A study on rats of the effects of chronic hypoxia from birth on respiratory and cardiovascular responses evoked by acute hypoxia

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1. Comparative studies were performed on eighteen rats 54 days old made chronically hypoxic from birth in an hypoxic chamber at 12% O_2 (CHB), and in eight weight-matched control rats (NB, 42 days old); both CHB and NB rats were anaesthetized with Saffan.

2. In NB rats, breathing 12 or 8% O_2 for 5 min induced a pattern of response comparable to that described in older rats (10–11 weeks old): an initial increase and secondary fall in minute volume ($\dot{V}_E$), a fall in arterial pressure (ABP), an increase in muscle vascular conductance, while cerebral blood flow (CBF) increased at the 1st minute in six animals and fell by the 5th minute in all animals. The adenosine receptor antagonist 8-phenyltheophylline (8-PT, 10 mg kg^{-1}) reduced the secondary fall in $\dot{V}_E$, the fall in ABP and muscle vasodilatation, indicating they were partly mediated by adenosine.

3. In CHB rats breathing 12% O_2, $\dot{V}_E$ was higher (277 ± 12 *vs.* 204 ± 18 ml min^{-1}), arterial partial pressures of O_2 (45 ± 2 *vs.* 88 ± 3 mmHg), CO_2 (32 ± 1 *vs.* 44 ± 1 mmHg) and ABP (105 ± 5 *vs.* 131 ± 5 mmHg) were lower, while muscle vascular conductance was higher (0·08 ± 0·01 *vs.* 0·03 ± 0·01 ml min^{-1} $mmHg^{-1}$) than in NB rats breathing air; these differences were reduced, but not abolished, when CHB rats acutely breathed air for 5 min.

4. In CHB rats, the smaller change from 12 to 8% O_2 for 5 min evoked a similar pattern of response to that evoked by 8% O_2 in NB rats, except that heart rate (HR) and CBF decreased progressively. However, 8-PT increased baseline $\dot{V}_E$ and reduced ABP in 12% O_2 and reduced the secondary decrease in $\dot{V}_E$ and HR evoked by 8% O_2, but had no effect on the fall in ABP, or change in muscle vascular conductance.

5. We propose that in CHB rats (i) there is accentuation of the components of the response to acute hypoxia (the fall in ABP, HR and CBF) that form a positive feedback loop which promotes central ventilatory depression and (ii) that adenosine exerts a tonic inhibitory influence on $\dot{V}_E$ and vasodilator influence in muscle and mediates the secondary fall in $\dot{V}_E$, but not the muscle vasodilatation induced by acute hypoxia.

During the neonatal period, there is a transition of the respiratory response to hypoxia from the 'fetal' response which is dominated by inhibition of breathing movements (Boddy, Dawes, Fisher, Pinter & Robinson, 1974) to the 'adult' response which is dominated by an increase in ventilation (Bonora, Marlot, Gaultier & Duron, 1984). Thus, neonates typically show a biphasic response; an initial increase in ventilation which wanes to a secondary fall towards, or even below the control level (see Eden & Hanson, 1987*a* for references). This second phase generally occurs in adults only if the hypoxia is severe or prolonged (Easton, Slykeman & Anthionsen, 1986, but see below). The neonatal development of the response has been attributed firstly to resetting of the sensitivity of the peripheral chemoreceptors from the fetal to the adult range of arterial partial pressure of O_2 (P_{a,O_2}) and to consequent facilitation of the initial increase in ventilation (Hanson, Kumar & McCooke, 1986). Secondly, there seems to be a reduction in the central, inhibitory influence of hypoxia upon respiratory neurones (Eden & Hanson, 1987*a*).

Chronic hypoxia from birth affects the neonatal development of the respiratory response to hypoxia: resetting of the peripheral chemoreceptors is delayed (Hanson, Kumar &

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Williams, 1989) and there may be persistence of the central inhibitory influence of hypoxia (Eden & Hanson, 1987*b*). Thus, by 5–10 weeks of age, the carotid chemoreceptors of rats that had been kept in an hypoxic chamber at 15% O_2 from birth showed a sensitivity to P_{a,O_2} comparable to that recorded in normoxic rats. However, the respiratory response to hypoxia included an obvious secondary fall in ventilation that was comparable to that normally seen on postnatal days 1–7 (Eden & Hanson, 1987*b*). This persistence of the local inhibitory effect of hypoxia on central respiratory neurones, and the positive feedback it implies, may render individuals who have been chronically hypoxic from birth more vulnerable to sudden infant death syndrome (SIDS, Eden & Hanson, 1987*b*).

In contrast, very little is known of the effect of chronic hypoxia from birth on the development of the cardiovascular response to systemic hypoxia. Our recent studies on the adult rat indicate that the pattern of respiratory and cardiovascular response evoked in this species by acute hypoxia shares features that are characteristic of the response pattern evoked in neonates (see Marshall & Metcalfe, 1988; Thomas & Marshall, 1994*b*, for discussion). Thus, under anaesthesia at least, there is a biphasic respiratory response to 3–5 min periods of breathing 8 or 6% O_2. Further, under anaesthesia, or in the conscious state, this is accompanied by an initial tachycardia and a secondary bradycardia, a gradual fall in arterial pressure and peripheral vasodilatation that is particularly pronounced in skeletal muscle (Marshall & Metcalfe, 1988, 1990; Neylon & Marshall, 1991; Thomas, Elnazir & Marshall, 1994; Thomas & Marshall, 1994*b*) as reported in neonates of larger species (Gootman, Buckley & Gootman, 1991). We have proposed that this pattern of response occurs because the local effects of hypoxia show greater predominance in the adult rat than in larger adult mammals. Therefore, not only can the secondary fall in ventilation be attributed to the central inhibitory effect of hypoxia on respiratory neurones, but the secondary bradycardia and peripheral vasodilatation can be mainly ascribed to the local effects of hypoxia on the sinoatrial node and vasculature (Marshall & Metcalfe, 1988). We have also shown that although hypoxia causes cerebral vasodilatation, the fall in systemic arterial pressure eventually leads to a fall in cerebral blood flow. We have, therefore, proposed that the cardiovascular response to acute hypoxia is part of the positive feedback loop mentioned above, in that the fall in cerebral blood flow would decrease further O_2 supply to the brain so accentuating the central hypoxia that causes respiratory depression (Thomas & Marshall, 1994*b*).

Our recent studies indicated that locally released adenosine plays a major role in the secondary fall in ventilation, bradycardia and muscle vasodilatation (Neylon & Marshall, 1991; Thomas *et al.* 1994). Moreover, when the central and peripheral adenosine receptors were blocked with 8-phenyltheophylline (8-PT) this broke the positive feedback loop and cerebral blood flow was well maintained (Thomas & Marshall, 1994*b*). Our evidence indicates that in skeletal muscle, adenosine acts in part by opening glibenclamide-sensitive K^+ channels on the skeletal muscle fibres, that are probably ATP-sensitive (K_{ATP}) channels, thereby releasing K^+ which then acts as a vasodilator (Marshall, Thomas & Turner, 1993).

As the neonatal components of the cardiovascular response to systemic hypoxia are readily identifiable in the rat and given the understanding we have of the mechanisms underlying them, the rat seemed an ideal species in which to investigate the effects of chronic hypoxia from birth on the development of the cardiovascular response to systemic hypoxia. Thus, this was the primary aim of the present study, which was performed on rats that were kept from birth in an hypoxic chamber at 12% O_2 and on a group of weight-matched controls. We also undertook to test whether adenosine and potassium play similar roles in these chronically hypoxic rats as in normoxic rats. Some of the results have been reported in brief (Thomas & Marshall, 1993).

METHODS

Experiments were performed on two groups of Wistar rats: a control group of rats (NB) that were born and reared in air, and a group of chronically hypoxic rats (CHB) that were born and reared in a normobaric, hypoxic chamber. The chamber (volume ~1·0 m^3) operated under a 12 h light–dark cycle. Gas circulated around the chamber, attached tubing and other units (see below) at ~15 l min^{-1} (i.e. about one complete change per hour). The O_2 concentration was continuously measured by an O_2 analyser (500D; P. K. Morgan, Gillingham, Kent, UK) and maintained at 12% (range, 11·75–12·25%) by a servo-controlled system such that deviations from the desired concentration were met by addition of N_2 or air through solenoid valves. Ambient CO_2 in the chamber was measured at regular intervals by using a Nova Stat Profile analyser (Stat 3; VA Howe, MA, USA): it was maintained at ~0·03% by circulating the gas through soda lime. The gas also circulated through a molecular sieve (Type 3A; Fisons, Loughborough, UK) so as to remove ammonia. Humidity was measured and maintained at 40–50% by circulating the gas through a freezer unit and silica gel. Ambient temperature was maintained at 22–24 °C.

