The effects of chronic hypoxia on renal function in the rat

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1. Studies were performed on rats that had been made chronically hypoxic (CH rats) in a normoxic chamber at 12% O_2 for 3–5 weeks. Under Saffan anaesthesia, respiratory and cardiovascular variables, renal haemodynamics and renal function were recorded while the rats spontaneously breathed 12% O_2 followed by a switch to air breathing for 20 min. Plasma renin activity was assessed by radioimmunoassay of angiotensin I. Plasma atrial natiruetic peptide (ANP) was indirectly assessed by measurement of cyclic GMP in urine.

2. When breathing 12% O_2, CH rats showed hyperventilation and raised haematocrit (52%) relative to normoxic (N) rats. But arterial pressure (ABP), renal blood flow (RBF), renal vascular conductance (RVC), mean right atrial pressure (mRAtP), urine flow, glomerular filtration rate (GFR) and absolute sodium excretion ($U_{Na}V$) were comparable to those recorded in N rats breathing air. Urinary cGMP was 40% greater than in N rats, but plasma renin activity was not significantly greater in CH than in N rats.

3. Air breathing in CH rats induced hypoventilation, a 12% increase in ABP, no change in mRAtP, RBF or GFR, but increases of 75 and 100% in urine flow and $U_{Na}V$, respectively. Neither urinary cGMP nor plasma renin activity changed. Such increases in urine flow and $U_{Na}V$ were absent when renal perfusion pressure (RPP) was prevented from rising during air breathing by using an occluder on the dorsal aorta.

4. We propose that by 3–5 weeks of chronic hypoxia renal function was normalized, principally because arterial O_2 content was normalized by the increase in haematocrit and because ABP and renal haemodynamics were normalized: acute hypoxia in N rats produces a fall in ABP. We suggest that plasma ANP was raised by the actions of hypoxia or erythropoietin on the atrium, rather than by atrial distension, but suggest that ANP had little direct influence on renal function and tended to limit the influence of the renin–angotensin system. We further propose that the diuresis and natriuresis seen during air breathing were mediated by the increase in RPP; neither plasma ANP nor renin activity change in the immediate short term.

It is evident from the literature that pronounced changes in renal function can occur during adaptation to chronic systemic hypoxia and it seems that the disturbances may be maintained when the hypoxia persists. Thus, some human subjects who ascend to high altitude show diuresis and natriuresis and tolerate the systemic hypoxia well, whereas others show antidiuresis and antinatriuresis and commonly show the symptoms of acute mountain sickness (Milledge & Catley, 1984; Honig, 1989). Further, whereas some chronically hypoxic patients have apparently normal renal function, others show reduced renal plasma flow and sodium and water retention (Aber, Bayley & Bishop, 1963) and may have a reduced ability to excrete a sodium load (Farber, Kiblawi, Strawbridge, Robertson, Weinberger & Manfredi, 1977).

The factors that determine these apparently opposing influences upon renal function in chronic hypoxia have received very little attention. However, the information that is available allows the hypothesis that systemic hypoxia leads to a re-setting of the balance between the antidiuretic and antinatriuretic influences of the renin–angiotensin system and the diuretic and natriuretic influences of atrial natriuretic peptide (ANP) and that this balance may vary between individuals. Thus, increased levels of plasma renin activity have been recorded in human subjects who climbed to high altitude (Milledge, Catley, Ward, Williams & Clarke, 1983; Shigeoka, Colice & Ramirez, 1985) and in patients with chronic obstructive lung disease (Farber *et al.* 1977). This might be explained by an increase in renal sympathetic activity induced by hypoxic stimulation of the peripheral chemoreceptors and/or by a reduction in renal perfusion

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pressure (Marshall, 1994). On the other hand, raised plasma levels of ANP have also been found in human subjects at high altitude (Milledge, Beeley, McArthur & Morice, 1989) in chronically hypoxic patients (Adnot *et al.* 1989) and in rats that were kept in a hypoxic chamber for 1, 7 or 21 days (McKenzie, Tanka, Inagami, Misono & Klein, 1986; Winter, Meleagros, Pervez, Jamal, Krausz, Polak & Bloom, 1989). Increased secretion of ANP in chronic hypoxia might be explained by right atrial distension secondary to hyperventilation, blood volume expansion or pulmonary hypertension (e.g. Adnot *et al.* 1989; Zhao, Winter, Krausz & Hughes, 1991) and/or by a direct action of hypoxia on the atrium (Baertschi, Hansmaninger, Walsh, Mentzer & Pence, 1986; for review see Brenner, Ballerman, Gunning & Zeidel, 1990). ANP not only has its own natriuretic and diuretic effects, but can also oppose the effects of activation of the renin–angiotensin system by inhibiting renin secretion from the juxta-glomerular apparatus and by antagonising the ability of angiotensin II to promote aldosterone secretion (Brenner *et al.* 1990).

