Interdependence of respiratory and cardiovascular changes induced by systemic hypoxia in the rat: the roles of adenosine

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1. In ten spontaneously breathing, Saffan-anaesthetized rats (group I), respiratory and cardiovascular responses evoked by 10 min periods of hypoxia (arterial partial pressure of O_2, P_{a,O_2}, 33 mmHg) were recorded before and after the administration of the adenosine receptor antagonist 8-phenyltheophylline (8-PT, 10 mg kg^{-1} I.V.). Similar experiments were performed on nine constantly ventilated rats (group II; P_{a,O_2}, 29 mmHg) with arterial partial pressure of CO_2 (P_{a,CO_2}) held constant.

2. In group I, hypoxia induced an initial increase and a secondary fall in ventilation ($\dot{V}_E$) with an accompanying secondary fall in heart rate (HR), arterial pressure (ABP) fell and cerebral vascular conductance (CVC) increased progressively. Cerebral blood flow (CBF) tended to fall with time during hypoxia. 8-PT abolished the secondary falls in $\dot{V}_E$ and HR and reduced the fall in ABP and increase in CVC, while CBF was better maintained.

3. In group II, hypoxia induced a similar cardiovascular response to that in group I, but at the 1st minute of hypoxia, the HR was lower and the increase in CVC was greater. 8-PT did not affect the hypoxia-induced changes in HR, ABP, CVC or CBF.

4. These results indicate specific ways in which the ventilatory and cardiovascular responses induced by hypoxia in the spontaneously breathing rat are interdependent. They also indicate that the influences of 8-PT on the cardiovascular changes induced by hypoxia during spontaneous ventilation are mainly a consequence of its ability to block the centrally mediated contribution of adenosine to the secondary fall in ventilation.

In the anaesthetized rat, 3 or 5 min periods of systemic hypoxia produce hyperventilation which wanes after the 2nd minute of hypoxia, tachycardia which wanes to bradycardia, a pronounced fall in arterial pressure and vasodilatation in hindlimb skeletal muscle; similar cardiovascular changes were recorded in the conscious rat (Marshall & Metcalfe, 1988*b*, 1990; Neylon & Marshall, 1991). Analysis of these changes led to the conclusion that the secondary fall in ventilation, bradycardia and peripheral vasodilatation reflect the local effects of hypoxia on central respiratory neurones, the sino-atrial node and peripheral vasculature, respectively (Marshall & Metcalfe, 1988*b*; Neylon & Marshall, 1991). Moreover, recordings of common carotid artery blood flow with all the branches ligated except the internal carotid artery, which supplies the brain, suggested that cerebral blood flow increased during the first 1–2 min of hypoxia, but then fell towards or below prehypoxic levels as arterial pressure reached its lowest level (Neylon & Marshall, 1991).

Considering these responses together raises the possibility that the respiratory and cardiovascular responses evoked by systemic hypoxia could become self-perpetuating. Thus, the secondary fall in ventilation would tend to exacerbate the fall in the arterial partial pressure of oxygen (P_{a,O_2}), so potentiating the bradycardia and peripheral vasodilatation that are caused by hypoxia and inducing a further fall in arterial pressure. This in turn might lead to a reduction in cerebral blood flow, particularly if arterial pressure fell below the autoregulatory range, and would thereby accentuate the reduction in O_2 transport to the brain and the respiratory depression, since the latter is thought to be centrally mediated (Dempsey & Forster, 1982).

In previous studies, we have suggested that adenosine plays a major role in the respiratory and cardiovascular changes observed during systemic hypoxia. Since the adenosine receptor antagonist 8-phenyltheophylline (8-PT) substantially reduced the secondary fall in ventilation, bradycardia and the vasodilatation in skeletal muscle induced by systemic hypoxia, we proposed that locally released adenosine mediates these changes by direct actions on central respiratory neurones, on the sino-atrial node and on the peripheral vasculature, respectively (Neylon & Marshall, 1991). However, if the respiratory and cardiovascular changes that occur during systemic hypoxia do form a positive feedback loop as proposed

above, then if an adenosine receptor antagonist were to block just one component of the loop, this might be expected to affect all the other components.

In order to investigate the interdependence of the respiratory and cardiovascular changes in more detail, and to elucidate further the role of adenosine, we have now compared the responses evoked during spontaneous respiration with those induced when ventilation was artificially held constant and have compared the effects of 8-PT on the responses induced by hypoxia under these two conditions. In order to accentuate the secondary fall in ventilation, bradycardia and peripheral vasodilatation that we propose reflect the local effects of hypoxia (Marshall & Metcalfe, 1988*b*), we used a 10 min period of hypoxia in the present study, rather than the 3 or 5 min periods chosen previously (Marshall & Metcalfe, 1988*b*; Neylon & Marshall, 1991). Some of the results have already been presented to the Physiological Society (Marshall & Thomas, 1993).

METHODS

Experiments were performed on Wistar rats, in which anaesthesia was induced with halothane, N_2O and O_2 and subsequently maintained with Saffan (7–12 mg kg^{-1} h^{-1} I.V.; Pitman-Moore Ltd, Uxbridge, UK) as described previously (Neylon & Marshall, 1991). The trachea was cannulated with a steel T-shaped cannula. Further, as described previously (Marshall & Metcalfe, 1988*a*; Neylon & Marshall, 1991), arterial pressure (ABP) and heart rate (HR) were recorded from the femoral artery, while carotid artery blood flow (CBF) was measured using a cuff-type electromagnetic probe placed around the common carotid artery after vascular isolation of the internal carotid artery supply. Carotid vascular conductance (CVC) was calculated on-line as a beat-by-beat division of CBF/ABP. All cardiovascular variables were displayed on a six-channel pen recorder (Gould Brush 2400, Gould Inc., Cleveland, OH, USA). The brachial artery was cannulated to allow 130 μl samples of arterial blood to be taken anaerobically for measurement of P_{a,O_2}, arterial partial pressure of carbon dioxide (P_{a,CO_2}) and arterial pH using a Nova Stat Profile Analyser (Stat 3, V. A. Howe, Waltham, MA, USA).