The CHB group were obtained from two pregnant Wistar rats which were placed in the chamber 3–4 days before the expected delivery. The litters, comprising eighteen newborn pups were kept together with the mother until they were weaned at day 21, after which they were housed in groups of three to four in the chamber. The animals were housed in standard cages and were supplied with standard rat chow and water *ad libitum*. They were continuously exposed to 12% O_2 except for two periods of approximately 20 min $week^{-1}$ when the chamber was opened to allow the cages to be cleaned and food and water to be replenished. At 6–7 weeks of age, each rat was removed from the chamber for an acute experiment. The body weight of the CHB group upon removal from the chamber was 180 ± 4 g (mean ± S.E.M.). The control NB group of eight rats were kept in the same room as the

hypoxic chamber and were used in acute experiments at approximately the same weight (186 ± 9 g) as the CHB group, by which time they were 42 ± 3·4 days old, compared with 53 ± 1·9 days for the CHB group.

For all acute experiments anaesthesia was induced with halothane, N_2O and O_2 and subsequently maintained with a continuous infusion of Saffan (7–12 mg kg^{-1} h^{-1} I.V.; Pitman Moore, Uxbridge, UK) as described previously (Neylon & Marshall, 1991). Recordings were also made as previously described (Marshall & Metcalfe, 1988; Neylon & Marshall, 1991; Thomas & Marshall, 1994*b*). Briefly, a T-shaped tracheal cannula was inserted and connected via a flow-head to an electrospirometer so that tidal volume (V_T) and respiratory frequency (R_F) could be continuously recorded; minute volume ($\dot{V}_E$) was calculated as $V_T \times R_F$. Arterial pressure (analysed as mean arterial pressure, ABP) and heart rate (HR) were recorded from the right femoral artery, and cerebral blood flow (CBF) was measured via an electromagnetic flow probe placed around the common carotid artery after vascular isolation of the internal carotid artery (see Thomas & Marshall, 1994*b*). Arterial blood flow (ABF) was also recorded from the aorta using a flow probe positioned distal to the renal arteries. In one litter of CHB rats, the tail and the blood supply to the testes were ligated so as to achieve isolation of blood flow to the skeletal muscles of the hindquarters. The baseline of aortic blood flow and the experimentally induced changes did not differ between this litter and the other, so for analysis they were considered together. Cerebral vascular conductance (CVC) and aortic vascular conductance (AVC) were calculated on-line by beat-by-beat division of CBF, or ABF, by ABP.

The right brachial artery was cannulated to allow 130 μl samples of arterial blood to be taken for measurement of P_{a,O_2}, arterial partial pressure of carbon dioxide (P_{a,CO_2}) and arterial pH by using the Nova Stat Profile analyser (see above). Arterial and venous blood samples, taken from the brachial artery and from a cannula placed in the right femoral vein with its tip in the vena cava, respectively, were used for determination of arterial and venous plasma concentrations of potassium ($[K^+]_a$ and $[K^+]_v$, respectively) by flame photometry (CIBA Corning Diagnostics Ltd, Halstead, UK) as described previously (Marshall *et al.* 1993).

Throughout surgery and thereafter, the CHB group routinely breathed 12% O_2, while the NB rats routinely breathed air. O_2 12% or air was blown across the end of the respiratory flow head by an air pump (see Marshall & Metcalfe, 1988).

Protocol

After an equilibration period of ~1 h, each CHB rat was exposed to a 5 min period of breathing 8% O_2 and to a 5 min period of breathing room air, before and after 8-PT (Sigma, Poole, Dorset, UK) at 10 mg kg^{-1} I.V. which produces effective blockade of adenosine receptors (Thomas & Marshall, 1994*b*). At least 10 min was allowed between stimuli and 15 min was allowed after 8-PT so that all cardiovascular and respiratory variables stabilized. Arterial samples for blood gas analysis were taken during 12% O_2, in the 5th minute of breathing 8% O_2 and in the 5th minute of breathing room air. Arterial and venous blood samples for plasma K^+ analysis were also taken at these times. Similar experiments were performed on NB rats; they were exposed to 5 min periods of breathing 12% O_2 and 8% O_2 before and after 8-PT administration, (10 mg kg^{-1} I.V.), arterial samples for blood gas analysis, and arterial and venous samples for plasma K^+ analysis being taken during air breathing and in the 5th minute of breathing 12 or 8% O_2. A test was made of responses evoked by an I.V. injection of adenosine (600 μg kg^{-1}) during 12% O_2 breathing, or air breathing in CHB and NB groups respectively, before and after 8-PT addition. This dose produced responses that were as large or larger than those induced by hypoxia.

Statistical analysis

All results are expressed as means ± S.E.M. Baseline values of each recorded variable were compared in CHB and NB rats by using Student's unpaired *t* test. Other comparisons were made by using ANOVA and a *post hoc t* test with Bonferroni correction for multiple comparisons. Within CHB and NB groups, we compared: (i) baseline values before and after 8-PT; (ii) the absolute values of each variable at the end of the 1st, 2nd and 5th minute of the periods when the inspirate was changed and the baseline levels before and after 8-PT; (iii) the peak responses to adenosine before and after 8-PT. We also made comparisons between the CHB and NB rats of the absolute values of all variables in (i) CHB rats breathing 12% O_2 and in NB rats at the 5th minute of acute exposure to 12% O_2; (ii) CHB rats at the 5th minute of acute exposure to air and in NB rats breathing air; (iii) CHB and NB rats at the 5th minute of breathing 8% O_2. In addition, comparisons were made between CHB and NB rats of (i) the absolute changes evoked when the inspirate of the CHB rats was changed from 12 to 8% O_2 and when the inspirate of the NB rats was changed from air to 8% O_2 and (ii) the peak responses evoked by adenosine before 8-PT. In all cases $P < 0.05$ was considered significant.

RESULTS

Group 1 (NB)

Responses evoked by acute hypoxia. The pattern of response evoked by breathing 12 or 8% O_2 in NB rats was similar to that described previously in older normoxic anaesthetized rats (Marshall & Metcalfe, 1988; Neylon & Marshall, 1991; Marshall *et al.* 1993; Thomas & Marshall, 1994*b*). Thus, both 12 and 8% O_2 evoked significant increases in R_F and V_T, although V_T waned by the 5th minute of hypoxia (Fig. 1). During 12 and 8% O_2, P_{a,O_2} and P_{a,CO_2} fell and pH was increased (Table 1). Concomitantly, there was a progressive fall in ABP during 12 and 8% O_2 and increases in AVC indicating vasodilatation in hindlimb skeletal musculature that reached statistical significance in 8% O_2 (Fig. 1). Any changes in HR did not reach significance (see Discussion). There were no significant changes in CVC or CBF. In fact, in six of the eight rats, CBF increased at 1 min in 8% O_2, but fell by the 5th minute as ABP reached its lowest value (Fig. 1). In the remaining two rats, CBF fell progressively during hypoxia. Thus, CBF fell between the 1st and 5th minute in all of the eight rats studied. Levels of $[K^+]_a$ tended to increase during 8% O_2 from 3·2 to 3·7 mM ($P = 0.06$; see Marshall *et al.* 1993); $[K^+]$ in the venous efflux from the hindlimbs, ($[K^+]_v$), was unaffected (Table 1; cf. Marshall *et al.* 1993).

Effects of 8-PT. Arterial blood gases, pH and levels of $[K^+]_a$ and $[K^+]_v$ taken during air breathing were unaffected by 8-PT (Table 1). Further, 8-PT had no significant effect on the baseline levels of any of the cardiovascular and respiratory variables during air breathing (Table 2), although V_T tended to increase ($P = 0.09$) and ABF tended to fall ($P = 0.058$).

