The roles of adenosine in regulating the respiratory and cardiovascular systems in chronically hypoxic, adult rats

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1. We have investigated the roles of adenosine in regulating the respiratory and cardiovascular systems of rats that were made chronically hypoxic for 3–4 weeks from 6 weeks of age (CH rats) in an hypoxic chamber at 12% O_2. They were studied under anaesthesia while breathing 12% O_2 and during acute hypoxia (breathing 8% O_2 for 5 min) before and after addition of the adenosine receptor antagonist 8-phenyltheophylline (8-PT, 10 mg kg^{-1}). The results were compared with those obtained from normoxic (N) rats in a previous study.

2. CH rats breathing 12% O_2 had greater minute ventilation ($\dot{V}_E$) than N rats breathing air, but their levels of arterial blood pressure (ABP), heart rate (HR), femoral vascular conductance (FVC) and cerebral vascular conductance (CVC) were fully comparable. 8-PT increased tidal volume (V_T) in CH rats indicating a greater tonic central inhibitory influence of adenosine on V_T than in N rats. However, 8-PT had no effect on cardiovascular variables, indicating no tonic cardioinhibitory or vasodilator influence of adenosine in CH rats.

3. Acute hypoxia in CH rats increased $\dot{V}_E$ such that at the 5th minute of 8% O_2 absolute $\dot{V}_E$ was comparable to that of N rats breathing 8% O_2. Moreover, in CH rats 8-PT increased V_T at the 5th minute of 8% O_2 indicating that the central inhibitory influence of adenosine limits the ability to maintain V_T in acute hypoxia as it does in N rats.

4. Eight per cent O_2 also produced a fall in ABP in CH rats that was comparable to that induced in N rats by the larger change from air to 8% O_2. However, the changes in HR were similar in CH and N rats while the increases in FVC and CVC were smaller in CH rats. This suggests that the ability of the secondary effects of hyperventilation and of the baroreceptor reflex to maintain cardiac output and thereby ABP is reduced in CH rats.

5. Whereas 8-PT substantially reduced the hypoxia-induced increases in FVC and CVC in N rats, it had a small effect in CH rats ($P = 0{\cdot}054$ and 0·06, respectively). Further, acute hypoxia in CH rats had no effect on the K^+ concentration in the venous efflux of hindlimb K^+ (K_v^+) before or after 8-PT treatment. We suggest that in CH rats, the dilator influence of adenosine in acute hypoxia occurs via actions on the blood vessel walls: there was no evidence that adenosine can release dilator concentrations of K^+ from skeletal muscle fibres in CH rats as proposed for N rats.

It is well established that changes in ventilation can influence the cardiovascular system by a variety of mechanisms (Daly, 1986; Marshall, 1994). These interactions are important in determining the observed cardiovascular response to acute systemic hypoxia, as the balance between the primary responses to hypoxic stimulation of peripheral chemoreceptors, the respiratory-dependent responses and the local effects of hypoxia varies according to the severity of the hypoxia and varies between species (Marshall, 1994). Our recent studies on the rat indicate that in this species, the initial increase in ventilation that arises from hypoxic stimulation of the peripheral chemoreceptors contributes, by secondary mechanisms, to the concomitant increase in heart rate, but limits the accompanying cerebral vasodilatation by reducing the arterial partial pressure of CO_2 (P_{a,CO_2}). On the other hand, the secondary fall in ventilation which occurs whilst hypoxia continues, contributes to the secondary fall in heart rate and facilitates the cerebral vasodilatation (Thomas & Marshall, 1994*b*). We have also shown that locally released adenosine plays important roles in these respiratory and cardiovascular responses to acute systemic hypoxia. By acting within the central nervous system, adenosine is at least partly responsible for the secondary fall in ventilation, its action in the heart contributes to the

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secondary fall in heart rate, it acts in skeletal muscle to produce vasodilatation and by the combination of these effects it contributes to the hypoxia-induced fall in arterial pressure (Neylon & Marshall, 1991; Thomas, Elnazir & Marshall, 1994; Thomas & Marshall, 1994*b*). Within skeletal muscle, adenosine apparently causes some dilatation by stimulating the release of dilator concentrations of K^+ from the skeletal muscle fibres via glibenclamide-sensitive K^+ channels, probably ATP-sensitive K^+ (K_{ATP}) channels (Marshall, Thomas & Turner, 1993). However, its dilator influence is largely dependent upon nitric oxide synthesis by the vascular endothelium (Skinner & Marshall, 1996).

Now, in a range of cardiovascular and respiratory disorders and at high altitude, systemic hypoxia exists on a chronic, rather than an acute, time base. Yet comparatively little is known of the influences of chronic hypoxia upon the cardiovascular system or upon respiratory–cardiovascular interactions, while the influences of adenosine in chronic hypoxia have received little attention. Our recent experiments on rats that were made chronically hypoxic from birth (CHB) by housing them in a chamber in 12% O_2 indicated that studies on the effects of chronic hypoxia on adult rats would be worthwhile. For example, with CHB conditions we found that adenosine exerted a tonic inhibitory influence upon ventilation, but had no tonic influence upon heart rate, cerebral or muscle vascular conductance or arterial pressure (Thomas & Marshall, 1995). Moreover, when they were exposed to acute hypoxia (8% O_2) this produced a similar pattern of response to that seen in control, normoxic rats except that the bradycardia and fall in arterial pressure were exacerbated. Also, whereas adenosine was partly responsible for the secondary decrease in ventilation and heart rate evoked by acute hypoxia, it apparently played no part in the muscle vasodilatation and caused no release of K^+ from skeletal muscle, even though muscle vasodilatation could be induced by exogenous adenosine (Thomas & Marshall, 1995). Thus these results suggested that chronic hypoxia from birth increased the local effects of acute hypoxia on the heart and circulation, but reduced the importance of adenosine and K^+ in mediating muscle vasodilatation.

