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INFLUENCES ON THE CARDIOVASCULAR RESPONSE TO GRADED LEVELS OF SYSTEMIC HYPOXIA OF THE ACCOMPANYING HYPOCAPNIA IN THE RAT

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SUMMARY

1. In spontaneously breathing, anaesthetized rats, a study was made of the effects upon the graded cardiovascular responses to systemic hypoxia (inspiratory fractional O_2 concentration, F_{i,O_2}: 0·15, 0·12, 0·08, 0·06) of maintaining arterial CO_2 pressure (P_{a,CO_2}) at the air-breathing level by adding CO_2 to the inspirate (eucapnic hypoxia), rather than allowing P_{a,CO_2} to fall (hypocapnia hypoxia).

2. At each F_{i,O_2}, maintenance of eucapnia significantly reduced the increase in respiratory frequency, but significantly accentuated the increase in tidal and minute volume: as a result the fall in P_{a,O_2} at each F_{i,O_2} was significantly reduced.

3. Concomitantly, maintenance of eucapnia reduced the increase in heart rate (HR) and fall in arterial pressure (ABP), the effects being significant at F_{i,O_2} 0·08 and/or 0·06. There was also a tendency for the increases in renal and femoral vascular conductances (RVC, FVC) to be reduced; at F_{i,O_2} 0·06 mean increases from control were 2 ± 10 *vs.* 16 ± 7% (eucapnia *vs.* hypocapnia) for RVC, and 62 ± 11 *vs.* 106 ± 27% for FVC.

4. As maintenance of eucapnia reduced the fall in P_{a,O_2} at each F_{i,O_2}, the above results were also considered as a function of P_{a,O_2}. Then, maintenance of eucapnia had similar significant effects on the changes in respiration and HR as described above and reduced the mean increase in RVC (16 ± 11 *vs.* 23 ± 10%, at P_{a,O_2} 31 mmHg, which was attained at F_{i,O_2} 0·06 with eucapnia and 0·08 with hypocapnia). However, maintenance of eucapnia had no effect on the falls in ABP and accentuated the mean increase in FVC ($74·9 \pm 13$ *vs.* 57 ± 10% at P_{a,O_2} 31 mmHg).

5. These findings indicate that, in the rat, the hypocapnia that accompanies the hyperventilatory response to systemic hypoxia facilitates the tachycardia and may accentuate the renal vasodilation, but attenuate the hypoxia-induced vasodilatation in skeletal muscle. Possible mechanisms are discussed.

INTRODUCTION

Our recent experiments on anaesthetized rats have shown that in this species graded levels of systemic hypoxia produce graded hyperventilation, tachycardia, fall in arterial pressure and vasodilatation in renal, mesenteric and muscle vasculature.

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Our analyses have so far suggested that the tachycardia is mediated by an increase in cardiac sympathetic activity, and that the peripheral vasodilatation and fall in arterial pressure is predominantly attributable to the local dilator effects of tissue hypoxia (Marshall & Metcalfe, 1988*b*). Superimposed upon these gradual changes there is an increase in the frequency of augmented breaths, each one being accompanied by a transient peripheral vasodilatation and fall in arterial pressure. We have proposed that these responses are all part of a reflex initiated by pulmonary irritant receptors and facilitated by peripheral chemoreceptors (Marshall & Metcalfe, 1988*a*).

However, during acute systemic hypoxia, arterial CO_2 pressure (P_{a,CO_2}) falls in association with the hyperventilation (Marshall & Metcalfe, 1988*b*). Thus, an obvious question that should be addressed is whether, and if so how, this fall in P_{a,CO_2} modulates the responses evoked by the fall in arterial O_2 pressure (P_{a,O_2})?

It is known that hypocapnia decreases the sensitivity of peripheral chemoreceptors to hypoxia (Cunningham, Robbins & Wolff, 1986), so this might be expected to attenuate the reflex effects exerted upon the cardiovascular system by the peripheral chemoreceptors. Further, a fall in P_{a,CO_2} may elicit a decrease in the sympathetic activity to the heart and vasculature by unloading the central chemoreceptors (e.g. Trzebski & Kubin, 1981; Lioy & Trzebski, 1984). On the other hand, given that a rise in P_{a,CO_2} can induce bradycardia and peripheral vasodilatation by local effects upon the heart and peripheral tissues (e.g. Lagneaux & Remacle, 1981; Marshall, 1986) it is reasonable to suppose that a fall in P_{a,CO_2} could produce opposite local effects. In addition, attenuation of both peripheral and central chemoreceptor activity would reduce central inspiratory drive, which in turn would be expected to facilitate cardiac vagal activity and to inhibit sympathetic activity to heart and vasculature, thus tending to reduce heart rate and produce vasodilatation (Spyer, 1981). Moreover, reduction of the hyperventilatory response might be expected to attenuate reflex tachycardia and vasodilatation elicited by pulmonary stretch receptors (Daly, 1986).

Experiments carried out by Koehler, McDonald & Krasney (1980) have already shown that in the conscious dog, the net effect of preventing the hypocapnia that accompanies the hyperventilatory response to graded systemic hypoxia, by adding CO_2 to the inspirate, is to attenuate the tachycardia and facilitate the increases in mesenteric and renal resistance that occur in this species. This implies that in the dog the predominating influence of the hypocapnia is to induce tachycardia and mesenteric and renal vasodilatation. In previous studies on the rat, systemic hypercapnia induced by adding CO_2 (3–10% CO_2) to the inspirate induced bradycardia, attributable to a predominance of the local myocardial depressant effect of CO_2, while the peripheral vascular effect depended on the severity of hypercapnia: net vasoconstriction in mild hypercapnia attributable to increased sympathetic activity, and vasodilatation in more severe hypercapnia due to the local effects of CO_2 (Lagneaux & Remacle, 1981; Hargreaves & Marshall, 1986). From this it could be predicted that in the rat, the hypocapnia that accompanies hypoxia would tend to induce tachycardia, but the peripheral vascular effects might be vasoconstrictor or vasodilator. It was against this background that the present investigation was performed on the rat to investigate the influence upon the response

to systemic hypoxia of the accompanying hypocapnia. Some of these results have been reported to the Physiological Society (Marshall & Metcalfe, 1987).