The effects of 8-PT on the responses evoked by acute hypoxia were similar to those described previously (Neylon & Marshall, 1991; Thomas & Marshall, 1994*b*). Thus, the secondary fall in V_T observed during acute hypoxia was reduced such that there was no longer a significant difference between V_T at the 1st and the 5th minute of 8% O_2 (Fig. 1). Associated with this, P_{a,CO_2} during 8% O_2 was lower than before 8-PT although levels of P_{a,O_2} and pH in 8% O_2 were not significantly affected (Table 1). In addition, the fall in ABP was reduced at the 5th minute of breathing 8% O_2 and in contrast to the situation before 8-PT, levels of AVC did not change significantly during 8% O_2 after 8-PT

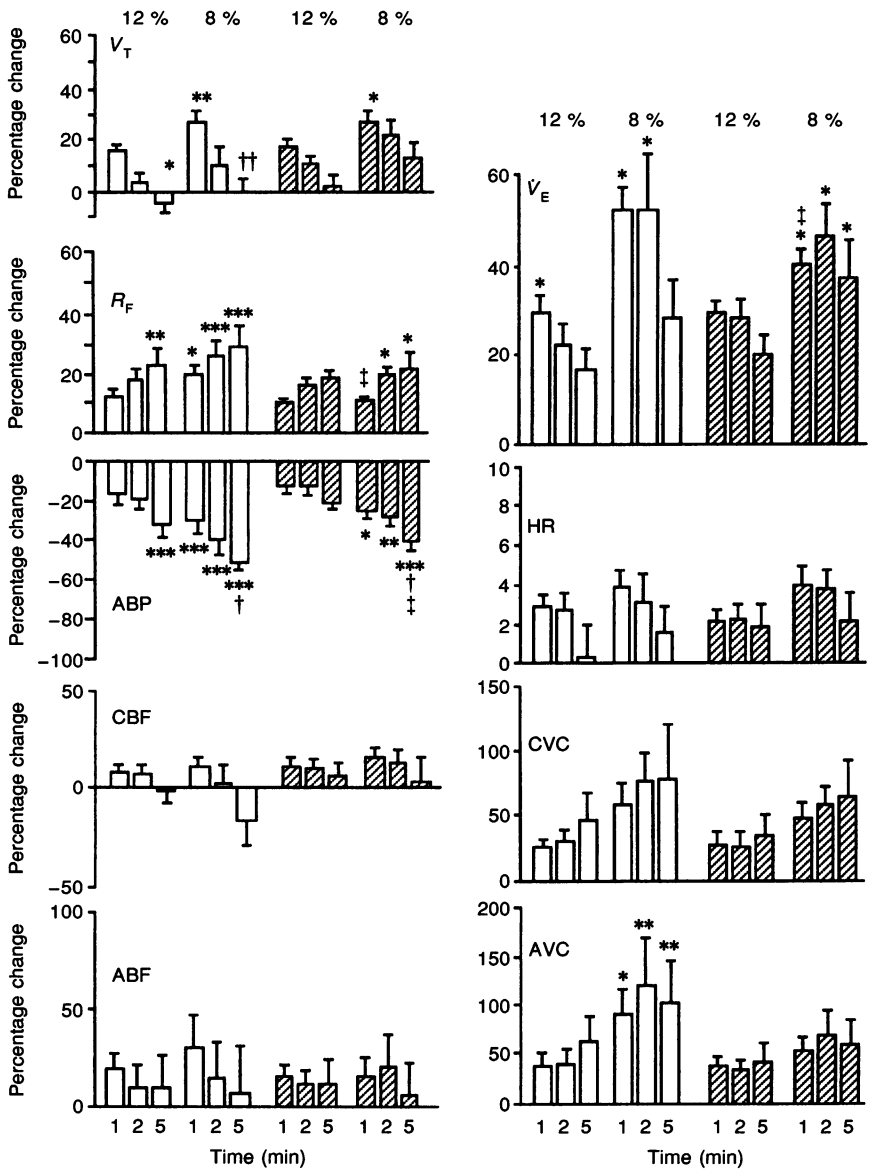

Figure 1. Percentage changes induced in respiratory and cardiovascular variables of NB rats by 5 min of breathing 12 and 8% O_2 before and after 8-PT

V_T, tidal volume; R_F, respiratory frequency; $\dot{V}_E$, minute volume; ABP, mean arterial blood pressure; HR, heart rate; CBF and CVC, cerebral blood flow and vascular conductance; ABF and AVC, aortic blood flow and vascular conductance. Each column represents mean percentage change from baseline ± S.E.M., recorded at the 1st, 2nd and 5th minute of hypoxia. Open columns, before 8-PT; hatched columns, after 8-PT. * Significant difference between values recorded during air breathing (control) and 12 or 8% O_2; † significant difference between change recorded at 1st and 5th minute; ‡ significant difference between change recorded before and after 8-PT at same time during hypoxia (in each case 1, 2 and 3 symbols represent $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively).

Table 1. Blood gas and plasma potassium values recorded during air breathing, 12 and 8% O_2 in NB, before and after 8-PT

	Before 8-PT					After 8-PT				
	P_{a,O_2}	P_{a,CO_2}	pH_a	$[K^+]_a$	$[K^+]_v$	P_{a,O_2}	P_{a,CO_2}	pH_a	$[K^+]_a$	$[K^+]_v$
Air	88 ± 3	44 ± 1	7·26 ± 0·0	3·2 ± 0·2	2·8 ± 0·1	89 ± 5	41 ± 1	7·26 ± 0·0	2·9 ± 0·2	3·0 ± 0·3
12% O_2	43 ± 1 ***	35 ± 1 ***	7·30 ± 0·0 *	3·3 ± 0·2	3·0 ± 0·2	46 ± 2 ***‡	33 ± 1 ***	7·32 ± 0·0 ***‡‡	3·0 ± 0·3	2·6 ± 0·2 ‡
8% O_2	32 ± 1 ***	32 ± 1 ***	7·32 ± 0·0 *	3·7 ± 0·2	3·1 ± 0·7	34 ± 1 ***	29 ± 1 *** ‡‡	7·34 ± 0·0 **	3·6 ± 0·3 **	2·2 ± 0·3 **

pH_a indicates pH in arterial blood. * Difference between values recorded during air breathing and 12 or 8% O_2; ‡ difference between values before and after 8-PT; the number of symbols indicates the degree of significant difference: 1, 2 and 3 symbols represent $P < 0·05$, 0·01 and 0·001, respectively.

(Fig. 1). 8-PT had no significant effect on the changes induced by hypoxia in CVC or CBF. However, the increase in CBF during 12 and 8% O_2 was greater after 8-PT in six of the eight rats, and CBF was better maintained than before 8-PT (see Fig. 1); CBF fell between the 1st and 5th minute in only two animals after 8-PT.

After 8-PT, the increase in $[K^+]_a$ during 8% O_2 reached significance (from 2·9 to 3·6 mM), whilst levels of $[K^+]_v$ were significantly reduced (from 3·0 to 2·2 mM; see Table 1).

Responses evoked by adenosine. Before 8-PT, a bolus injection of adenosine (600 μg kg^{-1} I.V.) evoked a significant increase in R_F, falls in both ABP and HR, increases in both CVC and AVC indicating vasodilatation in cerebral circulation and hindlimb skeletal muscle (Fig. 2). 8-PT reduced the fall in ABP and HR, and the increases in CVC and AVC (Fig. 2).

Group 2 (CHB)

Levels of V_T, R_F and $\dot{V}_E$ measured during 12% O_2 in CHB rats were significantly larger than those measured during air breathing in NB rats (Table 2). These levels of V_T in the CHB group were also significantly larger than those recorded in the 5th minute of acute breathing of 12% O_2 in the NB group, whilst levels of R_F and $\dot{V}_E$ measured during 12% O_2 did not differ between the two groups. P_{a,O_2} and P_{a,CO_2} were significantly lower and arterial pH was higher in CHB rats breathing 12% O_2 than in NB rats breathing

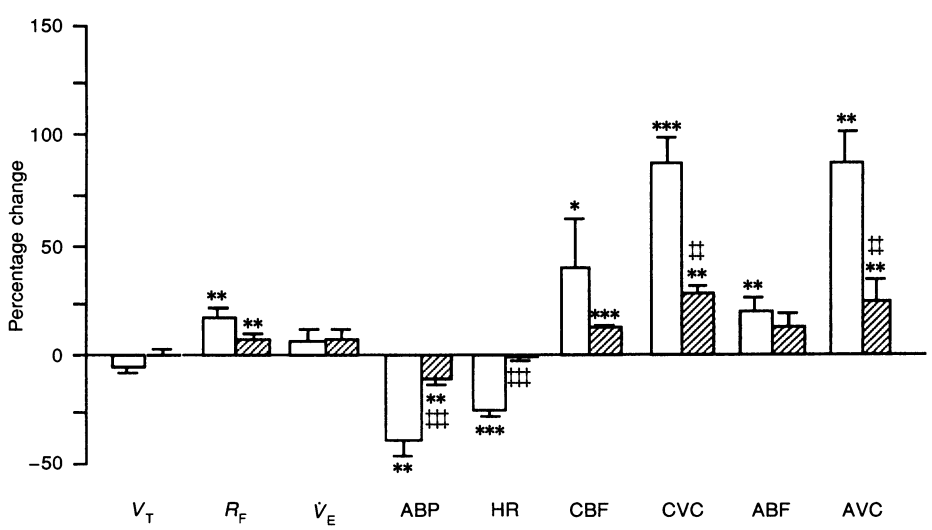

Figure 2. Peak percentage changes induced in respiratory and cardiovascular variables of NB rats by adenosine (600 μg kg^{-1} I.V.) before (open columns) and after (hatched columns) 8-PT

Columns and abbreviations as in Fig. 1. * Significant difference between value recorded before and after injection of adenosine; ‡ significant difference between change recorded before and after 8-PT; 1, 2 and 3 symbols represent $P < 0·05$, 0·01 and 0·001, respectively.