Group I. This group of ten rats (weighing 357 ± 10 g, mean $\pm$ S.E.M.) breathed spontaneously throughout. The side-arm of the tracheal cannula was connected via a flow head to an electrospirometer (see Marshall & Metcalfe, 1988*a*), so allowing tidal volume (V_T) and respiratory frequency (R_F) to be recorded on the pen recorder; minute volume ($\dot{V}_E$) was calculated as $V_T \times R_F$. Throughout the experiment air or a hypoxic mixture was blown across the end of the flow head by an air pump at a rate greater than 1 l min^{-1}. Following a 1 h equilibration period after surgery at the experimental level of anaesthesia (Saffan infusion at 7–9 mg kg^{-1} h^{-1}), continuous recordings of all cardiovascular and respiratory variables were made during air breathing and during a 10 min period of hypoxia (8% O_2), before and after 8-PT (10 mg kg^{-1} I.V.; Sigma, UK), 15 min being allowed after administration of 8-PT and before another hypoxic stimulus was given. Arterial blood samples for blood gas analysis were taken during normoxia and in the 10th minute of each period of hypoxia.

Group II. A further nine rats (weighing 417 ± 18 g) were artificially ventilated throughout the experiment. A bilateral pneumothorax was performed by puncturing the thorax between the 7th and 8th ribs, each incision being held open by means of a retractor. Artificial ventilation was maintained with a ventilator (Small Animal Ventilator, Ealing, UK) against an end-expiratory pressure of 1 cmH_2O. The ventilator frequency was set at a level that approximated to the spontaneous respiratory frequency and the delivery pressure was adjusted such that P_{a,O_2} and P_{a,CO_2} were comparable to those recorded in group I during air breathing. In practice we found that in order to achieve these levels of P_{a,O_2} and P_{a,CO_2}, it was necessary to ventilate the animals with air with added CO_2 ($2{\cdot}7 \pm 0{\cdot}1\%$), O_2 being reduced accordingly.

The animal was allowed to equilibrate at the experimental level of anaesthesia until cardiovascular variables and blood gases were stable (see above). It was then ventilated with a hypoxic mixture for 10 min. The mixture was adjusted so that the P_{a,O_2} was comparable with that recorded during 8% O_2 in the spontaneously breathing rats of group I, and so that P_{a,CO_2} was maintained at the control level; the gas mixture used was $6{\cdot}3 \pm 0{\cdot}1\%$ O_2 and $2{\cdot}9 \pm 0{\cdot}2\%$ CO_2. Then, 8-PT was administered (10 mg kg^{-1} I.V.) and the animal was ventilated with the same hypoxic mixture for a further 10 min period. Blood gas analysis showed that after 8-PT, the P_{a,O_2} attained during hypoxia was higher than that recorded before, even though the same hypoxic mixture was used (see Thomas & Marshall, 1993). Thus, extra N_2 was added to the hypoxic mixture and further 10 min periods of hypoxia were administered until the P_{a,O_2} measured during hypoxia was comparable with that obtained in the same animal during hypoxia before 8-PT. This required one or two further attempts; the hypoxic mixture used was $5{\cdot}7 \pm 0{\cdot}2\%$ O_2 and $2{\cdot}9 \pm 0{\cdot}2\%$ CO_2. Only the response evoked by the final hypoxic period producing a P_{a,O_2} comparable with that before 8-PT was considered for final analysis; the others were ignored.

To determine whether blood flow in the common carotid artery with all but the internal carotid artery ligated gave a reasonable estimate of cerebral blood flow, a solution of Methylene Blue was injected into the right common carotid artery at the end of the experiment. To this end, the right common carotid artery was cannulated towards the brain and a small clip was placed on the left jugular vein to impede blood flow out of the cerebral circulation (the right jugular vein was already ligated and cannulated towards the heart, see above). Immediately after the dye had been injected, a concentrated solution of potassium chloride was introduced through the right femoral vein to kill the animal.

Statistical analysis

In both groups I and II, the values of each variable under control conditions and during hypoxia were compared by using ANOVA. *Post hoc* comparisons were made by using Student's paired t test with the Bonferroni correction for multiple comparisons between: (i) baseline values and values recorded at the end of the 1st, 5th and 10th minute of hypoxia; (ii) the absolute values at the 1st minute and at the 10th minute of hypoxia; and (iii) the absolute changes evoked by hypoxia before and after 8-PT. Comparisons were also made between the baseline levels of cardiovascular and respiratory variables and arterial gases before and after 8-PT by Student's paired t test (groups I and II). In addition, the percentage changes induced in each variable at the end of the

1st, 5th and 10th minute of hypoxia in group I were compared with those evoked at the same times in group II by using Student's unpaired t test.