In order to test the hypothesis that there is a re-setting of the balance between the influences of the renin–angiotensin system and those of ANP in chronic hypoxia, we performed the present study on rats that were housed in a normobaric, hypoxic chamber at 12% O_2 for 3–5 weeks (CH rats). We recorded renal function together with a range of respiratory and cardiovascular variables, including right atrial pressure, whilst they were breathing 12% O_2. We also measured plasma renin activity and cGMP in the urine to provide an index of plasma ANP (Wong *et al.* 1980). We were able to compare these variables with those recorded in our previous studies on control normoxic rats (N rats) whilst they were breathing air or acutely breathing 12% O_2 (Neylon, Marshall & Johns, 1995, 1996). In addition, the CH rats were acutely switched to air breathing to test whether any disturbances in renal function, renin and ANP levels could be readily reversed. In our previous studies on N rats we had established that the changes in renal function induced by acute hypoxia were largely attributable to the hypoxia-induced changes in systemic arterial pressure and thereby in renal perfusion pressure (RPP). Therefore, in some CH rats of the present study we tested the extent to which the changes in renal function induced by the acute switch to air breathing could be prevented by avoiding the induced change in RPP by means of an aortic occluder. Some of these results have already been published in brief (Neylon, Johns & Marshall, 1994).

METHODS

Experiments were performed on two groups of male Wistar rats that were kept for a period of 3–5 weeks in a normobaric, hypoxic chamber at 12% O_2 that was temperature and humidity controlled (see Thomas & Marshall, 1995). Group 1 weighed 264 ± 2 g (mean ± S.E.M., $n = 9$), while Group 2 weighed 298 ± 15 g ($n = 8$). Prior to removal from the chamber and surgery the animals were fasted overnight but allowed water *ad libitum*.

After removal from the chamber, anaesthesia was induced using a halothane–O_2–N_2O mixture, followed by a bolus dose of Saffan (Pitman Moore, Uxbridge, UK; 4 mg kg^{-1} total steroids) given via the jugular vein. Anaesthesia was maintained by continuous I.V. infusion of Saffan at 12–20 mg total steroids kg^{-1} h^{-1} during surgery and at 8–14 mg kg^{-1} h^{-1} during the experimental period. A tracheotomy was then performed and an air pump was used to blow 12% O_2 in N_2 continuously across a flow head that was connected to the side-arm of the tracheal cannula (see Thomas & Marshall, 1995). The animal was prepared for measurement of respiratory, cardiovascular and renal function variables as previously described in detail (Neylon *et al.* 1995). Briefly, respiratory frequency (R_F) and tidal volume (V_T) were monitored via the flow head which was connected to an electrospirometer. Arterial pressure (ABP) was recorded from the right femoral artery and/or the right brachial artery and heart rate (HR) was derived from the ABP recording. Samples of arterial blood for measurement of blood gases and pH and haematocrit (Hct) were collected from the brachial artery: analysis was performed by using a Nova Stat 3 Profile Analyser (V. A. Howe, Waltham, MA, USA) and a microhaematocrit centrifuge, respectively.

Inulin was infused via the right femoral vein (see below). The bladder was cannulated and the left kidney was approached retroperitoneally to allow cannulation of its ureter and collection of urine. Renal blood flow (RBF) was recorded from the renal artery using an electromagnetic flow probe and meter (Carolina Medical Electronics Inc., NC, USA). A zero flow signal was obtained by transiently occluding the artery distal to the flow probe. Renal vascular conductance (RVC) was calculated on-line by dividing RBF by ABP. In all experiments care was taken to avoid damaging the renal nerves of the left kidney and a test was made to determine whether the renal nerves were functional: electrical stimulation (15 V, 0·2 ms duration, 10 Hz) of the coeliac ganglion that produced a blanching of the kidney was taken to indicate viable renal innervation. In the animals of Group 2, an occluder, consisting of a silk thread threaded through a piece of polythene tubing and attached to a screw device, was placed on the descending aorta, just rostral to the renal artery: it was used to control RPP (see below). Right atrial pressure (RAtP) was recorded via a cannula inserted through the external jugular vein so that its tip was just in the atrium: the position of the cannula tip was verified post mortem.