Table 2. Baseline values of respiratory and cardiovascular variables recorded in NB rats during air breathing (control), and in CHB rats in 12% O_2 (control), before and after 8-PT

	V_T (ml)	R_F (breaths min^{-1})	$\dot{V}_E$ (ml min^{-1})	ABP (mmHg)	HR (beats min^{-1})	CBF (ml min^{-1})	CVC (ml min^{-1} mmHg)	ABF (ml min^{-1})	AVC (ml min^{-1} mmHg)
NB rats									
Before 8-PT	1·8±0·1	110±3	204±18	131±3	445±9	2·0±0·2	0·02±0·001	4·3±1·1	0·03±0·01
After 8-PT	2·0±0·1	106±3	210±18	128±4	457±12	2·0±0·1	0·02±0·001	3·1±1·0	0·02±0·01
CHB rats									
Before 8-PT	2·2±0·1¶¶	125±3¶¶	277±12¶¶¶	105±5¶¶$	432±12	1·8±0·3	0·02±0·002	7·9±0·8¶$	0·08±0·01¶
After 8-PT	2·5±0·1‡‡‡	122±3	299±11‡‡‡	94±4‡‡	434±10	1·7±0·3	0·02±0·004	6·1±0·9‡‡	0·07±0·01

¶ Difference between values during 12% O_2 in CHB, and air breathing in NB; ‡ difference between values recorded before and after 8-PT; $ difference between values recorded in CHB and NB rats breathing the same gas mixture. Levels of significance as in Table 1.

air (Table 3, cf. Table 1). Comparing blood gas values in CHB rats breathing 12% O_2 and in NB rats in the 5th minute of acute breathing 12% O_2, P_{a,O_2} values did not differ, but P_{a,CO_2} was lower in the CHB than the NB group (Table 3, cf. Table 1).

In CHB rats breathing 12% O_2, levels of ABP were lower while levels of ABF and AVC were higher than in the NB rats breathing air. On the other hand, levels of HR, CBF and CVC in the CHB group breathing 12% O_2 did not differ from those in the NB group breathing air (Table 2). Comparing cardiovascular variables in CHB rats breathing 12% O_2 and in NB rats at the 5th minute of acute exposure to 12% O_2, ABP was lower and ABF was higher in the CHB group but the other variables were not significantly different.

Levels of $[K^+]_a$ and of $[K^+]_v$ measured in CHB breathing 12% O_2 were greater than in NB rats breathing air (cf. Tables 3 and 1).

Responses evoked by acute hypoxia. The respiratory response evoked when CHB rats breathed 8% O_2 was qualitatively similar to that evoked by 8% O_2 in NB; there was an initial increase in $\dot{V}_E$, but a significant waning of V_T.

Table 3. Blood gas and plasma potassium values recorded during air breathing, and in 12% O_2 (control) and 8% O_2 in CHB rats before and after 8-PT

	Before 8-PT					After 8-PT				
	P_{a,O_2}	P_{a,CO_2}	pH_a	$[K^+]_a$	$[K^+]_v$	P_{a,O_2}	P_{a,CO_2}	pH_a	$[K^+]_a$	$[K^+]_v$
Air	93 ± 2	35 ± 1	7·27 ± 0·0	3·7 ± 0·1	3·6 ± 0·2	96 ± 3	31 ± 1	7·25 ± 0·0	3·6 ± 0·3	3·3 ± 0·3
	***	***	***	*		***	***	***		
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12 % O_2	45 ± 2	32 ± 1	7·31 ± 0·0	4·2 ± 0·3	3·3 ± 0·3	50 ± 2	27 ± 1	7·32 ± 0·0	3·8 ± 0·2	3·6 ± 0·3
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8 % O_2	34 ± 2	26 ± 1	7·32 ± 0·0	4·3 ± 0·3	3·5 ± 0·3	39 ± 2	22 ± 1	7·33 ± 0·0	3·9 ± 0·3	3·2 ± 0·2
	***	***				***	***		**	
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Results recorded during 12% O_2 (control) and 8 % O_2 and air breathing in CHB rats, before and after 8-PT. * Difference between values recorded during 12% O_2 (control) or 8 % O_2; ‡ difference between values before and after 8-PT; ¶ difference between values during 12 % O_2 in CHB rats, and air breathing in NB rats; $ difference between values recorded in CHB and NB rats breathing the same gas mixture. Levels of significance as in Table 1.

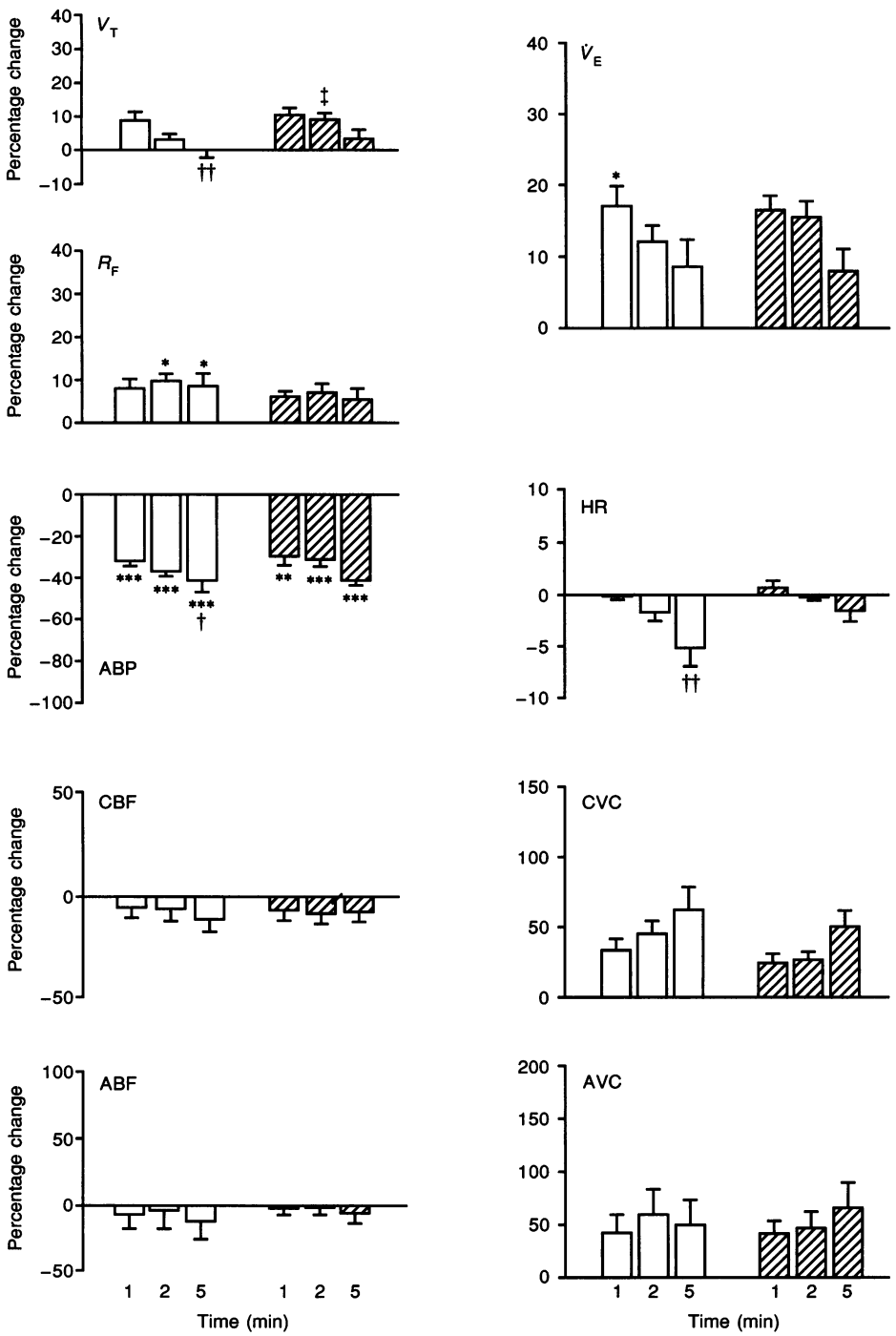

Figure 3. Percentage changes induced in respiratory and cardiovascular variables of CHB rats by 5 min of breathing 8% O_2 before and after 8-PT

Columns and abbreviations as in Fig. 1. * Difference between absolute values measured during 12% O_2 (control) and 8% O_2; † difference between change recorded at 1st and 5th minute; ‡ difference between change recorded before and after 8-PT at the same time during hypoxia. Levels of significance as in Fig. 1.