Therefore the primary aim of the present study was to investigate the influences of adenosine upon the respiratory and cardiovascular systems of mature rats made chronically hypoxic from 6 weeks of age when they are sexually mature (CH rats). To this end, we tested the effect of the adenosine receptor antagonist 8-phenyltheophylline (8-PT) which can cross the blood–brain barrier (Thomas & Marshall, 1994*b*; Thomas *et al.* 1994) both when they were breathing 12% O_2 and on the responses evoked when inspirate was acutely changed to 8% O_2 or to air. In view of our previous findings on normoxic rats (N), we recorded blood flow and vascular conductance of the cerebral circulation and hindlimb muscle and measured the K^+ concentrations in the arterial supply (K^+_a) and venous drainage of the hindlimb (K^+_v). Some of our findings have been reported in brief to The Physiological Society (Thomas & Marshall, 1994*a*).

METHODS

Experiments were performed on twelve male Wistar rats (CH rats) that were housed in small groups in a normobaric chamber at 12% O_2 for 3–4 weeks prior to the acute experiment. The chamber has been described in detail previously (Thomas & Marshall, 1995). Briefly, the O_2 concentration was kept between 11·75 and 12·15%, the CO_2 concentration was maintained at ~0·03%, while the humidity and temperature were kept at 40–50% and 22–24 °C, respectively, by appropriate servo-controlled systems, apart from two periods of ~20 min per week when the chamber was opened to allow the cages to be cleaned and food and water to be replenished. The mean body weight of the animals when they were removed from the chamber and taken into the acute experiment was 320 ± 12 g (mean ± S.E.M.).

The animals were prepared for the experiment as described previously (Neylon & Marshall, 1991; Thomas & Marshall, 1994*b*). Briefly, anaesthesia was induced with a halothane–nitrous oxide–oxygen mixture and maintained with 7–12 mg kg^{-1} h^{-1} Saffan (Pitman-Moore Ltd, Uxbridge, Middlesex, UK) given via a jugular vein. The trachea was cannulated with a T-shaped cannula so that tidal volume (V_T) and respiratory frequency (R_F) could be continuously recorded: minute volume ($\dot{V}_E$) was calculated off-line as the product of V_T and R_F. Mean arterial blood pressure (ABP) and heart rate (HR) were recorded from a femoral artery, while femoral blood flow (FBF) was recorded from the contralateral femoral artery via a cuff-type electromagnetic transducer. Carotid blood flow (CBF) was recorded as an index of cerebral (forebrain) blood flow (see Thomas & Marshall, 1994*b*) via a cuff-type transducer placed on a common carotid artery after vascular isolation of the internal carotid artery. Femoral vascular conductance and carotid vascular conductance (FVC and CVC, respectively) were calculated on-line by dividing FBF or CBF by ABP. All recorded variables were displayed on 6- and 4-channel pen recorders. The brachial artery was cannulated so that samples of arterial blood (130 μl) could be taken for measurement of the partial pressure of O_2 (P_{a,O_2}) and CO_2 (P_{a,CO_2}) and of arterial pH by using a Nova Stat Profile Analyser (Stat 3, V.A. Howe, Waltham, MA, USA). Blood samples (< 100 μl) were also taken from the brachial artery and from a cannula placed in the femoral vein with the tip at the point of convergence of the iliac veins (Marshall *et al.* 1993). The K^+_a and K^+_v in the venous blood that predominantly drained the hindlimb from which blood flow was recorded, were measured by flame photometry.

Throughout surgery and during the experimental period, unless otherwise stated below, CH rats breathed 12% O_2 which was blown across the end of the respiratory flow head by an air pump at > 1 l min^{-1} from a gas bag. During the experimental period, the depth of anaesthesia was such that the recorded cardiovascular and respiratory variables were stable between experimental stimuli, and strong mechanical stimulation of a paw evoked, at most, a weak withdrawal of that paw and a transient rise in arterial pressure. The depth of Saffan anaesthesia was greater than that at which mechanical stimulation, or hypoxic stimulation of peripheral chemoreceptors can evoke the characteristic cardiovascular components of the alerting or defence response (Marshall & Metcalfe, 1988). The criteria used for judging anaesthesia in the present study were the same as those used in our previous studies (Marshall *et al.* 1993; Thomas & Marshall, 1994*b*).

Table 1. Baseline values of respiratory and cardiovascular variables recorded in CH rats breathing 12% O_2 and N rats breathing air before and after 8-PT