All variables were displayed on an eight-channel pen recorder (Lectromed, Jersey, Channel Islands) and were also collected on an Apple Macintosh IIci computer by using LabVIEW software (Neylon *et al.* 1995): ABP, RAtP, RBF and respiratory air flow signals were digitized and from these signals mean ABP and RAtP (mABP and mRAtP, respectively), HR, and mean RBF, RVC, RF and VT, were derived. The LabVIEW system sampled at 100 Hz per channel and displayed a 2 s mean; the final value used for analysis from each clearance period was a 15 min average of the 2 s mean values (Neylon *et al.* 1995).

Experimental protocols

On completion of the surgery all animals were given a 2 ml primer of inulin (15 mg ml^{-1} inulin in saline) and then infusion of the same concentration of inulin begun at 3 ml h^{-1}. After a 2 h equilibration period, urine was collected during ten 15 min clearance periods. During this time, the Group 1 animals continuously breathed 12% O_2 except for two 20 min periods of air breathing during the third and the eighth clearance periods, air breathing beginning 5 min before the start of the clearance period. The two clearance periods either side of each air breathing period provided basal and recovery

periods. The Group 2 animals were subjected to a comparable ten clearance-period protocol except that during both periods of air breathing, the aortic occluder was adjusted in order to maintain femoral ABP constant. Femoral ABP gave an indication of ABP in the distal part of the body and therefore of RPP.

Blood gas and haematocrit samples were taken immediately before and during the 20th minute of air breathing. Plasma samples were taken before and after each basal and recovery period for measurement of electrolytes and inulin. Glomerular filtration rate (GFR) was determined by inulin clearance. Urinary and plasma sodium concentrations were measured by flame photometry. In addition, in Group 1, urine and plasma samples were taken before and in the 20th minute of air breathing for measurement of urinary cGMP using a radioimmunoassay kit (cGMP-[^{125}I] assay system; Amersham) and the measurement of plasma renin activity by radioimmunoassay of angiotensin I (SB-REN-2 radioimmunoassay; CIS (UK) Ltd, High Wycombe, Bucks, UK), respectively. At the end of the acute experiments the rats were killed by an overdose of anaesthetic.

Analysis of results

All the results are expressed as means ± standard error of the mean (S.E.M.). In both Groups 1 and 2 there was no difference between the values attained during the first and second period of air breathing (ANOVA). Nor was there any difference between the values recorded during the basal and recovery periods before and after the two periods of air breathing (ANOVA). Therefore, in both groups the responses evoked by air breathing were fully reproducible and so, for analysis, the values recorded during both basal periods were meaned as were the values recorded during the two periods of air breathing.

Within Groups 1 and 2, basal values of respiratory, cardiovascular and renal variables were compared with values recorded during air breathing by using ANOVA, and comparisons between Groups 1 and 2 were made by using a two-way ANOVA. In addition, the absolute differences between basal values and values recorded during air breathing in Group 1 were compared with the absolute differences recorded in Group 2 by using a one-way ANOVA. Blood gas and pH values within and between groups were compared using