Comparing absolute values in CHB and NB, V_T recorded in CHB at the 5th minute of 8% O_2 was greater than in NB at the 5th minute of 8% O_2 (2·2 ± 0·1 *vs.* 1·7 ± 0·1 ml min^{-1}), while $\dot{V}_E$ recorded at these times did not differ between CHB and NB (298 ± 17 *vs.* 253 ± 22 ml min^{-1}). The initial increase in $\dot{V}_E$ recorded at 2 min after they were switched from 12 to 8% O_2 (46 ml min^{-1}) was comparable to that calculated for NB rats, by using the difference between the increase in $\dot{V}_E$ recorded at the 2nd min of breathing 12 and 8% O_2 (60 ml min^{-1}). The levels of P_{a,O_2} and pH measured at the end of the 5th minute of 8% O_2 did not differ between CHB and NB rats, but P_{a,CO_2} was lower in CHB rats (Table 3).

As in NB, 8% O_2 in CHB rats induced a progressive fall in ABP and the percentage changes evoked in ABP by 8% O_2 were similar in both groups (cf. Figs 1 and 3). The absolute level of ABP at 5 min was not significantly different from that recorded in NB when they were switched from air to 8% O_2 . AVC tended to increase in CHB rats during 8% O_2 (as in NB), but the changes did not reach significance (cf. Figs 1 and 3). In contrast to NB rats, HR fell progressively in CHB rats so that at the 5th minute HR was significantly different from that recorded at 1 min (cf. Figs 1 and 3). Similarly CBF rats showed no tendency to increase initially (cf. NB in Fig. 1): in ten of eighteen CHB rats, CBF fell progressively during breathing of 8% O_2.

Levels of $(K^+)_a$ and $(K^+)_v$ were not changed during breathing 8% O_2 in the CHB group (Table 3).

Effects of 8-PT. After 8-PT, baseline levels of V_T and $\dot{V}_E$ during 12% O_2 were increased (Table 2) and consistent with this, P_{a,O_2} was higher and P_{a,CO_2} lower than before 8-PT (Table 3). Baseline ABP during 12% O_2 fell after 8-PT, as did ABF (Table 2). $[K^+]_a$ fell after 8-PT from 4·2 to 3·8 mM, but $[K^+]_v$ did not change (Table 3).

After 8-PT, the waning of V_T during 8% O_2 in CHB rats was reduced, as in NB rats (Fig. 4). Consistent with the more persistent hyperventilation, P_{a,O_2} recorded during 8% O_2 was higher and P_{a,CO_2} was lower after 8-PT (Table 3). Furthermore, after 8-PT, 8% O_2 no longer evoked a significant fall in HR (Fig. 3). In contrast to the NB group, the fall in ABP evoked by 8% O_2 in the CHB group was not affected by 8-PT. AVC was not significantly changed during 8% O_2 before or after 8-PT (Fig. 3) and CBF showed a similar tendency to fall during 8% O_2 after 8-PT as before (Fig. 3). $[K^+]_a$ increased during 8% O_2 after 8-PT; $[K^+]_v$ did not change (Table 3).

Responses evoked by adenosine. A bolus injection of adenosine (600 μg kg^{-1} I.V.) evoked a similar response to that seen in NB rats (see Fig. 2). Thus, R_F increased, ABP and HR fell and there were increases in CBF, CVC, ABF and AVC (Fig. 4). The changes evoked by adenosine were not different from those evoked in NB rats. As in NB rats, the falls in ABP and HR and the increases in CVC and AVC were significantly reduced by 8-PT (Fig. 3).

Responses evoked by air breathing. When CHB rats breathed room air for 5 min, there were significant falls in R_F and $\dot{V}_E$ (Fig. 5). In spite of this, the absolute level of $\dot{V}_E$ at the 5th minute of air breathing (245 ± 11 ml min^{-1}) was still greater than in NB rats during air breathing (204 ± 18 ml min^{-1}). Consistent with the fall in $\dot{V}_E$, P_{a,CO_2} was increased (Table 3). In fact, P_{a,O_2} rose to a level not significantly different from that observed in NB rats during air breathing, while P_{a,CO_2} remained significantly lower than that in NB rats during air breathing (compare Tables 1 and 3). Further, ABP increased significantly during air breathing (to 119 ± 3 mmHg) but did not reach the level recorded in NB breathing air (131 ± 3 mmHg; see Fig. 5 and Table 2). ABF, AVC, CBF and CVC all tended to fall, while HR

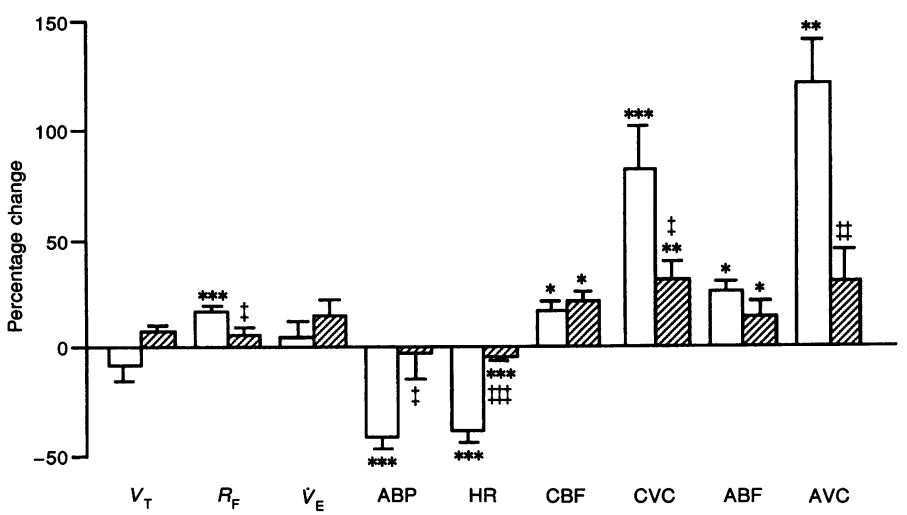

Figure 4. Peak percentage changes induced in respiratory and cardiovascular variables of CHB rats by adenosine (600 μg kg^{-1} I.V.), before and after 8-PT

Columns, symbols and abbreviations as in Fig. 2.

tended to increase (Fig. 5). There was a fall in $[K^+]_a$ (from 4·2 to 3·7 mM), but $[K^+]_v$ did not change (Table 3).

Effects of 8-PT. After 8-PT, when baseline levels of V_T and $\dot{V}_E$ were increased (see above), the absolute fall in $\dot{V}_E$ induced by air breathing was unaffected (Fig. 5). 8-PT had no effect on any of the cardiovascular changes evoked by air breathing (Fig. 5). As noted above, $[K^+]_a$ during 12% O_2 breathing was significantly lower after 8-PT; under these conditions there was no significant fall in $[K^+]_a$ during air breathing (Table 3). As before 8-PT, $[K^+]_v$ was not changed by air breathing (Table 3).

DISCUSSION

The present study indicates that chronic hypoxia from birth induces pronounced effects on baseline levels of cardiovascular and respiratory variables, on the cardiovascular and respiratory responses evoked by a further acute hypoxic challenge and on the role of adenosine in those responses.