	CH rats		N rats	
	Before 8-PT	After 8-PT	Before 8-PT	After 8-PT
Tidal volume (V_T) (ml)	2·38 ± 0·08	2·78 ± 0·07***	2·44 ± 0·07	2·59 ± 0·05
Respiratory frequency (R_F) (breaths min^{-1})	115·64 ± 5·19††	102·55 ± 4·14**	95·56 ± 3·59	91·78 ± 4·26
Minute volume ($\dot{V}_E$) (ml min^{-1})	274·18 ± 13·03†	284·11 ± 12·29	227·64 ± 10·44	237·79 ± 12·01
Mean arterial pressure (ABP) (mmHg)	125·62 ± 5·94	117·43 ± 4·14	130·83 ± 2·75	125·52 ± 3·64
Heart rate (HR) (beats min^{-1})	419·19 ± 9·97	422·55 ± 9·30	436·31 ± 8·65	429·14 ± 14·88
Femoral blood flow (FBF) (ml min^{-1})	1·62 ± 0·16	1·51 ± 0·14	2·02 ± 0·21	2·09 ± 0·22
Femoral vascular conductance (FVC) (ml min^{-1} $mmHg^{-1}$)	0·015 ± 0·001	0·014 ± 0·001	0·016 ± 0·001	0·017 ± 0·001
Carotid blood flow (CBF) (ml min^{-1})	2·22 ± 0·25	2·32 ± 0·20	2·20 ± 0·20	1·75 ± 0·1**
Carotid vascular conductance (CVC) (ml min^{-1} $mmHg^{-1}$)	0·02 ± 0·002	0·02 ± 0·002	0·02 ± 0·001	0·01 ± 0·001*

Values shown for N rats were obtained in our previous studies (see text). † Significant difference between value in CH and N rats; * significant difference between values recorded before and after 8-PT treatment. 1, 2 and 3 symbols represent $P < 0·05$, 0·01 and 0·001, respectively. $n = 12$ for CH rats and 8 for N rats except for the carotid blood flow and vascular conductance values in N rats when $n = 10$.

Protocol

In each rat, the inspirate was switched from 12% to 8% O_2 for 5 min before and after administration of 8-PT (10 mg kg^{-1} I.V., Sigma). In five CH rats, responses evoked by switching the inspirate from 12% O_2 to air for 5 min were also tested before and after 8-PT. A period of at least 10 min was allowed between test stimuli and at least 15 min was allowed after 8-PT treatment so that all cardiovascular and respiratory variables stabilized. Blood samples for analysis of arterial blood gases and arterial pH and of K_a^+ and K_v^+ were taken when the animal was breathing 12% O_2 and during the 5th minute of breathing 8% O_2 or air.

Statistical analyses

All results are expressed as the mean ± S.E.M., measurements being taken under control conditions and at the 1st, 2nd and 5th minute of breathing 8% O_2 or air. Within CH rats, absolute values during 12% O_2 and at the 5th minute of breathing 8% O_2 or air and before and after 8-PT were compared by Student's paired t test. In addition, absolute values and absolute changes induced by switching the inspirate in the present CH rats were compared, using Student's unpaired t test, with those recorded in our previous study on control, normoxic (N) rats (Marshall *et al.* 1993). Since CBF and CVC were not recorded in that study, comparisons for these variables in CH rats were made with those recorded in another study on N rats (Thomas & Marshall, 1994*b*). In all cases $P < 0·05$ was considered significant.

RESULTS

Baseline values

The V_T recorded in CH rats breathing 12% O_2 was comparable to that of N rats breathing air (Table 1). However, R_F and therefore $\dot{V}_E$ were greater in CH rats. Not surprisingly, P_{a,O_2} was lower in CH rats breathing 12% O_2 than in N rats breathing air and, associated with the greater ventilation in CH rats, P_{a,CO_2} was lower and arterial pH was higher than in N rats (Table 2). By contrast, there were no differences between the baseline values of the cardiovascular variables recorded in CH rats and N rats breathing air (Table 1). However, K_v^+ was greater in CH rats breathing 12% O_2 than in N rats, while K_a^+ did not differ significantly in CH and N rats (Table 2).

Responses to 8% O_2

The pattern of respiratory and cardiovascular responses evoked by 8% O_2 is shown in Fig. 1: it was quantitatively comparable to that evoked in N rats by switching the inspirate from air to 8% O_2 (Marshall *et al.* 1993; Thomas & Marshall, 1994*b*). Thus the increase in V_T tended to wane by the 5th minute of 8% O_2 (Fig. 1) as also occurred in N rats. Nevertheless, the P_{a,CO_2} recorded in CH rats in the 5th

Table 2. Arterial blood gases, arterial pH and arterial and venous potassium recorded in CH and N rats before and after 8-PT

	CH rats			N rats		
	12% O_2	8% O_2	Air	12% O_2	8% O_2	Air
Before 8-PT						
P_{a,O_2} (mmHg)	43·3 ± 0·6 †††	33·3 ± 0·7	94·4 ± 4·8	—	34·5 ± 0·8	85·6 ± 1·5
P_{a,CO_2} (mmHg)	33·2 ± 1·1 †††	27·7 ± 0·8	39·6 ± 2·6	—	32·8 ± 1·4	47·1 ± 0·7
pH_a	7·38 ± 0·01 †††	7·41 ± 0·01	7·30 ± 0·01	—	7·40 ± 0·01	7·30 ± 0·01
K^+_a (mM)	3·5 ± 0·2	3·7 ± 0·2	2·7 ± 0·2	—	4·2 ± 0·1	3·6 ± 0·1
K^+_v (mM)	3·1 ± 0·2 ††	3·1 ± 0·2	2·6 ± 0·2	—	2·6 ± 0·3	2·0 ± 0·3
After 8-PT						
P_{a,O_2} (mmHg)	47·1 ± 1·5**	33·8 ± 1·0	105·1 ± 1·9	—	37·8 ± 1·5*	90·4 ± 2·3
P_{a,CO_2} (mmHg)	29·1 ± 0·9***	23·8 ± 0·6***	33·8 ± 1·0*	—	30·0 ± 1·1	45·8 ± 1·4
pH_a	7·42 ± 0·01***	7·45 ± 0·01***	7·33 ± 0·2	—	7·42 ± 0·01*	7·30 ± 0·01
K^+_a (mM)	2·9 ± 0·2***	3·1 ± 0·2**	2·4 ± 0·1	—	4·1 ± 0·2	3·4 ± 0·2*
K^+_v (mM)	2·9 ± 0·1	2·7 ± 0·2	2·1 ± 0·1	—	2·2 ± 0·3*	2·2 ± 0·2