RESULTS

Measurement of carotid blood flow as an indication of cerebral blood flow

The immediate observation upon injection of a concentrated solution of Methylene Blue into the right common carotid artery (see Methods) was blue coloration of the right eye together with a little of the nostril on that side of the face. The roof of the mouth was also slightly coloured, whereas the tongue showed no coloration at all, confirming that the lingual artery was successfully ligated. There was no coloration in the skin of the cheek and removal of the skin revealed no coloration of the underlying muscles. This suggests that all of the branches of the external carotid artery had been successfully tied off. Upon opening the cranium, it was clear that the dye had gone to the cerebral hemispheres, cerebellum and rostral parts of the brainstem, and coloration was exclusively seen on the right side. It appears, therefore, that measurement of carotid blood flow under the conditions described provides a good index of forebrain blood flow, but is contaminated by blood flow to the orbit and upper buccal cavity. On this basis, we have used the terms cerebral blood flow and cerebral vascular conductance to refer to recordings made from and derived from (respectively) the common carotid artery.

Effects of systemic hypoxia during spontaneous ventilation (group I)

The pattern of cardiovascular and respiratory response evoked by spontaneously breathing 8% O_2 was consistent with that described previously in the rat (Marshall & Metcalfe, 1988*b*; Neylon & Marshall, 1991). There was an increase in $\dot{V}_E$ at 1 min of hypoxia, due to an increase in both V_T and R_F, but there was a secondary fall in V_T and therefore in $\dot{V}_E$ (Fig. 1). P_{a,O_2}, recorded at the end of the 10th minute of hypoxia, fell to 33·1 ± 1·1 mmHg and P_{a,CO_2} fell to 34·4 ± 2·6 mmHg because of the hyperventilation (Table 1). In addition, there was a progressive fall in ABP during hypoxia; HR tended to increase initially, but waned after about 2–3 min, such that the absolute HR recorded at the 10th min was significantly different from that recorded at the 1st minute (Fig. 1). There was a progressive increase in CVC, indicating cerebral vasodilatation. Cerebral blood flow did not change significantly. However, there was a tendency for CBF to increase initially and then fall towards or below control levels by the 10th minute. Indeed, in seven out of ten animals, CBF was lower at the 10th than at the 1st minute. (Fig. 1).

Effects of 8-PT

The dose of 8-PT we used (10 mg kg^{-1}) has previously been shown to achieve good blockade of adenosine receptors under conditions comparable with those of the present study; it attenuated the respiratory and cardiovascular effects of an intra-arterial infusion of adenosine (0·3 mg kg^{-1} min^{-1}) and of a bolus injection of the stable adenosine analogue 2-chloroadenosine at 30 μg kg^{-1} I.V. (see Marshall, Thomas & Turner, 1993).

After 8-PT during air breathing, baseline levels of V_T were significantly increased, whilst baseline levels of HR, CVC and CBF were reduced. 8-PT had no effect on the other respiratory or cardiovascular variables, or on arterial blood gases during air breathing (Tables 1 and 2).

After 8-PT, V_T was significantly increased at the end of both the 1st and 5th minute of hypoxia and the change in V_T recorded at the 5th minute after 8-PT was significantly greater than that evoked before 8-PT. Moreover, in contrast to the responses observed before 8-PT, the absolute value of V_T at the 1st and 10th minute were not significantly different (Fig. 1), i.e. there was no secondary waning of the increase in V_T or $\dot{V}_E$. P_{a,O_2} and P_{a,CO_2} recorded at the end of the 10th minute of hypoxia were not significantly affected by 8-PT (Table 1), although P_{a,CO_2} tended to be lower and arterial pH was significantly

Table 1. Blood gas values recorded during air breathing and during hypoxia before and after 8-phenyltheophylline, when ventilaton was spontaneous or held constant

	Spontaneous ventilation			Artifical ventilation		
	P_{a,O_2} (mmHg)	P_{a,CO_2} (mmHg)	pH	P_{a,O_2} (mmHg)	P_{a,CO_2} (mmHg)	pH
Before 8-PT						
Normoxia	93·5 ± 2·2	44·0 ± 3·5	7·24 ± 0·02	93·4 ± 2·6	39·8 ± 1·2	7·33 ± 0·01
Hypoxia	33·1 ± 1·1	34·4 ± 2·6	7·27 ± 0·03	29·3 ± 1·2	38·9 ± 1·7§§	7·19 ± 0·03
After 8-PT						
Normoxia	96·4 ± 3·5	42·6 ± 2·1	7·29 ± 0·03	89·1 ± 3·5	43·2 ± 1·8	7·27 ± 0·02
Hypoxia	33·6 ± 2·4	31·3 ± 2·1	7·38 ± 0·03‡‡	30·7 ± 0·9	38·6 ± 1·5§§	7·19 ± 0·01

Values of P_{a,O_2}, P_{a,CO_2} and arterial pH were recorded during the 10th minute of hypoxia. For further details of hypoxic mixtures, see text. Significant difference between value recorded when ventilation was spontaneous and held constant: §§ $P < 0·01$. Significant difference between value recorded before and after 8-PT: ‡‡ $P < 0·01$.

Table 2. Effects of 8-phenyltheophylline upon baseline levels of respiratory and cardiovascular variables during air breathing when ventilation was spontaneous

	V_T (ml)	R_F (breaths min^{-1})	$\dot{V}_E$ (ml min^{-1})	Mean ABP (mmHg)	HR (beats min^{-1})	CBF (ml min^{-1})	CVC (ml min^{-1})
Before 8-PT	2·2 ± 0·1	89·6 ± 4·3	195·1 ± 10·1	138·2 ± 4·1	393·4 ± 10·7	2·2 ± 0·2	0·02 ± 0·001
After 8-PT	2·4 ± 0·1‡	86·8 ± 3·5	206·6 ± 14·3	139·5 ± 4·5	381·5 ± 8·5‡	1·75 ± 0·1‡‡	0·01 ± 0·001‡‡

Significant difference between values recorded before and after drug: ‡ $P < 0·05$; ‡‡ $P < 0·01$.