Normoxia from birth

The pattern of response evoked by acute hypoxia (breathing 12 or 8% O_2 for 5 min) in NB rats of 6 weeks old was similar to that observed in older rats (10–11 weeks) during

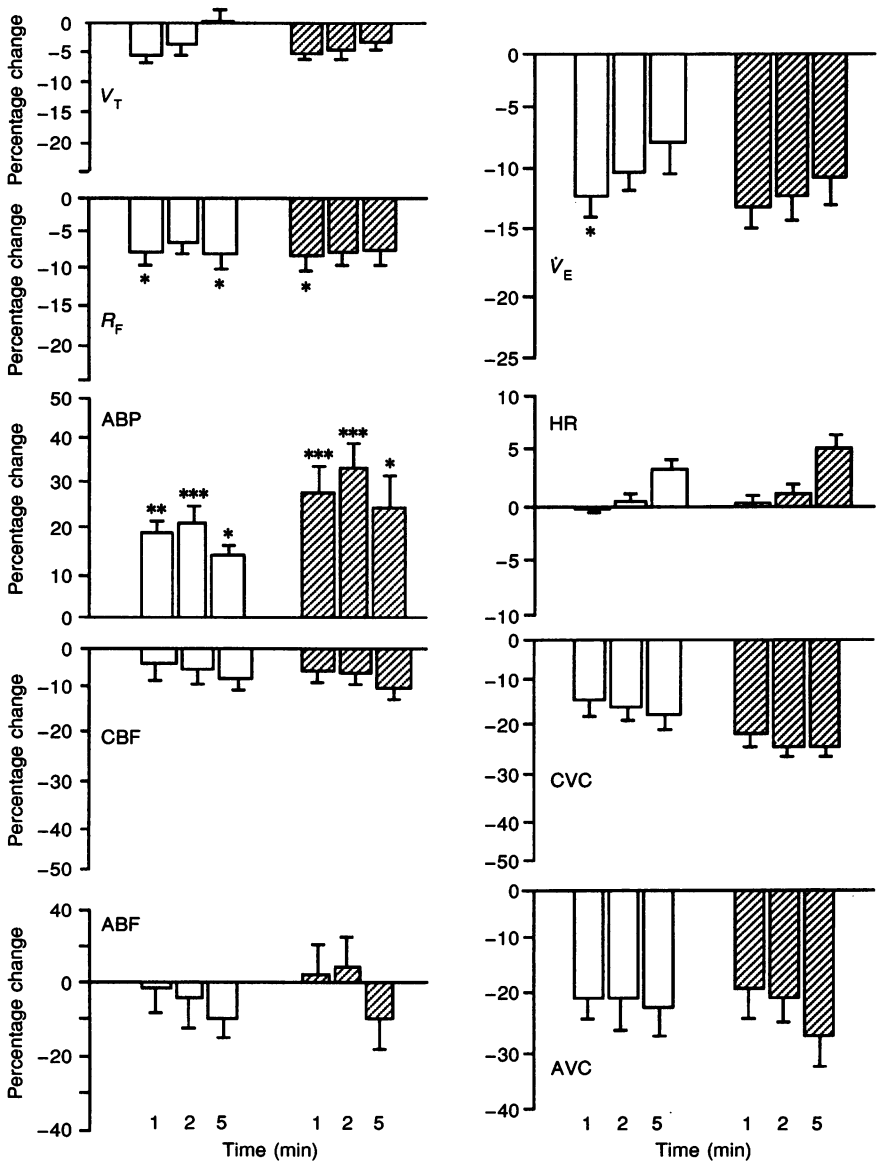

Figure 5. Percentage changes induced in respiratory and cardiovascular variables of CHB rats by 5 min of breathing air, before and after 8-PT

Columns and abbreviations as in Fig. 3. * Significant difference between absolute values measured during 12% O_2 (control) and air. Levels of significance as in Fig. 1.

10 min of breathing 8% O_2, viz. an initial increase in ventilation and heart rate followed by secondary falls in both variables, a gradual fall in arterial pressure and an increase in both aortic (muscle) and carotid (cerebral) vascular conductance, indicating vasodilatation in skeletal muscle and cerebral circulations, respectively, with a secondary fall in cerebral blood flow (Marshall & Metcalfe, 1998; Neylon & Marshall, 1991; Thomas & Marshall, 1994*b*). The fact that the fall in heart rate, increase in cerebral vascular conductance and secondary fall in cerebral blood flow did not reach statistical significance by the 5th minute of the present study may be explained by the relatively small group size and the shorter stimulus period. In our previous study these changes became greater from the 5th to the 10th minute of hypoxia (Thomas & Marshall 1994*b*). Thus, these changes are consistent with the proposal that the secondary respiratory and cardiovascular changes may develop into a positive feedback loop (see the introductory section and Thomas & Marshall, 1994*b*).

The effects of 8-PT upon the responses evoked by hypoxia were also similar to those observed in older rats: 8-PT reduced the secondary fall in ventilation and heart rate, the fall in arterial pressure and the increase in muscle vascular conductance, indicating that these responses were at least partly caused by locally released adenosine (see Neylon & Marshall, 1991; Thomas & Marshall, 1994*b*; Thomas *et al.* 1994). However, the present results contrast with our observations on older rats, in which the hypoxia-induced muscle vasodilatation was accompanied by an increase in the K^+ efflux from skeletal muscle ($[K^+]_v$) which was abolished by 8-PT (Marshall *et al.* 1993); in NB, hypoxia caused no change in $[K^+]_v$ before 8-PT and a significant fall of $[K^+]_v$ after 8-PT.

The present results on young rats, therefore, suggest that K^+ release via stimulation of adenosine receptors that are coupled to K_{ATP} channels (Marshall *et al.* 1993, see introductory section) played a smaller role in the muscle vasodilatation than in older rats. This may be age related, for K_{ATP} channels were not found in skeletal myotubules taken from neonate rats (Spruce, Standen & Stanfield, 1987). Given that in cardiac myocytes and coronary artery smooth muscle, it is the A_1 subtype of adenosine receptors that are coupled to K_{ATP} channels (Kirsch, Codina, Birnbaumer & Brown, 1990; Dart & Standen, 1993), it is possible that the hypoxia-induced vasodilatation that was blocked by addition of 8-PT in NB rats was mediated by A_2 receptors that induce vasodilatation by increasing cAMP levels in vascular smooth muscle (Olsson & Pearson, 1990). The hypoxia-induced fall in $[K^+]_v$ in NB rats after 8-PT could be explained by catecholamine-induced activation of the β_2-adrenoreceptor-mediated uptake mechanism for K^+, which is particularly avid in skeletal muscle (Clausen, 1986; Mian, Marshall & Kumar, 1990). The fact that 8-PT revealed this fall in $[K^+]_v$, supports the idea that in the absence of 8-PT, hypoxia did cause some release of K^+ from the skeletal muscle fibres by an adenosine-mediated mechanism.

In six out of eight NB rats, cerebral blood flow was better maintained during hypoxia when 8-PT had reduced the hypoxia-induced fall in arterial pressure and secondary fall in ventilation, as in older rats (Thomas & Marshall 1994*b*). Thus, it seems that in young, as in older, rats adenosine plays an important role in the proposed positive feedback loop that links arterial pressure, cerebral blood flow and ventilation.

Chronic hypoxia from birth

In the rats that were hypoxic for 3–4 days antepartum and from birth (CHB), there was reduced weight gain in that they were, on average, 12 days older when they reached the same weight as the NB rats that breathed air from birth. Growth retardation is a recognized effect of chronic hypoxia (Stickney & van Liere, 1953; Mortola, Morgan & Virgona, 1986; Eden & Hanson, 1987*b*). It can be explained by inadequate lactation by the mother (see Mortola *et al.* 1986) and by decreased appetite and food ingestion (Alippi, Barcelo, Rio & Bozzini, 1983).

Respiratory effects. The CHB group breathing 12% O_2 were hyperventilating relative to the NB group breathing air, judging from the minute ventilation and P_{a,CO_2}. Thus, one important question is whether chronic hypoxia from birth changed the respiratory sensitivity to hypoxia. This judgement is complicated by the biphasic nature of the response to hypoxia. As indicated in the introductory section the mechanisms underlying the two phases cannot be clearly distinguished, for although the initial increase in ventilation reflects the predominating effect of peripheral chemoreceptor stimulation, it may be somewhat offset by the central inhibitory effect of hypoxia. On the other hand, the secondary decrease in ventilation merely reflects the gradual predominance of this inhibitory effect over the ongoing stimulatory effect of the peripheral chemoreceptors. Nevertheless, the absolute value of minute volume recorded in CHB rats during 12% O_2, when they were in the quasi-steady state of the response to 12% O_2, was similar to that recorded in NB rats in the quasi-steady state at the 5th minute of breathing 12% O_2 (see Results). Further, both groups showed similar absolute levels of minute ventilation at the 5th minute of breathing 8% O_2. Finally, the initial increase in ventilation recorded in CHB rats, when they were switched from 12 to 8% O_2 was comparable to that calculated for NB rats from the difference between the increase in ventilation recorded at the 2nd minute of breathing 12 and 8% O_2 (see Results).