Values shown for N rats were obtained in previous studies. pH_a, arterial pH. †Significant difference between values recorded in CH rats breathing 12% O_2 and N rats breathing air; *significant difference between values recorded before and after 8-PT. 1, 2 and 3 symbols represent $P < 0·05$, $P < 0·01$ and $P < 0·001$, respectively. $n = 12$ for CH rats except for the values recorded during air breathing when $n = 5$. $n = 8$ for N rats. For simplicity, the P values for the effects of changing the inspirate on these variables in both CH and N rats have been included in the text where appropriate.

minute of 8% O_2 (Table 2) was lower than that recorded during 12% O_2 ($P < 0·01$). Moreover, in 8% O_2, the P_{a,CO_2} value was significantly lower than that recorded in N rats breathing 8% O_2 ($P < 0·001$, see Table 2), even though the absolute levels of $\dot{V}_E$ recorded at the 5th minute of 8% O_2 in CH and N rats were not significantly different (326 ± 11 *vs.* 356 ± 17 ml min^{-1}). The fall in ABP evoked by 8% O_2 was not significantly different from that evoked in N rats (34 ± 5·5% at 5 min in CH rats, see Fig. 1; and −47 ± 2·4% in N rats, Marshall *et al.* 1993). However, the increases in FVC and CVC (Fig. 1) were significantly smaller ($P < 0·05$) than those evoked in N rats (FVC and CVC increased by 144 ± 45 and 51 ± 18%, respectively, in N rats, Marshall *et al.* 1993; Thomas & Marshall, 1994*b*). HR and CBF tended to wane between the 1st and 5th minute of 8% O_2 (Fig. 1) as in N rats (Thomas & Marshall, 1994*b*), but the differences between values recorded at the 1st and 5th minute did not reach significance. Breathing 8% O_2 had no effect on either K^+_a or K^+_v (Table 2) even though in N rats, 8% O_2 increased K^+_a and K^+_v ($P < 0·01$ in both cases, Table 2).

Responses to air breathing

The pattern of responses evoked in CH rats by switching the inspirate from 12% O_2 to air were essentially the opposite of those evoked by 8% O_2 (cf. Figs 1 and 2). The P_{a,O_2} and P_{a,CO_2} values were higher ($P < 0·001$) and the arterial pH was lower ($P < 0·01$) at the 5th minute of air breathing than during 12% O_2. Moreover, the P_{a,CO_2} at the 5th minute of air breathing was lower than in N rats breathing air (Table 2, $P < 0·01$) even though the absolute $\dot{V}_E$ (227 ± 21 ml min^{-1}) was fully comparable to that recorded in N rats breathing air (228 ± 10 ml min^{-1}, Table 1). Air breathing increased ABP and decreased FVC and CVC by approximately 15% (Fig. 2). Air breathing had no significant effect on K^+_a or K^+_v in CH rats (Table 2).

Effects of 8-PT

In CH rats breathing 12% O_2, 8-PT increased V_T and reduced R_F (Table 1). There was no significant change in $\dot{V}_E$, but the increase in V_T led to an increase in P_{a,O_2}, a fall in P_{a,CO_2} and an increase in arterial pH (Table 2). These effects were greater than those induced by 8-PT in N rats breathing air (cf. Marshall *et al.* 1993), for in these animals there were no significant changes in P_{a,O_2}, P_{a,CO_2} or arterial pH (Table 2) and the ventilatory variables were not significantly changed (Table 1). The cardiovascular variables of CH rats breathing 12% O_2 were not changed by 8-PT. Similar findings were made in N rats breathing air, except that 8-PT decreased baseline CVC and CBF (Table 1).

The effects of 8-PT on the respiratory and cardiovascular responses evoked in CH rats breathing 8% O_2 were similar to those induced in N rats (cf. Marshall *et al.* 1993; Thomas & Marshall, 1994*b*). Thus the change in V_T induced at the 5th minute of breathing 8% O_2 was greater after 8-PT than before, both in absolute terms and as a percentage of the new baseline (Fig. 1). As a consequence, the P_{a,CO_2} fell to a lower level and arterial pH rose to a higher level during 8% O_2 after 8-PT treatment (Table 2). The fall in ABP induced

by 8% O_2 was also substantially smaller after 8-PT treatment (Fig. 1). The increases in FVC and CVC evoked by 8% O_2 tended to be smaller, although the differences between the changes evoked before and after 8-PT treatment did not quite reach significance ($P = 0{\cdot}054$ and 0·06, respectively): in N rats the increases in FVC and CVC evoked by CVC were significantly reduced, by ~65 and 30%, respectively.

The respiratory and cardiovascular responses evoked in CH rats by air breathing were not significantly altered by 8-PT (Fig. 2).