METHODS

Experiments were carried out on eight male Sprague–Dawley rats (388 ± 19 g body weight, mean ± S.E.M.) using techniques and equipment described recently (Marshall & Metcalfe, 1988*a*, *b*). 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 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. Arterial pressure was recorded from the left femoral artery and heart rate was electronically computed from the pressure recording. Blood flows were recorded simultaneously and continuously via electromagnetic flow transducers, from the right femoral artery with paw ligated so as to reflect mainly muscle blood flow, and from the left renal artery. Femoral and renal vascular conductances were electronically computed on-line as blood flow/arterial pressure. Ventilation was recorded from the tracheal cannula by means of a flow head and electrospirometer. Blood gases and arterial pH were measured in 140 μl samples taken from the right brachial artery during air breathing and at the end of the 2nd minute of each hypoxic period (see below). Each blood sample was replaced by an equal volume of saline: regular checks on haematocrit, comparing values obtained at the beginning and end of experiments, have revealed no significant or consistent trend.

After a 1–2 h equilibration period at the experimental level of anaesthesia (see above and Marshall & Metcalfe, 1988*b*), each animal was given each of the test hypoxic mixtures to breathe for 3 min. The mixtures were delivered by an air pump via a T-tube placed across the end of the flow head through which the animal breathed. They contained 15, 12, 8 and 6 % O_2 either in pure N_2, or in N_2 with sufficient CO_2 added (4·9–3·0 %) to maintain the P_{a,CO_2} at the end of the 2nd minute of hypoxia at the level pertaining during air breathing. In preliminary experiments CO_2 was added by trial and error, but in the experiments reported here we found that we could predict relatively easily how much CO_2 should be added to each of the hypoxic mixtures, presumably because there was little variation between animals in the magnitude of the changes in blood gas values or ventilation evoked by each O_2 percentage. On occasions when we did not achieve acceptable maintenance of P_{a,CO_2} at the first attempt, the test was repeated after a minor adjustment had been made to the CO_2 in the gas mixture. In each experiment the four pairs of hypoxic mixtures were administered in random order.

All results are expressed as mean ± S.E.M. Statistical tests were carried out using Student's paired *t* test.

RESULTS

The basic features of the respiratory and cardiovascular responses evoked by the hypoxic mixtures without CO_2 were the same as we have described previously (Marshall & Metcalfe, 1988*a*, *b*, see Fig. 1). There were gradual changes in each variable which were generally graded with the level of hypoxia. Superimposed upon these changes was an increase in the frequency of augmented breaths, defined as an additional inspiratory effort at the peak of inspiration, each being associated with transient femoral and renal vasodilatation and fall in arterial pressure. In order to quantify the gradual changes in each variable we have adopted the same procedure as previously (Marshall & Metcalfe, 1988*a*, *b*): a smooth line was drawn through the recording of the gradual changes and measurements were made at the end of the 2nd minute of hypoxia.

Addition of CO_2 to the hypoxic mixtures affected all of the respiratory and cardiovascular variables (see Fig. 1). The mean changes (± S.E.M.) evoked by each hypoxic mixture with and without CO_2 are shown in Fig. 2, as a function of the fraction of O_2 in the inspirate (F_{i,O_2}).