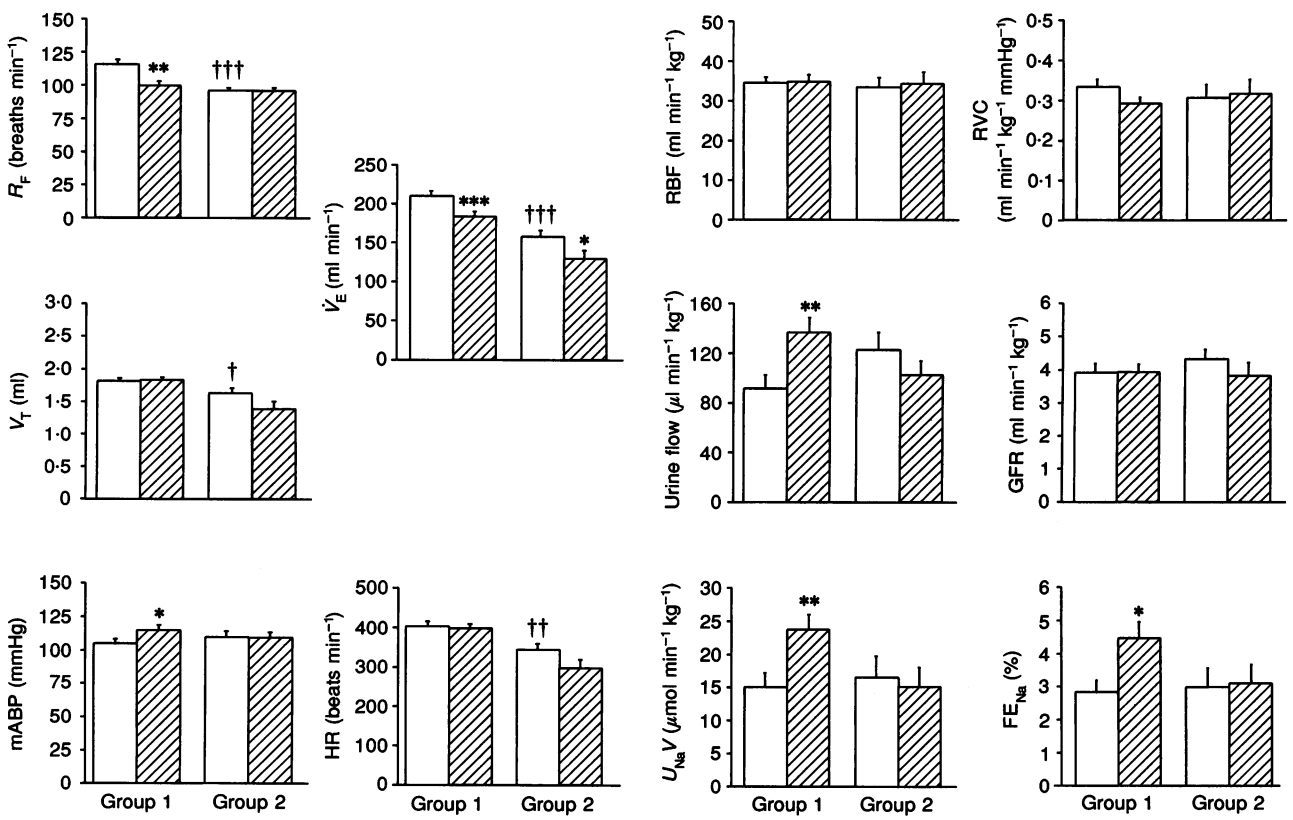

Figure 1. Effects of air breathing on chronically hypoxic animals with and without renal perfusion pressure controlled (Groups 1 and 2, respectively)

In both groups the open columns represent the basal values when the chronically hypoxic animals were breathing 12% O_2 and the hatched columns represent the mean value attained when the animals were allowed to breathe room air. The abbreviatons given represent: respiratory frequency (R_F); tidal volume (V_T); mean arterial pressure (mABP); respiratory minute volume ($\dot{V}_E$); heart rate (HR); renal blood flow (RBF); absolute sodium excretion ($U_{Na}V$); renal vascular conductance (RVC); glomerular filtration rate (GFR); and fractional sodium excretion (FE_{Na}). The number of animals in Groups 1 and 2 are 9 and 8, respectively. Asterisks represent a statistical comparison of the values during 12% O_2 and the values during air breathing: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Significant differences between the basal values attained in Groups 1 and 2 are indicated by † $P < 0.05$, †† $P < 0.01$ and ††† $P < 0.001$.

Table 1. Mean values of P_{a,O_2}, P_{a,CO_2}, arterial pH and haematocrit recorded during 12% O_2 and during air breathing for chronically hypoxic animals (Groups 1 and 2)

	12% O_2				Air breathing			
	pH	P_{a,O_2} (mmHg)	P_{a,CO_2} (mmHg)	Hct (%)	pH	P_{a,O_2} (mmHg)	P_{a,CO_2} (mmHg)	Hct (%)
Group 1	7·29 ± 0·02	50·85 ± 2·22	28·08 ± 0·97	52·2 ± 0·8	7·23 ± 0·02 ***	96·23 ± 1·34 ***	33·27 ± 1·26 ***	52·54 ± 0·81
Group 2	7·35 ± 0·01 †	51·83 ± 1·21	34·22 ± 0·01 ††	51·5 ± 1·1	7·27 ± 0·01 ***	101·11 ± 2·33 ***	42·11 ± 2·31 ***††	50·8 ± 1·1

Values are means ± S.E.M. pH, arterial pH; P_{a,O_2}, partial pressure of arterial O_2; P_{a,CO_2}, partial pressure of arterial CO_2; and Hct, haematocrit. Significant differences between the values attained during 12% O_2 and the values attained during air breathing are indicated by *** $P > 0·001$. Significant differences between the values attained in Groups 1 and 2 are indicated by † $P < 0·05$ and †† $P < 0·01$. The number of animals in Groups 1 and 2 are 9 and 8, respectively.