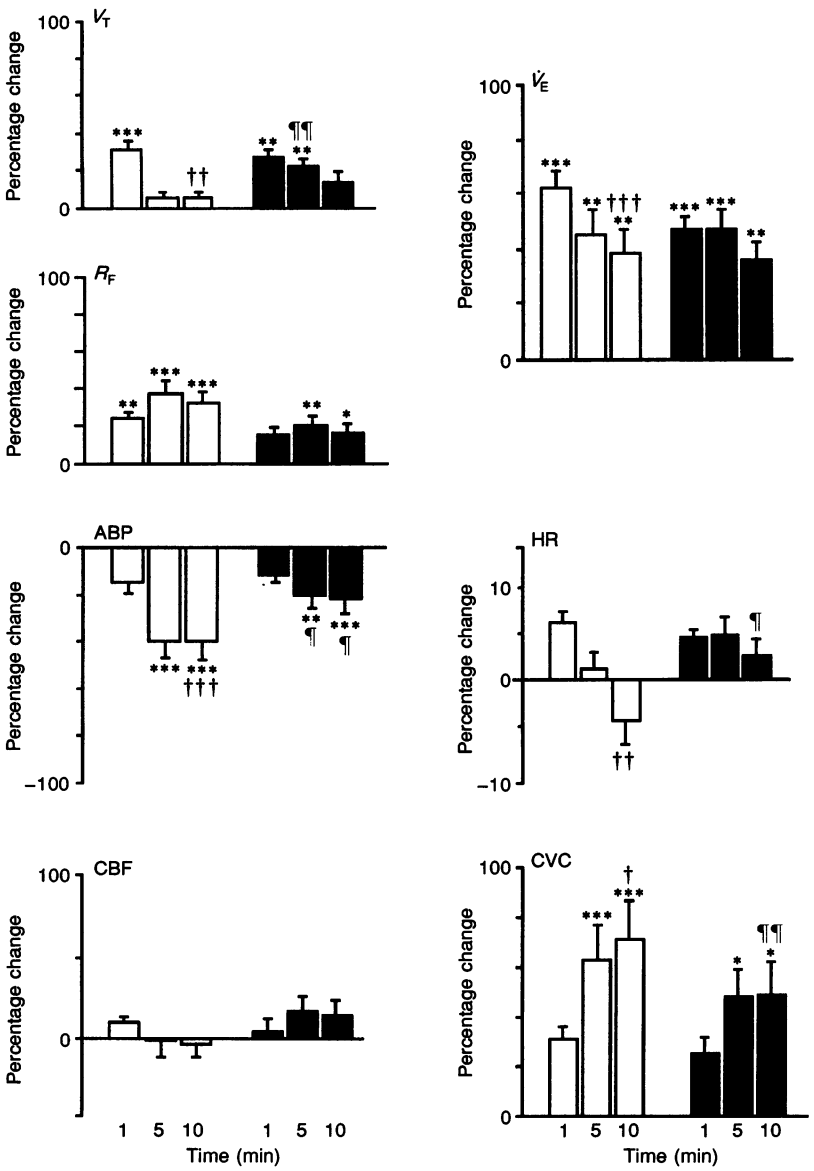

Figure 1. Effect of 8-PT upon mean cardiovascular and respiratory changes induced by systemic hypoxia (8% O_2) during spontaneous ventilation (group I)

Open and filled columns indicate changes before and after 8-PT, respectively. Each column indicates mean ± S.E.M. percentage change at the end of the 1st, 5th and 10th minute of hypoxia, as indicated below the columns. Significant difference between absolute values recorded during air breathing and hypoxia: * $P < 0·05$; ** $P < 0·01$; *** $P < 0·001$. Significant difference between absolute values at 1 and 10 min of hypoxia before and after 8-PT: † $P < 0·05$; †† $P < 0·01$; ††† $P < 0·001$. Significant difference between absolute changes evoked during hypoxia before and after 8-PT: ¶ $P < 0·05$; ¶¶ $P < 0·01$.

higher, consistent with the reduction in the secondary fall in V_T. Amongst the cardiovascular variables, the fall in ABP evoked by hypoxia was greatly reduced at the 5th and 10th minute of hypoxia, the secondary waning of HR between the 1st and 10th minute was abolished and the HR recorded at the 10th minute of hypoxia was significantly greater than that recorded at the same time before the drug (Fig. 1). The increase in CVC tended to be smaller after 8-PT, reaching significance at the 10th minute and, in contrast to the situation before 8-PT, the CVC recorded at the 10th minute was not significantly different from that recorded at the 1st minute of hypoxia (Fig. 1). There was also a tendency for CBF to be better maintained during hypoxia after 8-PT (Fig. 1); CBF was lower at the 10th than at the 1st minute of hypoxia in only one out of ten animals (cf. before 8-PT, see above).

Cardiovascular response evoked by systemic hypoxia during constant artificial ventilation (group II)

Baseline levels of cardiovascular variables recorded during artificial ventilation were not significantly different from those recorded in spontaneously breathing animals during air breathing (Table 2). Furthermore, when using the suitably adjusted gas mixture (see Methods) the P_{a,O_2} recorded during hypoxia in the artificially ventilated animals was fully comparable with that obtained during 8% O_2 in the spontaneously breathing rat (group I, Table 1), while the P_{a,CO_2} recorded during hypoxia was not significantly different from the control P_{a,CO_2} (Table 1).