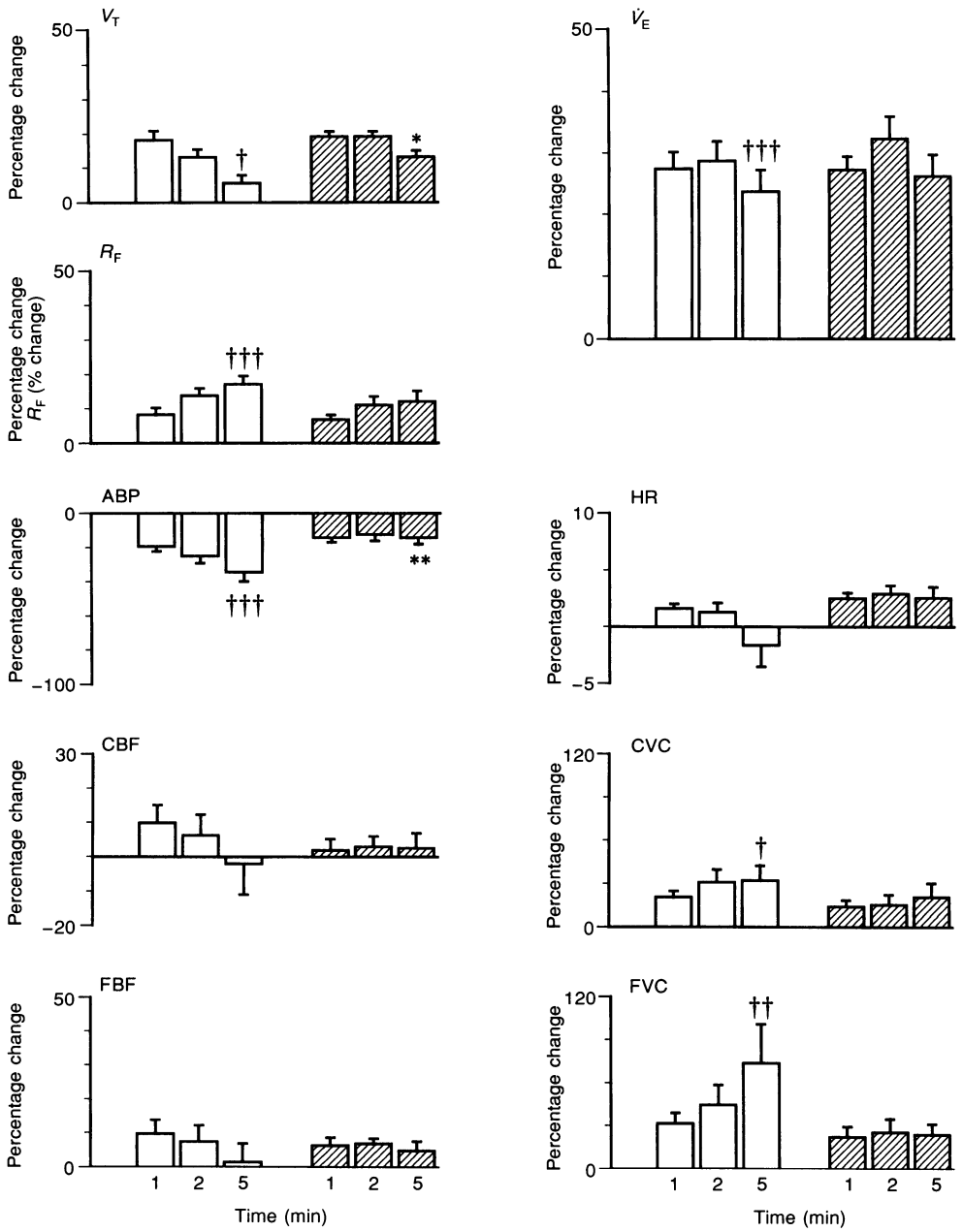

Figure 1. Percentage changes induced in respiratory and cardiovascular variables of CH rats by 5 min of breathing 8% O_2 before and after 8-PT

V_T, tidal volume; R_F, respiratory frequency; $\dot{V}_E$, minute volume; ABP, mean arterial blood pressure; HR, heart rate; FBF and FVC, femoral blood flow and vascular conductance; CBF and CVC, cerebral 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. □, before 8-PT; ▨, after 8-PT. † Significant difference between value recorded during 12% O_2 (control) and 5th minute of breathing 8% O_2. * Significant difference between change recorded before and after 8-PT at 5th minute of 8% O_2. In each case 1, 2 and 3 symbols represent $P < 0{\cdot}05$, $P < 0{\cdot}01$ and $P < 0{\cdot}001$, respectively).

When CH rats were breathing 12% O_2, 8-PT reduced K_a^+ but had no significant effect on K_v^+ (Table 2). Similarly, 8-PT reduced K_a^+ in N rats during under their baseline conditions (air breathing, Table 2). After 8-PT treatment as before, neither 8% O_2, nor air breathing, significantly affected K_a^+ or K_v^+ in CH rats (Table 2). This contrasts with N rats in which 8-PT abolished the increase in K_v^+ induced by 8% O_2 (Table 2, Marshall *et al.* 1993).