Table 2. Basal values of the respiratory, cardiovascular and renal function variables in Groups 1 and 2 when they were breathing 12% O_2

	Group 1	Group 2
R_F (breaths min^{-1})	117 ± 3	97 ± 4
V_T (ml)	1·8 ± 0·1	1·6 ± 0·1
$\dot{V}_E$ (ml min^{-1})	185·5 ± 13·0	156·4 ± 7·8
mABP (mmHg)	105 ± 4	111 ± 3
HR (beats min^{-1})	406 ± 11	347 ± 14
mRAtP (mmHg)	−0·85 ± 0·38	−1·0 ± 0·2
RBF (ml min^{-1} kg^{-1})	34·64 ± 1·44	33·4 ± 2·8
RVC (ml min^{-1} kg^{-1} $mmHg^{-1}$)	0·33 ± 0·02	0·31 ± 0·03
Urine flow (μl min^{-1} kg^{-1})	91·9 ± 10·8	123·7 ± 13·2
GFR (ml^{-1} min^{-1} kg^{-1})	3·9 ± 0·3	4·3 ± 0·3
$U_{Na}V$ (μmol^{-1} min^{-1} kg^{-1})	15·1 ± 2·0	16·6 ± 3·2
FE_{Na} (%)	2·8 ± 0·4	3·0 ± 0·6

The abbreviations represent: respiratory frequency (R_F); tidal volume (V_T); respiratory minute volume ($\dot{V}_E$); mean arterial pressure (mABP); heart rate (HR); mean right atrial pressure (mRAtP); renal blood flow (RBF); renal vascular conductance (RVC); glomerular filtration rate (GFR); absolute sodium excretion ($U_{Na}V$); and fractional sodium excretion (FE_{Na}). Values are expressed as means ± S.E.M. The number of animals in Groups 1 and 2, are 9 and 8, respectively.

Student's paired and unpaired t tests, respectively. In Group 1, both plasma renin activity and urinary cGMP measured under basal conditions and during air breathing were compared using ANOVA. Significance was considered when $P < 0·05$.

RESULTS

Baseline values during chronic hypoxia: Group 1 *vs.* Group 2

The baseline values of all the respiratory, cardiovascular and renal function variables in Groups 1 and 2 are shown in Tables 1 and 2 and Fig. 1. The baseline levels of R_F, V_T and therefore respiratory minute volume ($\dot{V}_E$, the product of R_F and V_T) were significantly lower in Group 2 than Group 1. In addition, the basal values of arterial P_{CO_2} (P_{a,CO_2}) and arterial pH were significantly greater in Group 2 than in Group 1, but there was no difference between Group 1 and 2 for either the arterial P_{O_2} (P_{a,O_2}) or Hct values (Table 2). The basal values of mABP, mRAtP, RBF and RVC were similar in Groups 1 and 2 as were the basal values of renal function, urine flow, absolute sodium excretion ($U_{Na}V$), fractional excretion of sodium (FE_{Na}) and glomerular filtration rate (GFR). The basal value of HR was significantly lower in Group 2 than in Group 1.

Air breathing

Group 1. When Group 1 breathed air for 20 min there was a reduction in R_F and $\dot{V}_E$ but there was no change in V_T (Fig. 1) such that P_{a,O_2} and P_{a,CO_2} increased and arterial pH fell; there was no change in the Hct (Table 1). There was also an increase in mABP, no change in HR, while RVC tended to fall, suggesting renal vasoconstriction: this meant that

Table 3. Plasma renin activity (PRA) and urinary cGMP levels attained in Group 1 animals during 12% O_2 and during air breathing

	PRA (ng ml^{-1} h^{-1})	cGMP (pmol min^{-1} kg^{-1})
12% O_2	6·17 ± 2·5 (6)	055·77 ± 3·64 (7)
Air breathing	11·14 ± 4·53 (6)	56·4 ± 4·6 (7)

The number of animals are shown in parentheses.

there was no change in RBF (Fig. 1). The mRAtP did not change significantly (−0·85 ± 0·38 and −0·94 ± 0·30 mmHg during 12% O_2 and air, respectively). Concomitantly, GFR showed no change but urine flow, $U_{Na}V$ and FE_{Na} increased substantially (Fig. 1).

Radioimmunoassays performed on plasma and urine samples taken from Group 1 revealed no significant change in either plasma renin activity or urinary cGMP levels upon exposure to air (Table 3).

Group 2. In contrast to Group 1, Group 2 showed no change in R_F during air breathing, and a tendency for a reduction in V_T (Fig. 1) such that $\dot{V}_E$ fell as in Group 1 (Fig. 1). As in Group 1 there were increases in P_{a,O_2} and P_{a,CO_2} and a fall in arterial pH, but the value of P_{a,CO_2} attained in Group 2 was significantly greater than in Group 1 (Table 1). Moreover, Hct did not change (Table 1).