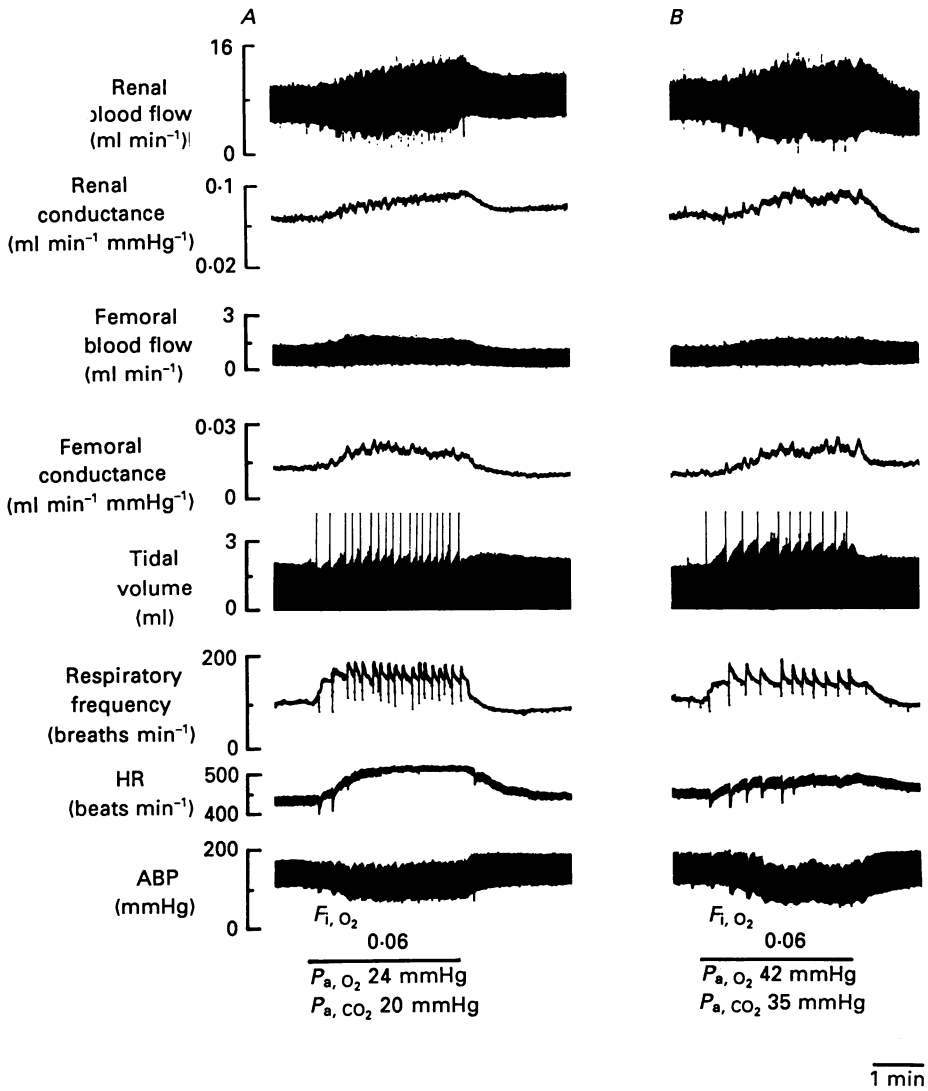

Fig. 1. Cardiovascular and respiratory responses evoked by hypoxia (F_{i,O_2} 0·06) without (*A*) and with (*B*) CO_2 added to inspirate to maintain P_{a,CO_2} at air-breathing level. Traces from above down: blood flow in renal artery, renal vascular conductance, blood flow in femoral artery, femoral vascular conductance, respiratory tidal volume and frequency, arterial pressure. Bar beneath each panel indicates 3 min period of breathing hypoxic mixture. The P_{a,O_2} and P_{a,CO_2} values are those measured at end of 2 min of hypoxia.

As can be seen from Fig. 2, at each F_{i,O_2} without CO_2, both P_{a,O_2} and P_{a,CO_2} fell, the magnitude of the change in each variable being graded with F_{a,CO_2}. But, by adding CO_2 to each of the hypoxic mixtures we successfully maintained P_{a,CO_2} at the air-breathing level across the full range of hypoxic mixtures. Addition of CO_2 also allowed arterial pH to remain constant; pH rose from 7·49 to 7·56 at F_{i,O_2} 0·15 and 0·06 without CO_2 respectively, but was 7·46 and 7·43 at F_{i,O_2} 0·15 and 0·06 with

CO_2, the air-breathing value being 7·47. Meanwhile P_{a,O_2} fell, but the values attained at each F_{i,O_2} with CO_2 were higher than with no added CO_2. The increase in respiratory frequency (F_r) recorded at each F_{i,O_2} was smaller when CO_2 was added (F_r increased from 110·6 ± 4·5 to 141·9 ± 7·2 breaths min^{-1} at F_{i,O_2} 0·21 and 0·06 without CO_2, respectively, and from 110 ± 5·2 to 123 ± 8·1 breaths min^{-1} at F_{i,O_2} 0·21 and 0·06 with CO_2, respectively), but the increases in tidal volume (V_t) were substantially greater in the presence of CO_2 (V_t increased from 1·9 ± 0·1 to 2·3 ± 0·1 ml at F_{i,O_2} 0·21 and 0·06 without CO_2, respectively, and from 1·7 ± 0·1 to 2·7 ± 0·1 ml at F_{i,O_2} 0·21 and 0·06 with CO_2, respectively). It was striking that the increase in F_r reached a peak at F_{i,O_2} 0·08 without CO_2 and at F_{i,O_2} 0·12 with CO_2, whereas V_t increased progressively as F_{i,O_2} was reduced, both with and without CO_2 in the inspirate. The result was that the product of F_r and V_t, minute volume ($\dot{V}_e$), increased significantly more when CO_2 was added to the inspirate at F_{i,O_2} 0·15, 0·08 and 0·06. The frequency of augmented breaths was graded with F_{i,O_2}, whether or not CO_2 was added to the inspirate, but the increase was smaller at each F_{i,O_2} when CO_2 was added, the difference being significant at F_{i,O_2} 0·08 and 0·06.

Arterial pressure (ABP) fell in a graded fashion as F_{i,O_2} was progressively reduced, but the fall at each F_{i,O_2} with added CO_2 tended to be smaller, the difference reaching significance at F_{i,O_2} 0·08: ABP fell from 132·4 ± 6·8 to 86·3 ± 10·8 mmHg at F_{i,O_2} 0·21 and 0·06 without CO_2, respectively, and from 129·7 ± 8·6 to 96·3 ± 4·0 mmHg at F_{i,O_2} 0·21 and 0·06 with CO_2, respectively. The increases in heart rate (HR) were also smaller when CO_2 was added; the differences between the with- and without-CO_2 values were significant both at F_{i,O_2} 0·08 and 0·06, and at F_{i,O_2} 0·06 with CO_2 the average change in HR was actually a fall to below control levels (from 434 ± 10·2 to 430 ± 18·7 beats min^{-1}) rather than a rise (to 466 ± 16·7 beats min^{-1}) seen at F_{i,O_2} 0·06 without CO_2.