Under these conditions, 10 min of hypoxia induced a pattern of cardiovascular changes that was similar to that described for the spontaneously breathing rats, as can be seen in Fig. 2, which illustrates the changes recorded both in the spontaneously breathing and artificially ventilated rats. Thus, there was a progressive fall in ABP, and a tendency for HR to increase initially, but to fall below the control level by the 10th minute of hypoxia. There was also a tendency for CVC to increase, this just missing statistical significance ($P = 0{\cdot}06$, 0·07 and 0·09 at the 1st, 5th and 10th minute of hypoxia, respectively). Furthermore, CBF tended to increase initially, but wane by the 10th minute (Fig. 2) such that CBF at the 10th minute was significantly different from that at the 1st

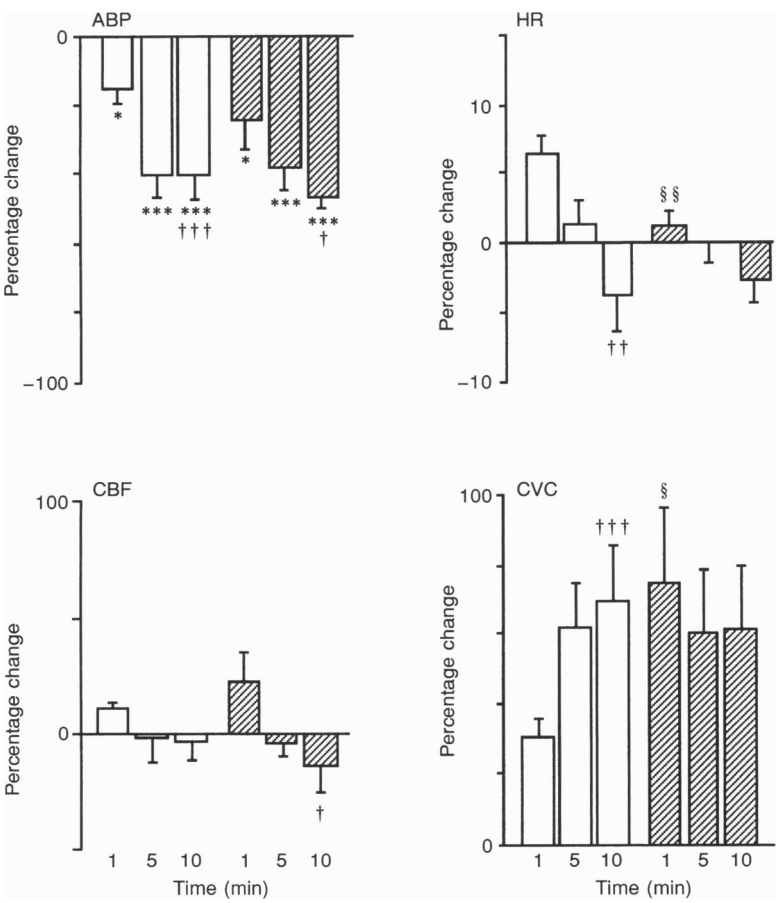

Figure 2. Mean cardiovascular changes evoked by hypoxia during spontaneous (group I) and constant artificial ventilation (group II)

Open and hatched columns indicate changes induced by hypoxia during spontaneous (group I as in Fig. 1) and artificial ventilation (group II), respectively. Significant difference between percentage changes evoked in hypoxia during spontaneous (group I) and artificial ventilation (group II): § $P < 0{\cdot}05$; §§ $P < 0{\cdot}01$. Other symbols as in Fig. 1.

Table 3. Effects of 8-phenyltheophylline upon baseline levels of cardovascular variables when ventilation was held constant

	Mean ABP (mmHg)	HR (beats min^{-1})	CBF (ml min^{-1})	CVC (ml min^{-1})
Before 8-PT	132·8 ± 4·1	429·4 ± 7·5	1·9 ± 0·3	0·014 ± 0·002
After 8-PT	131·5 ± 5·4	424·6 ± 8·3	1·5 ± 0·2‡	0·012 ± 0·001‡

Significant difference between values recorded before and after drug: ‡$P < 0·05$.

minute. However, the HR recorded at the 1st minute of hypoxia during constant ventilation was significantly lower than that evoked during spontaneous ventilation (Fig. 2). On the other hand, CVC at the 1st minute of hypoxia during constant ventilation was significantly greater than that recorded during spontaneous ventilation (Fig. 2).

Effects of 8-PT

When ventilation was held artificially constant, 8-PT reduced the baseline levels of CVC and CBF, but had no effect on the baseline levels of HR or ABP (Table 3). As indicated in the Methods, when the same hypoxic mixture was used before and after 8-PT, the P_{a,O_2} reached during hypoxia after 8-PT was greater in each animal than the level measured before 8-PT. The mean values of P_{a,O_2} reached in hypoxia before and after 8-PT were, respectively, 29·3 ± 1·2 and 32·0 ± 1·4 mmHg, and were significantly different ($P < 0·05$). However, when extra N_2 was then added to the hypoxic mixture (see Methods), the P_{a,O_2} and P_{a,CO_2} achieved in hypoxia after 8-PT were not significantly different from those attained before 8-PT (see Table 1). As can be seen from Fig. 3, under these conditions 8-PT had no effect upon the cardiovascular changes induced by hypoxia. Notably, HR showed the same tendency to fall between the 1st and 10th minute and CBF also tended to wane with time, the difference between the CBF values at the 1st and 10th minute showing a P value of 0·09.