The use of the aortic occluder rostral to the renal artery in Group 2 successfully prevented the increase in mABP as recorded from the femoral artery, from occurring during air breathing (cf. Groups 1 and 2, Fig. 1). This presumably meant that RPP was prevented from increasing (see above). Under these conditions, RBF and RVC remained constant during air breathing and there was no change in HR or mRAtP (−1·03 ± 0·23 and −0·856 ± 0·24 mmHg during 12% O_2 and air, respectively; Fig. 1). As in Group 1, there was no significant change in GFR, but the increases in urine flow, $U_{Na}V$ and FE_{Na} seen in Group 1 were completely eliminated (Fig. 1).

DISCUSSION

The present study has demonstrated that when healthy rats are made chronically hypoxic for 3–5 weeks by breathing 12% O_2, not only do they show respiratory and cardiovascular adaptations, as has been documented before (Dempsey & Forster, 1982; Kuwahira, Heisler, Piiper & Gonzalez, 1993), but their renal function adapts such that it is comparable to that recorded in control N rats breathing air. This is particularly remarkable given that acute hypoxia in the rat, induced by breathing 12% O_2 for 20–30 min, produces pronounced antidiuresis and antinatriuresis (Behm, Mewes, DeMuinck Keizer, Unger & Rettig, 1993; Neylon *et al.* 1995, 1996). Since the renal adaptations to chronic hypoxia occur against a background of respiratory and cardiovascular adaptations it is appropriate to consider them in relation to these respiratory and cardiovascular adaptations.

When studied under the control conditions of our experiments, i.e. anaesthetized with Saffan, breathing 12% O_2 and prepared for recording a range of variables, the CH rats of Group 2 had a lower $\dot{V}_E$ than those of Group 1. Consequently, the P_{a,CO_2} values of Group 1 were lower than in Group 2. Nevertheless, both groups of CH rats had lower P_{a,CO_2} values than those recorded in our previous studies on N rats that were similarly prepared but breathing air (Neylon *et al.* 1995, 1996). Thus, the CH rats were showing a resting hyperventilation as would be expected in animals that have adapted to chronic hypoxia (Dempsey & Forster, 1982; Bouverot, 1985). It is not clear why the magnitude of the hyperventilation was different in the two groups, for they were of the same strain and came from the same breeding station. We can only surmise that there is 'within-strain' variability in the responses evoked by hypoxia as suggested by our previous findings (Neylon *et al.* 1995, 1996).

Despite the discrepancies between the magnitudes of the resting hyperventilation in Groups 1 and 2, their P_{a,O_2} values were very similar (51 and 52 mmHg, respectively). Moreover, both groups showed the same increase in Hct (to 51 and 53%) relative to the value of 40% recorded in our previous studies on N rats (Mian & Marshall, 1996). Thus, the arterial O_2 content must have been very similar in the two groups of CH rats. Indeed, our recent studies (J. M. Marshall & W. R. Davies, unpublished observations) and those of Kuwahira *et al.* (1993) showed that the increase in Hct induced in CH rats was sufficient to raise their arterial O_2 content when breathing 12% O_2 to that of N rats breathing air. Thus, it seems very probable that the parity of the P_{a,O_2} values in Groups 1 and 2 and the normalization of their arterial O_2 content were at least partly responsible for the fact that in both groups the systemic ABP had normalized even though acute hypoxia produces a pronounced fall in mABP (see also Kuwahira *et al.* 1993; Mian & Marshall, 1996).

Previous studies on N rats have shown that the fall in mABP induced by acute hypoxia is largely due to vasodilatation occurring in skeletal muscle in response to the local effects of tissue hypoxia (see Neylon & Marshall, 1990). Our recent studies have shown that muscle blood flow is similar in CH rats breathing 12% O_2 and N rats breathing air (Davies, Thomas & Marshall, 1994). Thus, it seems that the adaptive increase in Hct and in arterial O_2 content help to ensure that by 3–5 weeks of chronic hypoxia, skeletal muscle of CH rats is no longer hypoxic and is therefore no longer under a significant tonic influence of vasodilator metabolites. Seen in this context, the fact that the levels of RBF and RVC recorded in CH rats were comparable to

those recorded in N rats by us and by others (Kuwahira *et al.* 1993; Neylon *et al.* 1995, 1996) is not surprising. Our previous studies indicated that the increase in RVC induced by acute hypoxia was largely a myogenic response to the hypoxia-induced fall in ABP and to a smaller extent reflected a dilator influence of tissue hypoxia (Neylon *et al.* 1995). It now seems reasonable to propose that during adaptation to chronic hypoxia, the normalization of ABP, the rise in Hct and arterial O_2 content, largely removed the hypoxic stimulus to the kidney and allowed renal haemodynamics to return to normal.