The increases in femoral vascular conductance (FVC) were similar whether or not CO_2 was added to the inspirate, except at F_{i,O_2} 0·06, when the average increase recorded with CO_2 was smaller than that recorded without CO_2 (74·9 ± 13·4 *vs.* 106 ± 27·4 % increase from control respectively): this difference did not reach significance. As we reported previously (Marshall & Metcalfe, 1988*b*), in the absence of CO_2 in the inspirate, the evoked increase in renal vascular conductance (RVC) increased progressively when F_{i,O_2} was reduced successively to 0·08, but at F_{i,O_2} 0·06 the increase in RVC was smaller than at F_{i,O_2} 0·08. This same pattern was observed when CO_2 was added to the inspirate, but the increase in RVC at each F_{i,O_2} tended to be smaller (Fig. 2).

Because the fall in P_{a,O_2} at each F_{i,O_2} was less when CO_2 had been added to the inspirate and because our previous studies (Marshall & Metcalfe, 1988*b*) indicated that at least some components of the response evoked by hypoxia were due to the local actions of the fall in P_{a,O_2}, the data discussed above are also presented as a function of P_{a,O_2} (Fig. 3). Since there was no significant difference between the levels of P_{a,O_2} recorded at F_{i,O_2} 0·15 without CO_2 and F_{i,O_2} 0·12 with CO_2 (58·8 ± 1·5 and 57·0 ± 2·9 mmHg, respectively), nor between those recorded at F_{i,O_2} 0·08 without CO_2 and F_{i,O_2} 0·06 with CO_2 (31·2 ± 1·2 and 30·8 ± 2·8 mmHg, respectively), we have tested for significant differences between the respiratory and cardiovascular changes recorded at these two levels of P_{a,O_2} with and without CO_2.

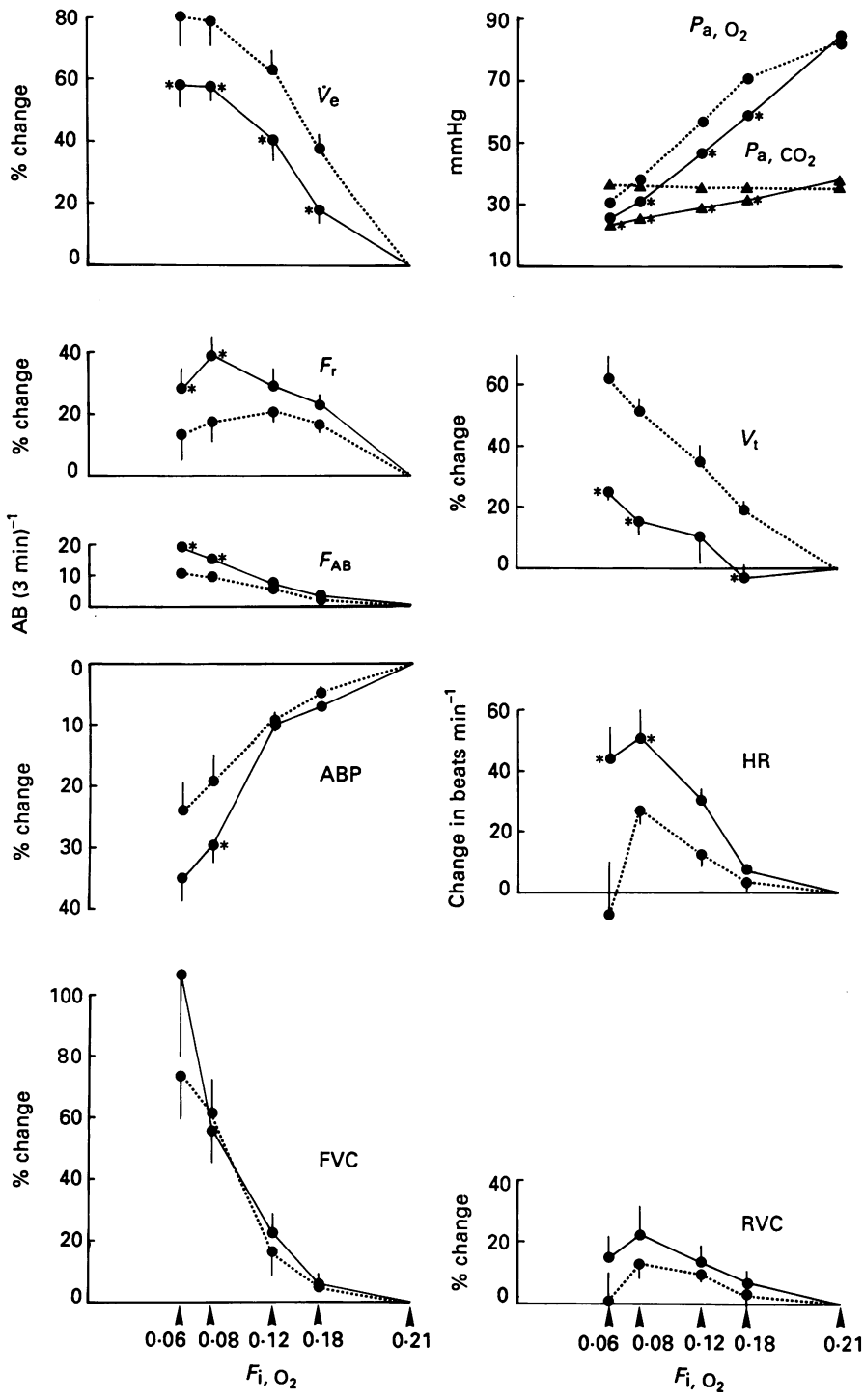

Fig. 2. For legend see facing page.