Thus, there is no reason to suggest that chronic hypoxia for 5–6 weeks from birth affected either the chemoreceptor-mediated hyperventilatory response to hypoxia, or the central inhibitory effect of hypoxia on ventilation. The former conclusion agrees with the finding of Eden & Hanson (1987*b*) that the carotid chemoreceptors of rats that

had been hypoxic for 5–10 weeks from birth showed a normal sensitivity to changes in P_{a,O_2}. However, the latter conclusion seems at variance with their finding that at 5–10 weeks the chronically hypoxic rats showed no ventilatory response when their inspirate was reduced from 15 to 12% O_2 and only a transient increase when the inspirate was reduced to 8% O_2, for this implied postnatal persistence of the central-inhibitory influence. The disparity may be related to differences in the concentrations of O_2 in the hypoxic chamber (15 and 12% O_2), and in the acutely imposed changes in the inspirate and/or to the fact that the rats studied by Eden & Hanson (1987*b*) were not anaesthetized.

A second question is whether chronic hypoxia from birth affects respiratory sensitivity to CO_2. Assuming the level of ventilation attained in response to a given inspired hypoxic mixture is a balance between any increase in ventilation caused by hypoxic stimulation of the peripheral chemoreceptors and the tendency for the resulting hypocapnia to reduce ventilation by unloading the central and peripheral chemoreceptors, then it seems that chronic hypoxia from birth may increase the sensitivity of the central chemoreceptors to CO_2. Thus, whereas both CHB and NB groups had similar values of P_{a,O_2} at the 5th minute of acute exposure to 8% O_2, the P_{a,CO_2} value was substantially lower in the CHB group. Moreover, when CHB rats were acutely exposed to air they achieved a similar P_{a,O_2} value, but at a lower P_{a,CO_2}, than that recorded in NB rats breathing air, i.e. they were still hyperventilating relative to NB. These observations are similar to those made in mature rats that had been made chronically hypoxic and were acutely exposed to 8% O_2 (Thomas & Marshall, 1994*a*), or air (Olson & Dempsey, 1978; Kuwahira, Heisler, Piiper & Gonzalez, 1993).

Qualitatively, adenosine exerted a similar influence on ventilation in CHB as in NB and older rats (see above and Thomas & Marshall, 1994*b*). Thus, the fact that 8-PT increased the baseline level of minute ventilation in CHB rats breathing 12% O_2, by increasing tidal volume, and that it reduced the secondary fall in tidal volume seen during 8% O_2 suggests that adenosine released centrally by the influence of hypoxia on the brain exerted a tonic inhibitory influence on the volume component of ventilation, which was accentuated by further acute hypoxia.

As an increase in $[K^+]_a$ can stimulate ventilation by acting on the peripheral chemoreceptors and as this effect is facilitated by concomitant hypoxia (Burgher, Estavillo, Kumar, Nye & Paterson, 1988), it is likely that the raised $[K^+]_a$ recorded in CHB rats breathing 12% O_2 relative to that recorded in NB rats breathing air, contributed to the tonic hyperventilation in CHB rats. Moreover, the fall in $[K^+]_a$ in CHB rats when they were acutely exposed to air may have contributed to the observed fall in ventilation.

Cardiovascular effects. The fact that CHB rats breathing 12% O_2 had a lower mean arterial pressure than NB rats breathing air could be attributed to a reduced peripheral resistance in the former, particularly as aortic vascular conductance in the CHB group was ~2·5 times greater than in the NB group. As aortic vascular conductance decreased substantially when CHB rats were acutely exposed to air, this implies a tonic dilator influence of hypoxia upon muscle vasculature. However, as acute exposure to air did not decrease muscle vascular conductance to the same level as that recorded in NB rats during air breathing (cf. Fig. 5, Table 2), there may have been a structural difference between the muscle vasculatures of the rats of CHB and NB. An increase in the capillary density in skeletal muscle has been reported in goslings exposed to chronic hypoxia (Snyder, Byers & Kayar, 1984).

Although CHB rats breathing 12% O_2 had raised ventilation and reduced arterial pressure relative to NB rats breathing air, their heart rates were similar. This is somewhat surprising as hyperventilation would be expected to lead to tachycardia (Thomas & Marshall, 1994*b*) as would a reduced arterial pressure, via the baroreceptor reflex. It may be that these relationships are altered by chronic hypoxia from birth, by the local depressant effect of hypoxia upon the sino-atrial node (Froldi & Belardinelli, 1990; Thomas *et al.* 1994; Thomas & Marshall, 1994*b*).

Given that chronic hypoxia (12% O_2) from birth has been associated with a reduction in the specific weight of the brain (Naeye, 1966), our findings that cerebral blood flow and vascular conductance were similar in CHB rats breathing 12% O_2 and NB rats breathing air suggest that blood flow per gram brain weight was proportionately higher in CHB rats than the NB group. It seems unlikely there was a tonic dilator influence of hypoxia on the cerebral vasculature of CHB rats as acute exposure to air did not significantly affect cerebral vascular conductance. Rather, hypoxia from birth may have increased the density of the vasculature in brain as reported in adult rats exposed to chronic hypoxia (LaManna, Vendel & Farrell, 1992).

A striking difference between CHB and NB rats was their cardiovascular response to acute hypoxia. Although both groups showed a fall in arterial pressure and increase in muscle vascular conductance, the fall in arterial pressure seen in the CHB group when their inspirate was switched from 12 to 8% O_2 was virtually the same as that seen in the NB group when they experienced the larger change, from air to 8% O_2 (cf. Figs 1 and 3). Moreoever, CHB rats, in contrast to NB rats, showed a progressive fall in heart rate and cerebral blood flow. Thus, in the CHB rats there was exacerbation of those components of the response that we have proposed can lead to the development of a positive feedback loop and ultimately to death (see above and Thomas & Marshall, 1994*b*). These observations are,

therefore, fully consistent with the report that at least some infants who were victims of SIDS showed evidence of chronic hypoxia (Naeye, 1980).

A further contrast between the CHB and NB groups was that 8-PT reduced arterial pressure in CHB rats breathing 12% O_2, but had no effect on baseline arterial pressure in NB rats breathing air. This suggests that in the chronically hypoxic state, adenosine exerted a tonic vasodilator influence; this was probably a small, widespread influence on the peripheral vasculature given that neither baseline muscle, nor cerebral vascular conductance, decreased significantly.

The fact that addition of 8-PT abolished the fall in heart rate that was evoked in the CHB group by acute hypoxia is in agreement with observations on older, normoxic rats and implicates adenosine in its mediation (Froldi & Belardinelli, 1990; Thomas & Marshall, 1994*b*). However, in complete contrast to our results in normoxic animals, addition of 8-PT had no effect on either the fall in arterial pressure, or muscle vasodilatation induced by acute hypoxia. Yet, in both CHB and NB rats, exogenous adenosine was fully capable of inducing a pronounced fall in arterial pressure and muscle vasodilatation that were attenuated by 8-PT. Therefore, an obvious conclusion is that chronic hypoxia from birth changes metabolism in muscle so as to greatly reduce the synthesis and/or release of adenosine that is caused by acute hypoxia. This may be associated with a greater oxidative capacity in the CHB group than the NB group as we have suggested for adult rats made chronically hypoxic (see Davies, Thomas & Marshall, 1994).

As $[K^+]_a$ and $[K^+]_v$ were both greater in CHB rats breathing 12% O_2 than in NB rats breathing air, it may be that K^+ contributed to the tonic vasodilatation in skeletal muscle in CHB rats breathing 12% O_2 (see Skinner & Powell, 1967). However, as $[K^+]_v$ did not increase in the CHB group when they acutely breathed 8% O_2 and as addition of 8-PT had no effect on $[K^+]_v$ when the CHB group acutely breathed 8% O_2, there is no reason to suggest that K^+ released from skeletal muscle through adenosine-coupled K_{ATP} channels, or via other mechanisms, contributed to the dilator response to further acute hypoxia (cf. NB rats above). The fact that $[K^+]_a$ in CHB rats was reduced by 8-PT, or by acute exposure to air, raises the possibility that K^+ was released from tissues other than skeletal muscle by a tonic influence of hypoxia and adenosine and exerted a tonic vasodilator influence in muscle and elsewhere. However, as there was no further increase in $[K^+]_a$ in CHB rats when they were acutely exposed to 8% O_2, the observed muscle vasodilatation cannot be ascribed to K^+ in the arterial blood. Rather, it may have been induced by catecholamines via β_2-adrenoreceptors on the vascular smooth muscle (Mian *et al.* 1990; Mian & Marshall, 1991) or prostaglandins (Busse, Forstermann, Matsuda & Pohl, 1984).

The fact that 8-PT had no effect on the fall in arterial pressure induced in CHB rats by breathing 8% O_2, and did not affect the accompanying fall in cerebral blood flow is fully consistent with the view that these changes are causally related components of a positive feedback loop (see above). Overall, it seems that adenosine plays a smaller role in the positive feedback loop in CHB rats than in NB rats in that 8-PT was far less effective in altering its individual components.