Since renal haemodynamics had returned to normal, it might be argued that renal function would have been expected to return to normal. This was indeed the case; the values for GFR, urine flow, $U_{Na}V$ and FE_{Na} in CH rats breathing 12% O_2 were within the ranges we have previously recorded in N rats breathing air (Neylon *et al.* 1995, 1996). The fact that there was no sign of the antidiuresis and antinatriuresis recorded in N rats breathing 12% O_2 might be explained given the evidence that these changes were largely due to the hypoxia-induced fall in RPP (Neylon *et al.* 1995).

However, the situation is not this straightforward, for the influences of hormones on the kidney must be considered. The level of cGMP recorded in the urine of CH rats breathing 12% O_2 was ~40% higher than we recorded in N rats breathing air ($40{\cdot}8 \pm 2{\cdot}5$ pmol min^{-1} (kg body weight)$^{-1}$; $P < 0{\cdot}01$ ANOVA; M. Neylon, J. M. Marshall & E. J. Johns, unpublished observations). If we assume that urinary cGMP directly reflects plasma ANP (Wong *et al.* 1980) then this indicates that plasma ANP was raised in CH rats. We have to accept that an increase in urinary cGMP may have reflected, at least in part, an increase in the actions of nitric oxide (NO) within the kidney given that cGMP is the second messenger for NO. However, our proposal is consistent with previous evidence that plasma ANP was raised by 60% in rats exposed for 7 days to a more severe level of hypoxia than we used (10% O_2; Winter *et al.* 1989).

Winter *et al.* (1989) demonstrated that this increase in plasma ANP was due to release from the right atrium rather than the right ventricle, even though right ventricular ANP content was raised concordant with right ventricular hypertrophy and pulmonary hypertension. Now, the level of RAtP recorded in the present CH rats breathing 12% O_2 was fully comparable to that recorded in N rats breathing air ($-1{\cdot}5 \pm 0{\cdot}6$ mmHg; M. Neylon, J. M. Marshall & E. J. Johns, unpublished observations), which is consistent with the finding that plasma ANP was raised in chronically hypoxic patients even though they had normal RAtP (Morice, Pepke-Zaba, Brown, Thomas & Higgenbottam, 1990). Indeed, if we assume the increase in ANP in plasma arose from the atrium, it may have been caused, not by atrial distension, but by a direct action of hypoxia upon the atrium (Baertschi *et al.* 1986). Although the arterial O_2 content had normalized in CH rats despite the reduced P_{a,O_2} (see above), the partial pressure of O_2 in the mixed venous blood must have been much lower in CH rats, assuming the body O_2 consumption of CH and N rats was not reduced in CH rats (see Davies *et al.* 1994). Therefore, the unloading of O_2 to atrial tissue from the endocardial surface may have been impaired. Another possibility is that ANP formation and release was stimulated in CH rats by erythropoietin (Porat *et al.* 1996), for plasma erythropoietin levels have been reported to increase in rats within a day of chronic hypoxia and to reach a peak at 3–5 weeks (see Ou *et al.* 1992, for discussion).

On the other hand, although plasma renin activity of CH rats breathing 12% O_2 tended to be higher than in N rats breathing air ($3{\cdot}02 \pm 0{\cdot}68$ ng ml^{-1} h^{-1}; see Neylon *et al.* 1996), the difference did not reach statistical significance. Further, the renin activity of CH rats was certainly not as high as in N rats that were acutely breathing 12% O_2 ($8{\cdot}36 \pm 1{\cdot}8$ ng ml^{-1} h^{-1}; Neylon *et al.* 1996). Since the levels of ABP were comparable in CH rats breathing 12% O_2 and in N rats breathing air, the present results are consistent with our evidence that the increase in renin activity evoked by acute hypoxia is predominantly due to the hypoxia-induced fall in RPP, rather than to increased renal sympathetic activity resulting from peripheral chemoreceptor stimulation. Moreover, any maintained effect of renal sympathetic activity on renin secretion in the CH rats may well have been inhibited by the action of ANP on the juxtaglomerular apparatus (Brenner *et al.* 1990).

Our previous studies on N rats indicated that even the 2·5-fold increase in renin activity recorded in acute hypoxia was not sufficient to produce significant antidiuresis and antinatriuresis by direct actions of angiotensin II on the renal tubules (Neylon *et al.* 1996). Further, the longer-term effects of angiotensin II on aldosterone release from the adrenal cortex that might have been expected in CH rats, given the tendency for renin levels to be raised, would in any case have been reduced by the raised plasma levels of ANP (Brenner *et al.* 1990). On the other hand, the experiments of Zhao *et al.* (1991) showed that infusion of ANP into N rats at rates chosen to produce plasma levels comparable to or higher than those measured during chronic hypoxia (increases of 25–100%) were not sufficient to produce natriuretic and diuretic effects. Overall therefore, it seems reasonable to conclude that in CH rats that have been breathing 12% O_2 for at least 3 weeks, any effects of increased renin and ANP secretion upon renal function not only counterbalance one another, but are small.