The effect of adding CO_2 to the inspirate upon the gradual respiratory changes showed the same trends when plotted against P_{a,O_2} as when plotted against F_{i,O_2}; with addition of CO_2 the evoked increases in F_r were smaller and the increases in V_t and $\dot{V}_e$ were larger (cf. Figs 2 and 3). However, there was no clear trend for the effect of CO_2 upon the frequency of augmented breaths when the results were plotted against P_{a,O_2}; at the more moderate level of hypoxia, the frequency of augmented breaths was higher when CO_2 had been added, but at the more severe level of hypoxia this situation was reversed (Fig. 3).

When the changes in ABP were considered as a function of P_{a,O_2} they were virtually identical whether or not CO_2 was added to the inspirate. However, for HR plotting the changes against P_{a,O_2} revealed the same trend as when they were plotted against F_{i,O_2}; at the most severe level of hypoxia, the average HR change was bradycardia when CO_2 was added, rather than tachycardia when no CO_2 was added. Plotting the RVC changes against P_{a,O_2} accentuated the differences noted when they were plotted against F_{i,O_2} because of the shape of the response curve (cf. Figs 2 and 3); at the lowest level of P_{a,O_2} tested, the increase in RVC was smaller when CO_2 was added in seven out of eight experiments though this did not reach statistical significance. On the other hand, in contrast to the tendency noted when FVC was plotted against F_{i,O_2}, when plotted as a function of P_{a,O_2} FVC tended to be larger when CO_2 was added to the inspirate. The average increases in FVC were $74{\cdot}9 \pm 13{\cdot}4$ and $56{\cdot}8 \pm 9{\cdot}7$ % from control at P_{a,O_2} 31 mmHg, with and without CO_2, respectively; this reflected a greater increase when CO_2 was added in six out of the seven experiments, but the differences did not reach statistical significance.

In addition to the changes so far described, hypoxia sometimes evoked a short-lasting episode of further tachycardia, a rise in arterial pressure, a decrease in RVC and an increase in FVC which was superimposed upon the gradual changes (see Fig. 3*A* in Marshall & Metcalfe, 1988*b*), accompanied by pupillary dilatation and exophthalmus. We have concluded that such episodes represent the autonomic components of the alerting–defence response (Marshall & Metcalfe, 1988*b*). In keeping with the observations made in that study, this pattern of response usually occurred when the level of anaesthesia was light, as judged by the paw withdrawal reflex, and was most readily elicited by 8 and 6% O_2. Neither the frequency of

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Fig. 2. Effects of graded levels of hypoxia upon blood gases and respiratory and cardiovascular variables, without and with P_{a,CO_2} maintained, plotted as a function of F_{i,O_2}. Graphs from above down show: P_{a,O_2} (●), P_{a,CO_2} (▲) and respiratory minute volume ($\dot{V}_e$); respiratory frequency (F_r), frequency of augmented breaths (F_{AB}), and respiratory tidal volume (V_t); arterial pressure (ABP) and heart rate (HR); femoral and renal vascular conductance (FVC, RVC). For each graph abscissa is F_{i,O_2}, ordinate is percentage change from baseline levels, with the exception of P_{a,O_2}, P_{a,CO_2} and F_{AB} which are shown as absolute values, and HR which is shown as change in beats min^{-1}. Each point represents mean of measurements made at end of 2nd minute of hypoxia except in the case of F_{AB} when F = number of absolute breaths in a 3 min hypoxic period (AB (3 min)$^{-1}$). S.E.M. indicated by bar except when encompassed within symbol. Continuous and dashed lines joining points indicate values without and with P_{a,CO_2} maintained, respectively. * indicates significant difference between values without and with P_{a,CO_2} maintained ($P < 0{\cdot}05$). Number of pairs of values tested in each case was eight, except in case of FVC when $n = 7$.