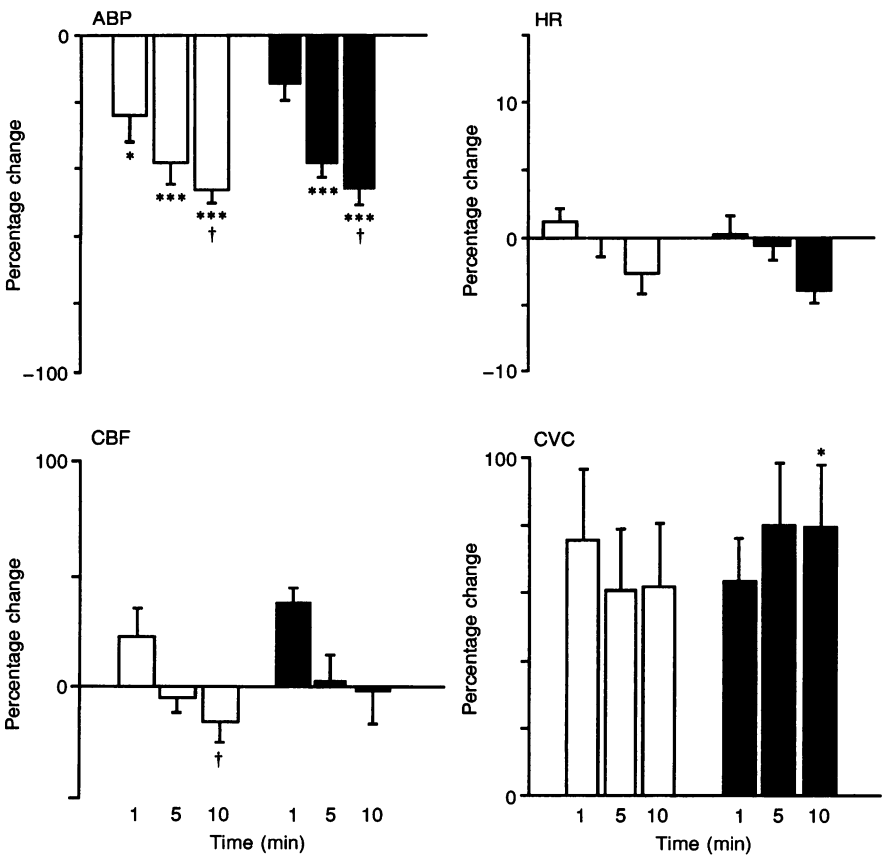

Figure 3. Mean cardiovascular changes induced by systemic hypoxia during constant artificial ventilation (group II) before 8-PT, and the effect of 8-PT on changes induced when extra N_2 was added to the hypoxic inspirate

Open and filled columns indicate changes before 8-PT, and with extra N_2 added after 8-PT, respectively. Symbols as in Fig. 1.

DISCUSSION

Responses evoked by hypoxia during spontaneous ventilation

In the spontaneously breathing rat, the pattern of respiratory and cardiovascular response induced by 10 min of systemic hypoxia (8% O_2) comprised a hyperventilation and increased heart rate, both of which tended to wane towards or below control levels at about 3 min, and a fall in arterial pressure. This pattern of response was comparable with that recorded in our previous studies in rats under similar conditions (Marshall & Metcalf, 1988*b*; Neylon & Marshall, 1991). The present recordings of blood flow from the common carotid artery with all but the internal carotid ligated showed that during spontaneous breathing, hypoxia induced an increase in vascular conductance in the region supplied by this artery. Judging from the regions that appeared blue after injecting Methylene Blue into the common carotid artery, it appears that such vascular isolation did allow assessment of blood flow to the forebrain (cerebrum and cerebellum), although there was some contamination by blood flow to the orbit and buccal cavity. It seems reasonable, therefore, to consider the changes in carotid blood flow and vascular conductance as changes in blood flow and vascular conductance to forebrain regions.

The magnitudes of these increases in cerebral vascular conductance (32% at 1 min) are consistent with those estimated previously in Saffan-anaesthetized rats (35%, Neylon & Marshall, 1991) by using the same technique. They were also comparable with those recorded in the cerebral hemispheres and cerebellum in the Saffan-anaesthetized rat by using radiolabelled microspheres (~60% increase at 2 min; Marshall & Metcalfe, 1990) and comparable with the 40% increase in vascular conductance of the whole brain of the rat when P_{a,O_2} was lowered to 45 mmHg (Morii, Ngai, Ko & Winn, 1987).

Although the increase in cerebral vascular conductance became greater as the period of hypoxia progressed, indicating progressive vasodilatation, there was no significant increase in cerebral blood flow. In fact, cerebral blood flow tended to increase initially and fall towards or below control levels when arterial blood pressure reached its lowest level (see below).

Comparison between responses evoked during spontaneous and artificial ventilation

In the animals of group II, when control levels of P_{a,O_2} and P_{a,CO_2} were similar to those recorded during air breathing in the spontaneously breathing rat and when the P_{a,O_2} reached during hypoxia was comparable with that attained during 8% O_2 in spontaneously breathing animals, the heart rate recorded at the 1st minute of hypoxia was significantly lower than that seen during spontaneous ventilation. This suggests that the hyperventilation normally induced by systemic hypoxia in spontaneously breathing rats facilitates an increase in heart rate. Marshall & Metcalfe (1988*a*, *b*) provided evidence that the initial increase in heart rate is not attributable to reflexes initiated by the pulmonary stretch receptors secondary to the hyperventilation. However, when P_{a,CO_2} was prevented from falling during hypoxia in the spontaneously breathing rat, the tachycardia was reduced or reversed to a bradycardia even though the hyperventilation was potentiated (Marshall & Metcalfe, 1989). This suggested that hypocapnia contributes to the tachycardia by antagonizing the stimulation of peripheral chemoreceptors produced by hypoxia, thereby attenuating the primary reflex bradycardia initiated by the peripheral chemoreceptors and allowing predominance of tachycardia mediated by the influence of hypoxia on the central nervous system and by a direct action of hypocapnia on the heart. The results of the present study are entirely consistent with that view. The secondary fall in heart rate during spontaneous ventilation may be attributed to alleviation of the hypocapnia simultaneous with the secondary reduction in hyperventilation, to a primary reflex response to chemoreceptor stimulation (Marshall, 1987) and/or to a result of the local effects of hypoxia on the sino-atrial node, which are known to be inhibitory (Belardinelli, Belloni, Rubio & Berne, 1980). The last two factors would contribute to a secondary fall in heart rate during constant artificial ventilation.