Acute return to air breathing

The fall in $\dot{V}_E$ and increase in ABP recorded in Group 1 CH rats when they acutely breathed air is consistent with the results of previous studies (Kuwahira *et al.* 1993; Mian & Marshall, 1996). The fall in $\dot{V}_E$ can be ascribed to removal of the hypoxic drive to peripheral chemoreceptors (Dempsey & Forster, 1982). It might be argued that the rise in ABP was due to removal of a tonic vasodilator influence of tissue hypoxia. However, our previous evidence indicates this is

unlikely (Mian & Marshall, 1996). Indeed, the increase in Hct means that when P_{a,O_2} is normalized by air breathing, the arterial O_2 content becomes higher than normal. Thus, the increase in ABP can be ascribed to the influence in skeletal muscle and elsewhere of a vasoconstrictor substance(s) released as a consequence of tissue *hyperoxia*: (Mian & Marshall, 1996).

Although the decrease in RVC that occurred during air breathing in Group 1 did not reach statistical significance, it was sufficient to keep RBF and GFR constant. Nevertheless, there was a substantial diuresis and natriuresis. This cannot be attributed to the hormones we monitored, for urinary cGMP and therefore, presumably, plasma ANP, did not change during air breathing while any change in plasma renin activity was in the upward direction. Rather, it is probable that air breathing caused a pressure diuresis and natriuresis. This suggestion is fully compatible with the finding that when the air breathing-induced increase in RPP was prevented in Group 2, there was no change in urine flow or sodium excretion. An increase in RPP can produce a decrease in sodium reabsorption in the proximal tubule in the absence of a change in GFR by increasing interstitial hydrostatic pressure throughout the kidney (Cowley, Roman & Krieger, 1991; Granger, 1992).

If our assumption, that plasma ANP did not change on acute return to air breathing, is correct then this is compatible with the finding that correction of hypoxia with supplemental O_2 in patients with pulmonary hypertension did not change plasma ANP in those in whom plasma ANP was raised. On the other hand, Winter *et al.* (1989) found that when CH rats were returned to air breathing for 24 h, plasma ANP returned to normal and the content of ANP in the right atrium and the number of ANP granules per cell fell to that seen in N rats. It, therefore, seems that the increase in ANP synthesis cannot be re-set in the immediate short term after removal of the chronic hypoxic stimulus, but can be re-set within 24 h. Our own results show that RAtP did not change on return to air breathing, while mixed venous P_{O_2} and O_2 content must have increased concomitant with the increase in arterial P_{O_2} and O_2 content. Thus, any direct hypoxic stimulus to ANP secretion was apparently removed on return to air breathing and was not replaced by an atrial-distending stimulus. It may be that the re-setting process requires up to 24 h after removal of hypoxia, or that ANP secretion is driven by raised erythropoietin (see above) and that its levels take up to 24 h to return to normal. The last possibility cannot be assessed given the uncertainty over the time course of plasma erythropoietin levels during chronic hypoxia and the factors that determine them (Ou *et al.* 1992).

In summary, the present study on the rat demonstrates that within 3–5 weeks of chronic hypoxia, renal function is normalized despite evidence of raised plasma ANP. It seems that the normalization of renal function can be explained by: (i) normalization of the arterial O_2 content due to the raised Hct; and (ii) normalization of ABP and renal haemodynamics. We propose that the increase in plasma ANP was triggered by hypoxia or by the action of erythropoietin, rather than by atrial distension. However, plasma ANP was apparently below the level at which it could exert significant natriuretic and diuretic effects on the kidney, even though it may have inhibited the anti-diuretic and antinatriuretic effects expected from hypoxic stimulation of the renin–angiotensin system. To this extent it may be argued that in rats that were acclimatized to chronic hypoxia the influences of ANP and the renin–angiotensin system were re-set. Further experiments will be required to determine how the balance between the influences of ANP and the renin–angiotensin system is changed earlier during the process of acclimatization, before the arterial O_2 content and haemodynamics have normalized. The present study also showed that an acute return to air breathing for just 20 min produced a diuresis and natriuresis that were attributable to the evoked increased in ABP and renal perfusion pressure. Since there were no accompanying changes in ANP or renin secretion we propose that the hypoxia-induced changes in the balance of these hormones cannot be reversed in the immediate short term.

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