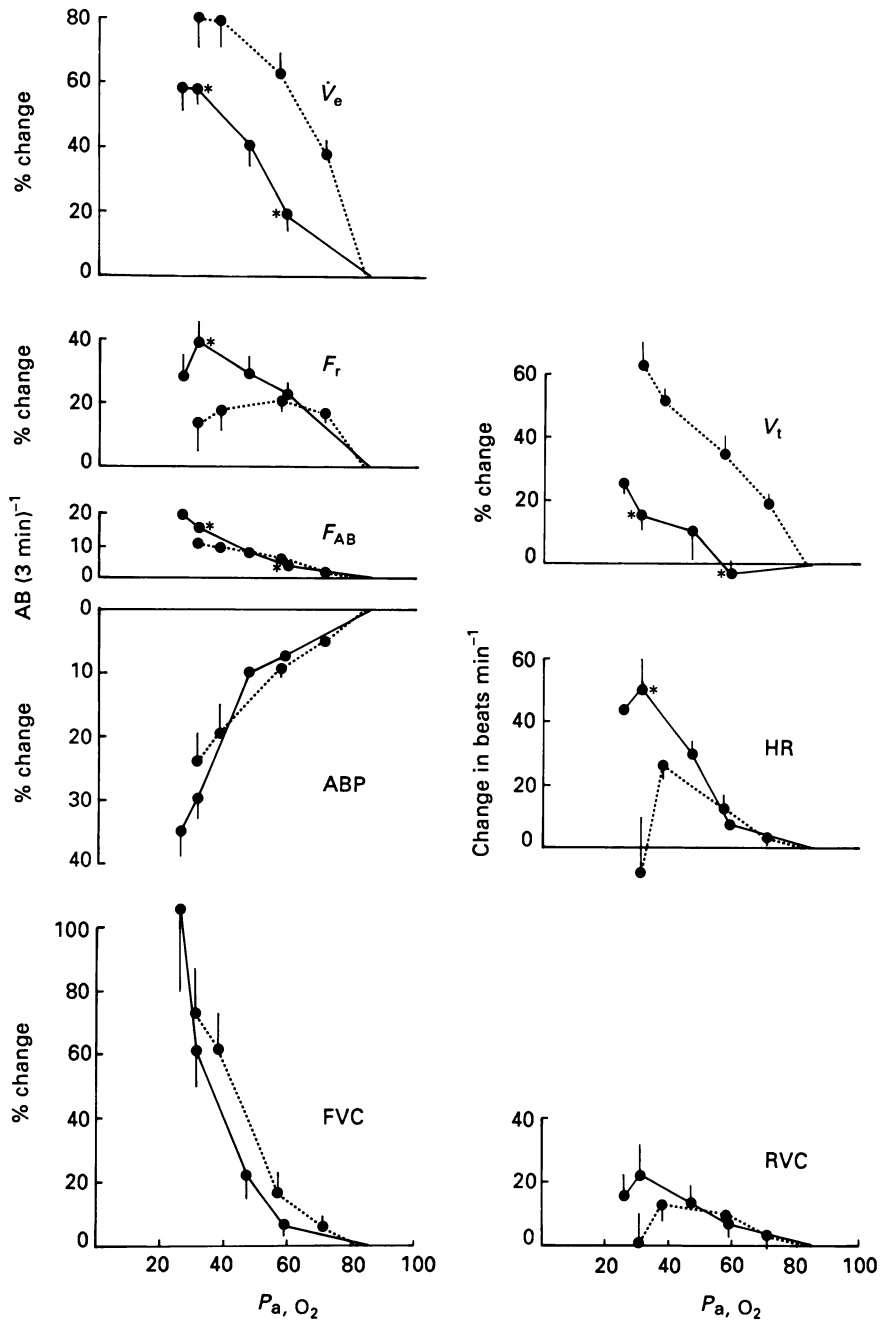

Fig. 3. Effects of graded levels of hypoxia upon respiratory and cardiovascular variables without and with P_{a,CO_2} maintained plotted as a function of P_{a,O_2}. Graphs are presented in same order as in Fig. 2. Ordinates and symbols as in Fig. 2, but abscissa in each case is P_{a,O_2} measured at end of 2nd minute of hypoxia.

occurrence nor the magnitudes of the response were obviously affected by adding CO_2 to the inspirate.

DISCUSSION

In the present study, when no attempt was made to control P_{a,CO_2}, graded levels of systemic hypoxia induced graded hyperventilation and a fall in P_{a,CO_2} (hypocapnic hypoxia). This was accompanied by an increase in the frequency of augmented breaths with associated transient cardiovascular changes, which were superimposed upon gradual tachycardia, vasodilatation in renal and femoral vascular beds and fall in arterial pressure, which in each case was graded with the level of hypoxia. Furthermore, at light levels of anaesthesia hypoxia sometimes evoked a short-lasting episode of changes which are characteristic of the pattern of the alerting–defence response, viz. tachycardia, rise in arterial pressure, vasodilatation in skeletal muscle and vasoconstriction in kidney. All of these changes are comparable to those we have described previously (Marshall & Metcalfe, 1988*a*, *b*).

By adding CO_2 to the hypoxic mixtures, we successfully maintained P_{a,CO_2} at the end of the 2nd minute of hypoxia at the level seen during air breathing. Although we were unable to follow P_{a,CO_2} continuously, it seems reasonable to assume that the air-breathing level was reached at least by the end of the 1st minute of hypoxia, since by this time the ventilatory response had usually reached its peak. For the sake of convenience we have referred to this stimulus as eucapnic hypoxia, whilst recognizing that this is not strictly accurate.

It is clear from Fig. 2 that at each F_{i,O_2}, the increase in minute volume was greater in eucapnic hypoxia than in hypocapnic hypoxia. This was due to a much larger increase in tidal volume during eucapnic hypoxia, given that the increase in respiratory frequency was actually smaller during eucapnic hypoxia than during hypocapnic hypoxia. These results can be explained since a fall in P_{a,CO_2} not only inhibits the response of peripheral chemoreceptors to hypoxia (Cunningham *et al.* 1986), but unloads the central chemoreceptors (Lioy & Trzebski, 1984). The differential effects on F_r and V_t may be a reflection of the strength of the drive to respiration, for Cragg & Drysdale (1983) found in their experiments on the rat, that when the drive to respiration was increased above a certain high level, as when P_{a,CO_2} was raised and P_{a,O_2} was lowered simultaneously, tidal volume increased progressively, but respiratory frequency reached a limit of 110–120 breaths min^{-1} and then remained steady or even fell (cf. Fig. 2). When plotted against F_{i,O_2} the changes in frequency of augmented breaths parallelled the changes in respiratory frequency in that the frequency of augmented breaths was higher during hypocapnic hypoxia than eucapnic hypoxia over the full range of hypoxia. Our recent results (Marshall & Metcalfe, 1988*a*) supported the accepted view (Glogowska, Richardson, Widdicombe & Winning, 1972; Widdicombe, 1982), that the increase in frequency of augmented breaths in hypoxia is initiated by rapidly adapting, pulmonary irritant receptors and facilitated by peripheral chemoreceptors, the hyperventilation evoked by the peripheral chemoreceptors tending to increase the velocity of air flow and thereby to stimulate the irritant receptors. The present results also accord with that view: the increase in respiratory frequency presumably stimulates the irritant receptors breath-by-breath and the frequency of augmented breaths actually

reached is governed by the threshold and refractoriness of their central pattern generator (Glogowska *et al.* 1972; Cherniack, von Euler, Glogowska & Homma, 1981; St. John, Bledsoe & Sokol, 1984).