DISCUSSION

Although there have been previous studies in which the influences of chronic hypoxia upon the respiratory and cardiovascular systems were separately investigated, to our knowledge this is the first study in which they have been investigated together. We can therefore comment on the ways in which chronic hypoxia in adult life may change the known interactions between the respiratory and cardio-

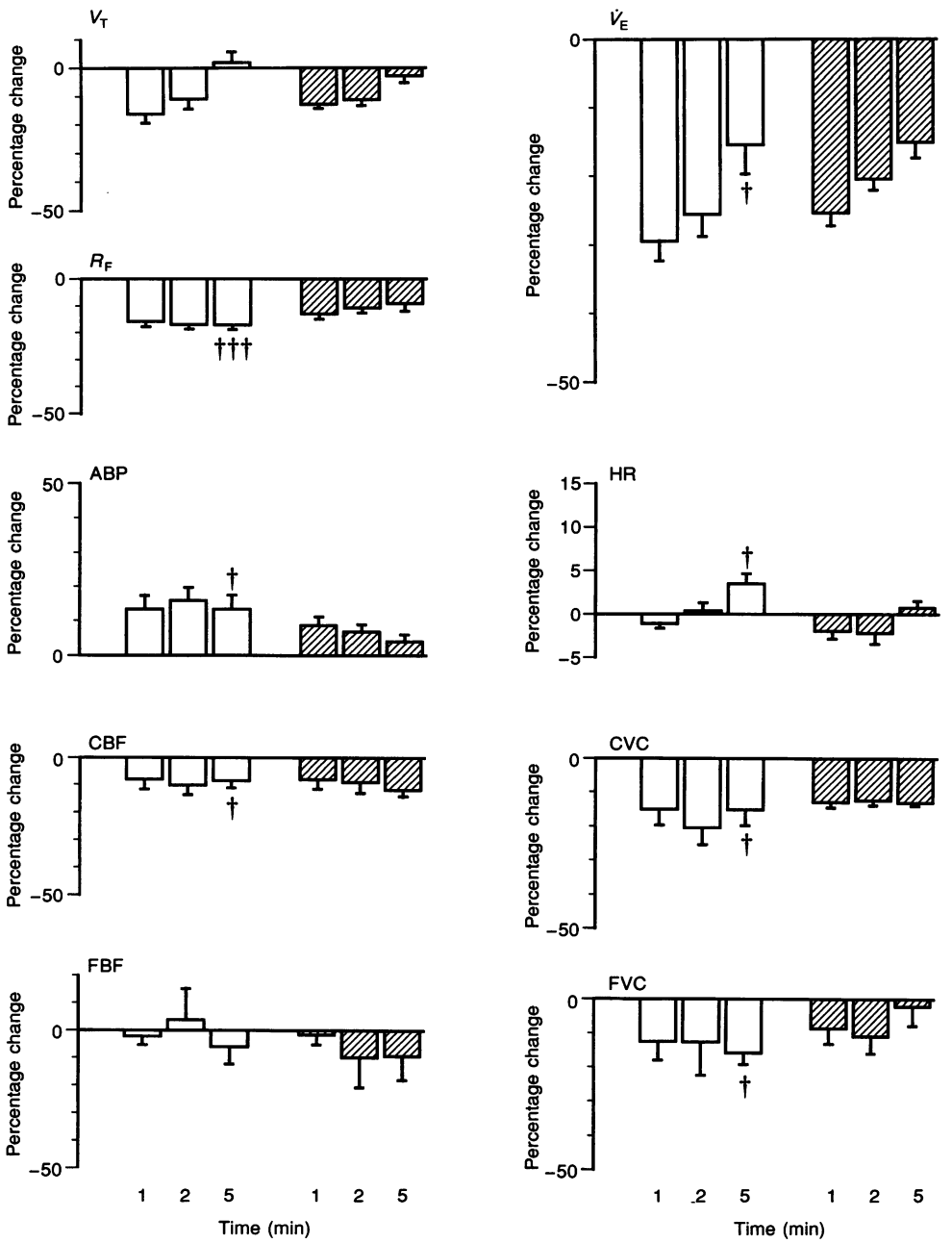

Figure 2. Percentage changes induced in respiratory and cardiovascular variables of CH rats by 5 min of breathing air

Columns and abbreviations as in Fig. 1. Symbols and levels of significance as in Fig. 1.

vascular systems. Further, our observations on the influences of the adenosine receptor antagonist 8-PT allow us to make new proposals on the influences of adenosine on the respiratory and cardiovascular systems in chronic hypoxic, adult rats. Since we have already carried out similar experiments on rats made chronically hypoxic from birth (CHB) we can directly compare the effects of chronic hypoxia imposed from birth and in adult life.

Tonic influences upon CH rats breathing 12% O_2

Respiratory influences. In the present study, the resting ventilation of CH rats when they were breathing 12% O_2 was substantially greater than in N rats breathing air, reflecting a greater R_F value in the CH rats. These results are similar to those reported by Kuwahira, Heisler, Piiper & Gonzalez (1993) and Olson & Dempsey (1978) and to our observations on CHB rats, except that in the latter, V_T was also increased (Thomas & Marshall, 1995). The fall in ventilation seen on acute switch to air breathing and which brought $\dot{V}_E$ close to values recorded in N rats breathing air is also consistent with previous results of other studies (Olson & Dempsey, 1978; Kuwahira *et al.* 1993; Thomas & Marshall, 1995) and indicates that the ventilation of CH rats was driven by the low P_{a,O_2} acting via the carotid chemoreceptors (Marshall, 1994). As the P_{a,CO_2} recorded in CH rats breathing air was lower than in N rats breathing air, this suggests chronic adaptation of respiratory sensitivity to CO_2, probably reflecting an increase in the sensitivity of the central chemoreceptors (Olson & Dempsey, 1978).

A new finding of the present study is that 8-PT increased baseline V_T and reduced R_F in CH rats breathing 12% O_2, so implying a tonic influence of adenosine upon ventilation. It is not clear exactly how adenosine was acting. It could be that adenosine contributed a tonic drive to the peripheral chemoreceptors which primarily affects R_F rather than V_T (McQueen & Ribeiro, 1981) and that the increase in V_T after 8-PT was the consequence of the respiratory cycle being lengthened by removal of the tonic influence on R_F. However, it is very likely that 8-PT also blocked a tonic action of adenosine upon central respiratory neurones which was limiting V_T (Wessberg, Hedner, Hedner, Persson & Jonasson, 1985). This tonic influence on V_T was much larger than that seen in N rats breathing air (see Table 1).