Since the increase in cerebral vascular conductance was significantly greater at the 1st minute of hypoxia during constant ventilation than during spontaneous ventilation, it seems that the hyperventilation normally seen during hypoxia attenuates the hypoxia-induced vasodilatation of cerebral vessels in some way. It may be that the hypocapnia induced by the hyperventilation exerted a cerebral vasoconstrictor influence (see Heistad, Marcus & Abboud, 1978) and so attenuated the cerebral vasodilatation induced by the local influence of hypoxia upon the cerebral vasculature and as a myogenic response to the fall in arterial pressure. If this is the case, then the fact that the increase in cerebral vascular conductance seen in the spontaneously breathing animal was greater at the 10th than at the 1st minute can be attributed to a smaller degree of hypocapnia at this time, concomitant with the secondary decrease in tidal volume.

During constant ventilation, the tendency for cerebral blood flow to be lower at the 10th than at the 1st minute of hypoxia and therefore to fall with time reached statistical significance. Although cerebral blood flow did not fall significantly below the baseline as we had hypothesized (see Introduction) during either constant or spontaneous ventilation (see above), we can certainly conclude that the increase in cerebral vascular conductance was not sufficient to *increase* cerebral blood flow when arterial perfusion pressure was falling. Therefore, O_2 delivery to the brain must have fallen and, during constant

ventilation at least, must have fallen with time during hypoxia. This is consistent with the hypothesis put forward in the Introduction, in that a progressive fall in O_2 delivery to forebrain structures would be likely to facilitate the development of a positive feedback loop during hypoxia, by potentiating the cerebral hypoxia and accentuating the secondary fall in ventilation that is thought to be mediated by the effect of hypoxia at a supra-pontine level (Dempsey & Forster, 1982).

As we have noted before, the pattern of response evoked by acute hypoxia in the rat, notably the secondary fall in ventilation and heart rate and fall in arterial pressure, is more comparable with that seen in neonates than in the adults of larger animal species. This may be because the local effects of hypoxia exert a greater influence in small mammals in general, whether neonate or adult, because they have a higher rate of O_2 consumption per gram body weight (Marshall & Metcalfe, 1988*b*). On the basis of the present results, we suggest that in the neonate, the development of the positive feedback loop we have proposed, during an acute hypoxic episode would provide an explanation for sudden infant death syndrome, which has already been associated with apnoea in hypoxia (Naeye, 1980).

Effects of 8-PT during spontaneous and artificial ventilation

Following administration of 8-PT during spontaneous air breathing, there was a significant increase in the baseline level of tidal volume with no change in respiratory frequency. This indicates that adenosine exerts a tonic inhibitory influence on respiration during air breathing, as has been suggested by others (Mueller, Widerlov & Breese, 1984; Wessberg, Hedner, Hedner, Persson & Jonasson, 1985). Furthermore, the baseline levels of cerebral blood flow and cerebral vascular conductance were reduced by 8-PT, both when ventilation was spontaneous and when it was held artificially constant. Given that neither the baseline levels of arterial pressure nor P_{a,CO_2} was affected by 8-PT under either condition, these results indicate that adenosine exerts a tonic vasodilator influence on the cerebral circulation. This was probably mediated by adenosine receptors on the central side of the blood–brain barrier; 8-PT can cross the blood–brain barrier, whereas 8-sulphophenyltheophylline (8-SPT) does *not* cross the blood–brain barrier (Daly, 1982), and did *not* affect resting cerebral vascular conductance in the rat during air breathing (Thomas, Elnazir & Marshall, 1994). In previous studies, both theophylline and 8-PT have been shown to reduce resting cerebral vascular conductance in anaesthetized rats (Morii *et al.* 1987: Neylon & Marshall, 1991).

Resting heart rate also fell after 8-PT when breathing was spontaneous, which is consistent with previous observations (Kellett, Bowmer, Collis & Yates, 1989; Neylon & Marshall, 1991). The mechanism responsible for this bradycardia is unclear. Given that exogenous adenosine evokes bradycardia (Neylon & Marshall, 1991) 8-PT would have been expected to induce tachycardia if it had removed a tonic influence of endogenous adenosine. Moreover an increase in heart rate might also have been expected given that the baseline level of ventilation was increased after 8-PT (see above).

Turning to the effects of 8-PT upon the responses evoked by hypoxia when breathing was spontaneous, 8-PT abolished the secondary fall in respiratory tidal volume and heart rate and reduced the fall in arterial pressure, as described by Neylon & Marshall (1991). As discussed in the Introduction, these results do not differentiate between two possibilities: (i) that 8-PT acted directly, by blocking receptors in the heart and peripheral tissues; and (ii) that the effects of 8-PT on the cardiac and blood pressure changes were secondary to its effect on the ventilatory response to hypoxia.