Thus, the results so far discussed indicate that the effect of maintaining P_{a,CO_2} constant is that at each F_{i,O_2}, central and peripheral chemoreceptor activity is higher leading to a greater increase in central inspiratory drive. Further, as is clear from Fig. 2, the resulting enhancement of the hyperventilation ensures that at each F_{i,O_2}, P_{a,CO_2} falls less than during hypocapnic hypoxia. All of these factors must be taken into consideration when interpreting the influence of CO_2 upon the cardiovascular response since the cardiovascular system can be affected by the local influence of CO_2 upon the heart and vasculature, by reflexes initiated by changes in central and peripheral chemoreceptor activity, by the central actions of changes in central inspiratory drive upon sympathetic and vagal activity, by reflexes secondary to hyperventilation and by the local actions of changes in P_{a,CO_2} (see Introduction). In their analysis of the effects of changes in P_{a,CO_2} upon the cardiovascular response evoked by graded hypoxia in the dog, Koehler *et al.* (1980) plotted the data as a function of P_{a,O_2}, rather than F_{i,O_2}, because, they argued, this allowed assessment of the threshold level of P_{a,O_2} required to produce the various components of the response. This statement is not correct when experiments are carried out under closed-loop conditions, i.e. when the receptors are exposed not only to the 'stimulus', but to the 'response', as was the case in their study and indeed in ours. Nevertheless we have presented our cardiovascular data in relation to P_{a,O_2}, as well as to F_{i,O_2}, because our recent study suggested that in the rat, the cardiovascular response to systemic hypoxia is strongly influenced by the local effects of changes in P_{a,O_2}. Since the changes in respiratory frequency, tidal volume and minute volume discussed above showed the same trends when considered as a function of P_{a,O_2} as when considered in relation to F_{i,O_2} (cf. Figs 2 and 3), the comments already made concerning possible respiratory-dependent influences upon the cardiovascular system resulting from changes in P_{a,CO_2} are appropriate in both cases. The fact that when plotted against P_{a,O_2} there was no longer a clear relationship between frequency of augmented breaths and respiratory frequency, in that at moderate levels of hypoxia maintenance of P_{a,CO_2} increased the frequency of augmented breaths, but reduced respiratory frequency, is consistent with the view that, in addition to the positive interaction between peripheral chemoreceptors and pulmonary irritant receptors discussed above, increased stimulation of peripheral chemoreceptors can itself initiate augmented breaths (Glogowska *et al.* 1972).

Before considering the gradual cardiovascular changes, it may be noted that in the conscious dog, behavioural arousal was far more common during eucapnic hypoxia than during hypocapnic hypoxia, which Koehler *et al.* (1980) attributed to a greater stimulation of peripheral chemoreceptors in eucapnic hypoxia. This would be consistent with our evidence that peripheral chemoreceptor stimulation can activate the brain stem defence areas to produce the cardiovascular components of the alerting–defence response (Marshall & Metcalfe, 1988*b*). That we could discern no obvious difference between the frequencies of occurrence, nor the magnitudes of this response in hypocapnic and eucapnic hypoxia, whether the observations were considered as a function of P_{a,O_2} or F_{i,O_2}, is not inconistent with Koehler *et al.*'s

results, nor with the general hypothesis. For whether or not a particular stimulus will evoke the cardiovascular components of the alerting–defence response under Saffan anaesthesia is unpredictable, being apparently dependent upon the depth of anaesthesia, which is difficult to control with precision (Marshall, 1987; Marshall & Metcalfe, 1988*b*).

Turning to the gradual cardiovascular changes, at the more severe levels of hypoxia the evoked fall in arterial pressure was greater during hypocapnic than eucapnic hypoxia when considered as a function of F_{i,O_2}, but not when considered as a function of P_{a,CO_2}. This is consistent with our proposal that the fall in arterial pressure is attributable mainly to vasodilatation in skeletal muscle and that this is induced by the local dilator action of hypoxia (Marshall & Metcalfe, 1988*b*). The changes we recorded in femoral vascular conductance accord with that view in that the increase in femoral vascular conductance evoked at F_{i,O_2} 0·06 tended to be greater when P_{a,CO_2} was allowed to fall than when it was maintained, whereas when plotted against P_{a,O_2} the mean increases in femoral vascular conductance were larger during eucapnic than hypocapnic hypoxia, this being so in all but one experiment at the most severe level of hypoxia. The fact that at comparable levels of P_{a,O_2} the differences between the increases in femoral vascular conductance recorded in eucapnic hypoxia and hypocapnic hypoxia were small, even though the concomitant hyperventilation was substantially greater in the former condition, is consistent with our previous conclusion that reflex vasodilatation evoked in skeletal muscle by pulmonary stretch receptors is weak in the rat and plays no significant part in the response to systemic hypoxia (Marshall & Metcalfe, 1988*a*, *b*). Rather, we suggest that any accentuation of the muscle vasodilatation in eucapnic hypoxia may have reflected the local vasodilator action of CO_2 upon the vasculature of skeletal muscle (Daugherty, Scott, Dabney & Haddy, 1967). If this is so, then it is reasonable to propose that the fall in P_{a,CO_2} that occurs in response to systemic hypoxia in the absence of experimental intervention may attenuate vasodilatation in skeletal muscle by exerting a local vasoconstrictor effect on the vascular smooth muscle.