In summary, the present study indicates that 6-week-old rats show a pattern of respiratory and cardiovascular response to acute hypoxia that is very similar to that observed in older rats of 10–11 weeks old. Moreover, in young, as in older, rats adenosine apparently contributes to components of the response that can be attributed to the local effects of hypoxia (the secondary fall in ventilation, the fall in arterial pressure and muscle vasodilatation), although an adenosine-dependent release of K^+ seems less important in mediating the muscle vasodilatation in young rats. On the other hand, the present study suggests that in young rats that have been chronically hypoxic from birth, there is greater predominance of the local effects of hypoxia that are potentially life threatening. Further, whilst adenosine contributes to the secondary fall in ventilation and bradycardia induced by acute hypoxia in these animals, neither adenosine, nor potassium released from skeletal muscle, contributes to the muscle vasodilatation.

Allipi, R. M., Barcelo, A. C., Rio, M. E. & Bozzini, C. E. (1983). Growth retardation in the early developing rat exposed to continuous hypobaric hypoxia. *Acta Physiologica Pharmacologica et Therapeutica Latinoamericana* **33**, 5–9.

Boddy, K., Dawes, G. S., Fisher, R., Pinter, S. & Robinson, J. S. (1974). Fetal breathing movements, electrocortical and cardiovascular responses to hypoxemia and hypercapnia in sheep. *Journal of Physiology* **243**, 599–618.

Bonora, M., Marlot, D., Gaultier, H. & Duron, B. (1984). Effects of hypoxia on ventilation during post-natal development in conscious kittens. *Journal of Applied Physiology* **56**, 1464–1471.

Burgher, R. E., Estavillo, J. A., Kumar, P., Nye, P. C. G. & Paterson, D. J. (1988). Effects of potassium, oxygen and carbon dioxide on the steady state of cat carotid body chemoreceptors. *Journal of Physiology* **401**, 519–531.

Busse, R., Forstermann, K., Matsuda, H. & Pohl, U. (1984). The role of prostaglandins in the endothelium-mediated vasodilatory response to hypoxia. *Pflügers Archiv* **401**, 77–83.

Clausen, T. (1986). Regulation of active Na^+–K^+ transport in skeletal muscle. *Physiological Reviews* **66**, 542–581.

Dart, C. & Standen, N. B. (1993). Adenosine-activated potassium current in smooth muscle cells isolated from pig coronary artery. *Journal of Physiology* **420**, 431–446.

Davies, W. R., Thomas, T. & Marshall, J. M. (1994). The effects of acute and chronic systemic hypoxia upon muscle oxygen supply in anaesthetised rats. *Journal of Physiology* **477.P**, 34*P*.

EASTON, P. A., SLYKERMAN, L. J. & ANTHONISEN, N. R. (1986). Ventilatory response to sustained hypoxia in normal adults. *Journal of Applied Physiology* **61**, 906–911.

EDEN, G. J. & HANSON, M. A. (1987*a*) Maturation of the respiratory response to acute hypoxia in the newborn rat. *Journal of Physiology* **392**, 1–9.

EDEN, G. J. & HANSON, M. A. (1987*b*). Effects of chronic hypoxia from birth on the ventilatory response to acute hypoxia in the newborn rat. *Journal of Physiology* **392**, 11–19.

FROLDI, G. & BELARDINELLI, L. (1990). Species-dependent effects of adenosine on heart rate and atrioventricular node conduction: mechanisms and physiology implications. *Circulation Research* **67**, 960–978.

GOOTMAN, P. M., BUCKLEY, N. M. & GOOTMAN, N. (1991). Post-natal maturation of neural control of the circulation. In *Reviews in Perinatal Medicine*, vol. 3, ed. SCARPELLI, E. M. & COSMI, E. V., pp. 1–72. Raven Press, New York.

HANSON, M. A., KUMAR, P. & MCCOOKE, H. B. (1986). Post-natal resetting of carotid chemoreceptor sensitivity in the lamb. *Journal of Physiology* **383**, 57.

HANSON, M. A., KUMAR, P. & WILLIAMS, B. A. (1989). Effects of chronic hypoxia upon development of respiratory reflexes in newborn kittens. *Journal of Physiology* **411**, 563–574.

KIRSCH, G. E., CODINA, J., BIRNBAUMER, L. & BROWN, A. M. (1990). Coupling of ATP-sensitive potassium channels to A_1 receptors by G-proteins in rat ventricular myocytes. *American Journal of Physiology* **259**, H820–826.

KUWAHIRA, I., HEISLER, N., PIIPER, J. & GONZALEZ, N. C. (1993). Effect of chronic hypoxia on haemodynamics organ bloodflow and O_2 supply in rats. *Respiration Physiology* **92**, 227–238.

LAMANNA, J. C., VENDEL, L. M. & FARRELL, R. N. (1992). Brain adaptation to chronic hypobaric hypoxia in rats. *Journal of Applied Physiology* **72**, 2238–2243.

MARSHALL, J. M. & METCALFE, J. D. (1988). Analysis of the cardiovascular changes induced in the rat by graded levels of systemic hypoxia. *Journal of Physiology* **407**, 385–403.

MARSHALL, J. M. & METCALFE, J. D. (1990). Effects of systemic hypoxia on distribution of cardiac output in the rat. *Journal of Physiology* **426**, 335–353.

MARSHALL, J. M., THOMAS, T. & TURNER, L. (1993). A link between adenosine, ATP-sensitive K^+ channels potassium and muscle vasodilatation in the rat in systemic hypoxia. *Journal of Physiology* **472**, 1–9.

MIAN, R. & MARSHALL, J. M. (1991). The role of catecholamines in responses evoked in arterioles and venules of rat skeletal muscle by systemic hypoxia. *Journal of Physiology* **436**, 499–510.

MIAN, R., MARSHALL, J. M. & KUMAR, P. (1990). Interactions between K^+ and β_2-adrenoreceptors in determining muscle vasodilatation induced in the rat by systemic hypoxia. *Experimental Physiology* **70**, 407–410.

MORTOLA, J. P., MORGAN, C. A. & VIRGONA, V. (1986). Respiratory adaptation to chronic hypoxia in newborn rats. *Journal of Applied Physiology* **61**, 1329–1336.

NAEYE, R. L. (1966). Organ and cellular development in mice growing at simulated high altitude. *Laboratory Investigation* **15**, 700–706.

NAEYE, R. L. (1980). Sudden infant deaths. *Scientific American* **245**, 52–58.

NEYLON, M. & MARSHALL, J. M. (1991). The role of adenosine in the respiratory and cardiovascular response to systemic hypoxia in the rat. *Journal of Physiology* **440**, 529–545.

OLSON, E. B. & DEMPSEY, J. A. (1978). Rat as a model for humanlike ventilatory adaptation to chronic hypoxia. *Journal of Applied Physiology* **44**, 763–769.

OLSSON, R. A. & PEARSON, J. D. (1990). Cardiovascular purinoceptors. *Physiology Reviews* **70**, 761–845.

SKINNER, N. S. & POWELL, W. J. (1967). Action of oxygen and potassium on vascular resistance of dog skeletal muscle. *American Journal of Physiology* **212**, 533–540.

SNYDER, G. K., BYERS, R. L., KAYAR, S. R. (1984). Effects of hypoxia on tissue capillarity in geese. *Respiratory Physiology* **58**, 151–160.

SPRUCE, A. E., STANDEN, N. B. & STANFIELD, P. R. (1987). Studies of the unitary properties of adenosine-5′-triphosphate-regulated potassium channels of frog skeletal muscle. *Journal of Physiology* **382**, 213–236.

STICKNEY, J. C. & VAN LIERE, E. J. (1953). Acclimatization to low oxygen tension. *Physiological Reviews* **33**, 13–34.

THOMAS, T., ELNAZIR, B. K. & MARSHALL, J. M. (1994). Differentiation of peripherally from centrally mediated actions of adenosine during hypoxia in the rat. *Experimental Physiology* **79**, 809–822.

THOMAS, T. & MARSHALL, J. M. (1993). The influence of chronic hypoxia from birth on the respiratory and cardiovascular systems studied in the rat under anaesthesia. *Journal of Physiology* **473**, 201*P*.

THOMAS, T. & MARSHALL, J. M. (1994*a*). The influence of adenosine on respiration and the cardiovascular system in chronically hypoxic rats under anaesthesia. *Journal of Physiology* **479.P**, 16–17*P*.

THOMAS, T. & MARSHALL, J. M. (1994*b*). Interdependence of the respiratory and cardiovascular changes induced by systemic hypoxia in the rat: the roles of adenosine. *Journal of Physiology* **480**, 627–636.

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