Cardiovascular influences. The cardiovascular variables (ABP, HR, FVC and CVC) of CH rats breathing 12% O_2 were not different from those recorded in N rats breathing air (Table 1). The same comment was made of ABP in our studies of muscle microcirculation in CH rats and N rats (Mian & Marshall, 1996) and of ABP and brain and muscle blood flow as recorded with radiolabelled microspheres (Kuwahira *et al.* 1993). These findings are different from those made in CHB rats (Thomas & Marshall, 1995), for they had a substantially lower ABP and higher muscle vascular conductance when breathing 12% O_2 than their controls breathing air. The HR was not only comparable in CH rats breathing 12% O_2 and N rats breathing air, but also in CHB rats and their controls (Thomas & Marshall, 1995) despite the greater $\dot{V}_E$ values in the chronically hypoxic rats. This suggests that ability of hyperventilation to induce tachycardia (see Thomas & Marshall, 1994*b*) is generally reduced by chronic hypoxia.

The increase in ABP and decrease in FVC seen on acute switch to air breathing is consistent with previous findings on muscle blood flow (Kuwahira *et al.* 1993) and with the vasoconstriction seen in individual arterioles of muscle microcirculation (Mian & Marshall, 1996). These findings raise the possibility of a tonic dilator influence of hypoxia in CH rats breathing 12% O_2. However, this idea must be treated with caution (see Mian & Marshall, 1996), for the raised haematocrit in CH rats (~56 *vs.* ~42% in N rats) means that the arterial O_2 content of CH rats breathing 12% O_2 was comparable to that of N rats breathing air, despite the lower P_{a,O_2} in CH rats (Davies, Thomas & Marshall, 1994). Thus if arterial O_2 supply is the important factor rather than P_{a,O_2}, then the peripheral tissues of CH rats may not be hypoxic. In other words, the muscle vasoconstriction observed when CH rats were switched to air breathing may reflect the effects of hyperoxia, rather than return to normoxia. Certainly, the finding that 8-PT did not affect ABP or FVC in CH rats breathing 12% O_2 and did not affect the responses evoked by air breathing confirms that adenosine was not exerting the tonic dilator influence that might have been expected if the tissues were hypoxic.

The decrease in CVC seen in CH rats in response to air breathing may similarly reflect the effect of *hyperoxia* on cerebral circulation. However, it is also possible, given that the cerebral circulation shows good pressure autoregulation that there was myogenic vasoconstriction in response to the accompanying increase in ABP. The lack of effect of 8-PT on CVC in CH rats breathing 12% O_2 is surprising, for 8-PT decreased CVC and CBF in N rats breathing air implying a tonic dilator influence of adenosine even under normoxic conditions (Thomas & Marshall, 1994*b*). Moreover, since 8-PT decreased P_{a,CO_2} in CH rats this might also have been expected to cause cerebral vasoconstriction (see Thomas & Marshall, 1994*b*). Indeed, since 8-PT also had no effect on CVC in CHB rats (Thomas & Marshall, 1995), it is now reasonable to make the general proposal that the tone of cerebral vessels of rats that have become acclimatized to breathing 12% O_2 is dominated by factors other than adenosine and P_{a,CO_2}.

Responses evoked by acute hypoxia

Respiration. Clearly, when CH rats were acutely switched from breathing 12 to 8% O_2, the fall in P_{a,O_2} was much smaller than occurred in N rats when they were switched from air to 8% O_2. Correspondingly, the initial increase in $\dot{V}_E$ during 8% O_2 was much smaller in CH rats than in N

rats (27 ± 3 *vs.* $62 \pm 5\%$, respectively; see Thomas & Marshall, 1994*b*). Nevertheless, the absolute value of $\dot{V}_E$ recorded at the 5th minute of breathing 8% O_2, when the stimulatory effect of the peripheral chemoreceptors on ventilation had reached a quasi-steady equilibrium with the central inhibitory effect of hypoxia, was similar in CH and N rats. As the P_{a,CO_2} recorded at the 5th minute of breathing 8% O_2 was lower in CH than in N rats, this is consistent with an increased central chemosensitivity to CO_2 in the CH rats as suggested above. In these respects, the CH rats were comparable to CHB rats (cf. Thomas & Marshall, 1995). Further, as the increase in V_T at the 5th minute of 8% O_2 was greater in CH rats after 8-PT than before, we can conclude that the action of adenosine within the central nervous system limits the ability to maintain an increase in V_T in acute hypoxia. This is comparable to the findings made in CHB rats and in N rats (Thomas & Marshall, 1994*b*, 1995).

ABP. The observation that the fall in ABP induced in CH rats by 8% O_2 was similar to that evoked in N rats by the change from air to 8% O_2 is particularly noteworthy given the fall in P_{a,O_2} was so much smaller in the CH rats (a fall of 10 *vs.* 51 mmHg). The secondary fall in HR seen by the 5th minute of 8% O_2 was not accentuated in CH rats relative to N rats, which contrasts with the accentuation seen in CHB rats relative to their controls (Thomas & Marshall, 1995). Furthermore, the increases in vascular conductance evoked by 8% O_2 in two large vascular beds, skeletal muscle and brain (FVC and CVC), were substantially smaller in CH than N rats. Thus it seems that the ability of hyperventilation (see above) and of the baroreceptor reflex to raise cardiac output and oppose the fall in ABP (Marshall, 1994; Thomas & Marshall, 1994*b*), was less effective in CH rats. This may be partly explained by downregulation of the influences of cardiac sympathetic activity on β-adrenoreceptors as reported in chronically hypoxic human subjects (Roach, Calbet, Olsen, Poulsen, Vissing & Saltin, 1996).