By contrast, the increase in renal vascular conductance was generally greater during hypocapnic than eucapnic hypoxia, particularly at the more severe levels of hypoxia, whether the changes are considered in relation to F_{i,O_2} or P_{a,O_2}. This is compatible with our suggestion that the local vasodilator effects of hypoxia make little contribution to vasodilatation in the kidney (Marshall & Metcalfe, 1988*b*). We previously proposed that the renal vasodilatation was largely due to a myogenic response induced by the fall in systemic arterial pressure. But, at least at comparable levels of P_{a,O_2}, the changes induced in arterial pressure were the same in eucapnic and hypocapnic hypoxia. Thus, it is necessary to invoke an additional vasoconstrictor influence upon the kidney during eucapnic hypoxia. This could be explained by an increase in renal sympathetic activity due to greater stimulation of central chemoreceptors (Trzebski & Kubin, 1981), of peripheral chemoreceptors (Daly, 1986) and/or to the effects of a greater increase in central inspiratory drive (Spyer, 1981). Our results do not allow us to distinguish the relative importance of these effects, but it is reasonable to propose that when P_{a,CO_2} is allowed to fall in systemic hypoxia the opposite is true, i.e. the renal vasodilatation is facilitated by inhibitory influences upon renal sympathetic activity. Taken together, the suggestions that the fall in

P_{a,CO_2} may exert a vasoconstrictor influence upon skeletal muscle, but a vasodilator effect on the kidney, are consistent with previous observations that the effects of a rise in P_{a,CO_2} in the rat depend upon the balance between vasodilator and vasoconstrictor influences (Lagneaux & Remacle, 1981; Hargreaves & Marshall, 1986).

The increases in heart rate were significantly smaller in eucapnic hypoxia than in hypocapnic hypoxia at the most severe levels, whether considered in relation to F_{i,O_2} or P_{a,CO_2}. This suggests that any increase in cardiac sympathetic activity due to greater central chemoreceptor activity, or any increase in cardiac sympathetic and decrease in vagal activity attributable to the greater increase in central respiratory drive that would be expected to accompany eucapnic hypoxia (see Spyer, 1981, and above), made no significant contribution to the changes in heart rate. Further, tachycardia initiated as a reflex by pulmonary stretch receptors secondary to the greater hyperventilation (Daly, 1986), apparently played no significant role, in accord with our evidence that the cardiac as well as the vascular component of this reflex is weak in the rat (Marshall & Metcalfe, 1988*a*, *b*). Rather, it seems that maintenance of P_{a,CO_2} and consequent greater stimulation of the peripheral chemoreceptors by the fall in P_{a,O_2} may have allowed a greater manifestation of the bradycardia initiated as a primary reflex by the peripheral chemoreceptors (Daly, 1986; Marshall, 1987). This leads to the conclusion that when P_{a,CO_2} was allowed to fall during hypoxia, this attenuated peripheral chemoreceptor activity sufficiently to allow the reflex bradycardia to be completely dominated by some influence/s that induce tachycardia. If it is accepted that the effects of central inspiratory drive and pulmonary stretch receptors are relatively unimportant, as is implied above, then it seems that tachycardia mediated by the excitatory effects upon sympathetic activity of central nervous hypoxia (Downing, Mitchell & Wallace, 1963) may have made the dominant contribution. This would be in accord with our previous evidence that the tachycardia observed in hypocapnic hypoxia is largely due to increased sympathetic activity (Marshall & Metcalfe, 1988*b*). However, another possibility is that tachycardia produced by the direct influence of a fall in P_{a,CO_2} upon the cardiac pacemaker plays a part, given the fact that the opposite effect predominates in the rat when P_{a,CO_2} is raised (Lagneaux & Remacle, 1981; Hargreaves & Marshall, 1986).

In summary, the present study suggests that the hypocapnia that accompanies the hyperventilatory response to systemic hypoxia substantially attenuates the hyperventilation by reducing the stimulation of peripheral and central chemoreceptors. Further, that the reduced stimulation of the peripheral chemoreceptors allows the bradycardia initiated by these receptors to be overcome by factors that induce tachycardia, which may include the direct excitatory action of a fall in P_{a,CO_2} upon the cardiac pacemaker. In addition, reduced stimulation of peripheral and central chemoreceptors, together with the resulting reduction in central inspiratory drive, may contribute to facilitate the renal vasodilatation by their inhibitory influence upon renal sympathetic activity. On the other hand, the fall in P_{a,CO_2} may slightly attenuate the vasodilatation in skeletal muscle by a local vasoconstrictor influence upon the muscle vasculature. We have already discussed the fact that the cardiovascular response evoked by hypocapnic hypoxia in the rat differs in several

important respects from that evoked in larger species, like the dog (Marshall & Metcalfe, 1988*b*). Thus, in the latter, arterial pressure rises, the fall in total peripheral resistance is much less marked and almost entirely attributable to vasodilatation in muscle, which in the dog, at least, can be ascribed largely to the reflex elicited by pulmonary stretch receptors (see Koehler *et al.* 1980). However, when Koehler *et al.* (1980) maintained eucapnia during hypoxia in the dog, the tachycardia was substantially attenuated and although renal and mesenteric vascular resistance showed no change in hypocapnic hypoxia, both increased in eucapnic hypoxia, indicating that the fall in P_{a,CO_2} had been exerting a vasodilator influence: no measurements were made of muscle vascular resistance. Thus, it seems that at least with respect to heart rate and splanchnic vascular resistance, hypocapnia exerts directionally similar effects during hypoxia in the rat as in the dog although the peripheral vascular effects are much less pronounced in the rat.

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