The experiments performed under constant artificial ventilation were intended to elucidate this issue. During constant ventilation when the P_{a,O_2} reached during hypoxia was adjusted so that it was the same before and after 8-PT, 8-PT did not affect the hypoxia-induced fall in arterial pressure and did not alter the tendency for heart rate to fall between the 1st and 10th minute. This strongly suggests that the effects of 8-PT on the arterial pressure and heart rate changes during spontaneous ventilation were a consequence of its effects on the secondary fall in ventilation. As far as the heart rate changes are concerned, this is fully compatible with the proposals made above about the interdependence of the ventilatory and cardiac changes. Since the P_{a,O_2} values attained in hypoxia during spontaneous respiration were not significantly altered when the secondary fall in ventilation was alleviated by 8-PT, it cannot be argued that the secondary fall in heart rate occurred because the secondary fall in ventilation exacerbated the fall in P_{a,O_2} (see Introduction). Rather, as the respiratory alkalosis was accentuated by 8-PT, it seems likely that changes in P_{a,CO_2} caused by the ventilatory changes played a major role in determining the heart rate changes (see above).

Of course, the second implication of the lack of effect of 8-PT on the heart rate response to hypoxia during constant ventilation is that locally released adenosine did not produce significant slowing of heart rate. This is compatible with our finding that 8-SPT, which cannot cross the blood–brain barrier (see above) and did not affect the ventilatory response to hypoxia, also had no effect on the secondary bradycardia (Thomas *et al.* 1994). However, adenosine deaminase, which breaks down endogenous adenosine, did reduce the secondary fall in heart rate during hypoxia in Saffan-anaesthetized rats, without affecting the secondary fall in ventilation (Thomas *et al.* 1994). Moreover, there is substantial evidence that locally released adenosine makes a dominant contribution to the bradycardia induced by hypoxia in isolated rat hearts (Froldi & Bellardinelli, 1990). Thus, we cannot exclude the

possibility that adenosine contributed directly towards a secondary bradycardia during spontaneous or constant ventilation, but must surmise that either its effects are resistant to adenosine receptor antagonists (Thomas *et al.* 1994) or they are small relative to the influence of other factors (see above).

The fact that the hypoxia-induced fall in arterial pressure was unaffected by 8-PT during constant ventilation can probably be explained by a progressive fall in cardiac output that persisted after 8-PT, rather than by a lack of effect of 8-PT on the hypoxia-induced fall in total peripheral resistance. For, in our previous studies on spontaneously breathing rats, both adenosine deaminase, which had no effect on the hypoxia-induced changes in ventilation but abolished the secondary bradycardia, and 8-SPT, which had no effect on either ventilatory changes or the secondary bradycardia, significantly reduced the vasodilatation in hindlimb skeletal muscle and the fall in arterial pressure evoked by hypoxia, suggesting that the local influence of adenosine on skeletal muscle contributes to these changes (Thomas *et al.* 1994).

Finally, the effect of 8-PT on the hypoxia-induced changes in cerebral vascular conductance in the spontaneously breathing rat was equivocal. The fact that cerebral vascular conductance no longer increased progressively and was lower at the 10th minute of hypoxia than before 8-PT may be attributed to blockade of the effect of locally released adenosine upon receptors on cerebral blood vessels (see Morii *et al.* 1987), but might also reflect a smaller myogenic dilatation in response to the smaller fall in arterial pressure. On the other hand, during constant ventilation, when 8-PT did not affect the hypoxia-induced fall in arterial pressure, 8-PT had no significant effect on the change in cerebral vascular conductance induced at any time during hypoxia. Thus, it seems that locally released adenosine was not a dominant factor in producing the hypoxia-induced vasodilatation in forebrain. This contrasts with the results of Morii *et al.* (1987) on hypoxia-induced cerebral vasodilatation in the rat, but is consistent with other studies showing that adenosine antagonists had no effect on hypoxia-induced cerebral vasodilatation in cats and humans (Haller & Kuschinsky, 1987; Bowton, Haddon, Prough, Adair, Alford & Stump, 1988).

The important point, in view of our hypothesis of a positive feedback loop, is that in spontaneously breathing animals, when 8-PT reduced the secondary fall in tidal volume and heart rate and the fall in arterial pressure, there was an obvious tendency for cerebral blood flow to be better maintained during hypoxia (in 9 out of 10 animals), whereas this was not the case when ventilation was held constant and 8-PT had no effect on the secondary fall in heart rate or fall in arterial pressure.

In summary, the present findings considered together with those of our previous studies (Marshall & Metcalfe, 1988*a*, *b*, 1989; Neylon & Marshall, 1991) indicate that the respiratory and cardiovascular responses evoked in the spontaneously breathing rat by systemic hypoxia are interdependent in the following ways: (i) the hypocapnia that arises from the initial hyperventilatory response to peripheral chemoreceptor stimulation makes a substantial contribution to an initial tachycardia, but attenuates the initial cerebral vasodilatation; (ii) alleviation of the hypocapnia concomitant with the secondary reduction in ventilation contributes to the secondary fall in heart rate and facilitates the cerebral vasodilatation; and (iii) the secondary fall in heart rate together with the vasodilatation induced in skeletal muscle and other peripheral tissues lead to a progressive fall in arterial pressure, which is ultimately responsible for a secondary fall in cerebral blood flow and a fall in O_2 delivery to the brain. These interrelationships are fully compatible with the hypothesis put forward in the Introduction that these changes form a positive feedback loop, since a progressive fall in O_2 delivery to the brain would be expected to exacerbate the central neural hypoxia and potentiate the secondary fall in ventilation. The present findings also indicate that adenosine plays a major role in the overall pattern of respiratory and cardiovascular response to hypoxia by acting at a central site to reduce respiration, to the extent that when the secondary hypoventilation was blocked by an adenosine receptor antagonist, this effectively broke the positive feedback loop.

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