Since the fall in ABP induced by 8% O_2 in CH rats was substantially smaller after 8-PT treatment, we can deduce that it was partly attributable to the cardioinhibitory and vasodilator influences of adenosine, as in N rats (see Thomas & Marshall, 1994*b*, and below). This finding contrasts directly with the fact that 8-PT had no effect on the fall in ABP induced by 8% O_2 in CHB rats (Thomas & Marshall, 1995).

Muscle vasculature. The observations that the increase in FVC evoked by 8% O_2 was smaller in CH rats than N rats and that the effect of 8-PT on the increase in FVC did not quite reach statistical significance were surprising since the increases in the diameter of individual arterioles of muscle that were evoked by switching from 12 to 8% O_2 in CH rats were comparable in magnitude to those induced in N rats by the switch from air to 8% O_2, and in both cases the dilator responses were greatly reduced by adenosine receptor blockade (Mian & Marshall, 1996). Given our recent evidence that chronic hypoxia causes an increase in the density of the arterial tree of skeletal muscle by inducing angiogenesis (Smith & Marshall, 1997), a possible explanation for this apparent disparity is that a given change in the diameter of individual arterioles produced by acute hypoxia and adenosine does not have such a large effect on gross muscle vascular conductance in CH as in N rats.

Nevertheless, if we assume that adenosine does contribute to the muscle vasodilatation induced by 8% O_2 in CH rats, it must be acknowledged that it acted differently to the effect in N rats. Since the cannula used for sampling K_v^+ collected blood from tail, testis and skin as well as muscle, the influence of muscle upon the arteriovenous difference for K^+ may be complicated by the influences of these other tissues. Nevertheless, as K_v^+ was greater in CH rats breathing 12% O_2 than in N rats breathing air, this raises the possibility of greater tonic release of K^+ by skeletal muscle of CH rats. However, as K_v^+ was not affected by 8-PT and as 8% O_2 did not increase K_v^+ there is no reason to suggest that K^+ release was tonically stimulated by the action of adenosine on K_{ATP} channels, nor that adenosine released in acute hypoxia caused further release of K^+ via these channels. Rather, in CH rats, the dilatation induced by adenosine in acute hypoxia must be attributed to its direct actions on the blood vessels (see Introduction). On the other hand, as K_a^+ in CH rats breathing 12 or 8% O_2 was reduced by 8-PT, it is possible that K^+ was released from tissues other than skeletal muscle by an action of adenosine on K_{ATP} channels as suggested for N rats (Marshall *et al.* 1993).

These findings are similar to those obtained in CHB rats (Thomas & Marshall, 1995). Indeed, it seems that chronic hypoxia, whether imposed from birth or in adult animals, increases the importance of factors other than adenosine in producing the muscle vasodilatation of acute hypoxia. These factors may include β-adrenoreceptor stimulation by adrenaline and the stimulation of NO synthesis, both of which are upregulated in CH rats (Shaul, Muntz, DeBeltz & Buja, 1990; Bartlett & Marshall, 1996).

Cerebral vasculature. CBF showed no greater tendency to fall below baseline during 8% O_2 in CH rats than it did in N rats (Thomas & Marshall, 1994*b*): this effect was not exacerbated as it was in CHB rats (Thomas & Marshall, 1995). In fact, considering the respiratory and cardiovascular response to acute hypoxia as a whole, chronic hypoxia in adult life did not accentuate those components that we proposed can lead to a positive feedback loop when chronic hypoxia is imposed from birth, namely the secondary fall in CBF, ventilation and HR and the fall in ABP (Thomas & Marshall, 1995).

The tendency for 8-PT to reduce the increase in CVC that was evoked by 8% O_2 in CH rats is comparable to the finding made in N rats (Thomas & Marshall, 1994*b*), in CHB rats (Thomas & Marshall, 1995) and with our

observations on red cell flux in the cerebral cortex of CH rats (Coney & Marshall, 1995). It may reflect blockade of the action of locally released adenosine on cerebral vessels (Coney & Marshall, 1995) but could also represent a smaller myogenic dilatation to a smaller fall in ABP.

In summary, the present study has shown that chronic hypoxia (12% O_2) imposed in adult rats affects both the respiratory and the cardiovascular system and the influences of adenosine. Thus there was evidence that CH rats showed an increased respiratory sensitivity to CO_2 relative to N rats and that adenosine acts tonically to limit V_T when breathing 12% O_2 as well as to limit the increase in V_T induced by acute hypoxia (8% O_2). On the other hand, CH rats were adapted to chronic hypoxia such that the values of ABP, HR, FVC and CVC recorded when breathing 12% O_2 were comparable to those of N rats breathing air: there was no evidence of a tonic cardioinhibitory, or vasodilator influence of adenosine. Further, acute hypoxia induced a similar pattern of response in CH rats as in N rats, but the increases in FVC and CVC evoked by 8% O_2 were smaller in CH than in N rats. We suggest the increase in FVC was partly attributable to adenosine, but via its direct actions on blood vessels rather than via its ability to release K^+ from skeletal muscle.

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