tends to offset this vasodilatation and may even overcome it in some individuals, so explaining the occasional net vasoconstrictor responses. Furthermore, the constrictor influence of circulating hormones like vasopressin may contribute to the vasoconstrictor influence and could explain the decreases in the regional vascular conductances seen after sympathetic blockade.

The secondary bradycardia and hypoventilation

The fact that the tachycardia was abolished by guanethidine supports the idea that it was mediated by an increase in sympathetic activity. By contrast, the bradycardia which commonly developed after the 2nd minute of hypoxia persisted after guanethidine and was even more pronounced. This finding, together with the observation that such secondary bradycardia in the intact animal was most commonly seen at deeper levels of anaesthesia, when sympathetic activity might be expected to be inhibited, strongly suggests that this response reflected the direct inhibitory action of hypoxia upon cardiac pacemaker activity (Rosen & Kjellmer, 1975). Using similar arguments, the tendency for hyperventilation to wane in the intact animal during the period of hypoxia, particularly at deeper levels of hypoxia, could be explained at least in part by gradual manifestation of the known ability of hypoxia of the central nervous system to inhibit respiration by an action on suprapontine structures (Dempsey & Forster, 1982). The reduced magnitude of the initial *hyper*ventilation and the accentuation of the secondary *hypo*ventilation after phentolamine or guanethidine, could then be ascribed to the relative reductions in O_2 supply to the central nervous system caused by the influences of these drugs upon the cardiac and vascular responses to hypoxia.

Thus, the rat seems to be far more susceptible to the direct effects of hypoxia than larger species like the dog and rabbit in whom the cardiovascular response has been analysed in detail, for in those species these same direct effects only become apparent when the reflex effects of hypoxia have been blocked (Uther *et al.* 1970; Koehler *et al.* 1980). This disparity may be related to the fact that while larger mammals, including cats, dogs, sheep and man, show well-maintained O_2 consumption during hypoxia (Acheson, Dawes & Mott, 1957), small mammals – not only the rat (Adams, Dieleman & Cain, 1982), but also the newborn of large mammalian species – have a much higher O_2 consumption/body weight which may be substantially reduced by hypoxia (Hill, 1959). Thus, small mammals in general may be more susceptible to the ability of reduced O_2 supply to cause release of vasodilator metabolites and to influence directly the processes concerned with maintaining heart rate and respiration. It may be noted that in newborn mammals, as in the rat, systemic hypoxia induces a fall in arterial pressure accompanied by tachycardia and hyperventilation which give way to bradycardia and hypoventilation (e.g. Sidi, Kuipers, Teitel, Heymann & Rudolph, 1983; Blanco, Hanson, Johnson & Rigatto, 1984).

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