The role of the renin–angiotensin system in the renal response to moderate hypoxia in the rat

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1. In two groups of Saffan-anaesthetized rats, we studied the role of the renin–angiotensin system in mediating the antidiuresis and antinatriuresis induced by moderate systemic hypoxia.

2. In both groups, a first period of hypoxia (breathing 12% O_2 for 20 min) induced a fall in arterial partial pressure of O_2 (P_{a,O_2}; to 42 mmHg), a fall in mean arterial pressure (MABP), no change in renal blood flow (RBF) due to an increase in renal vascular conductance (RVC = RBF/MABP) and falls in urine flow and absolute sodium excretion ($U_{Na}V$). Concomitantly, plasma renin activity increased from $3{\cdot}08 \pm 0{\cdot}68$ (mean ± S.E.M.) to $8{\cdot}36 \pm 1{\cdot}8$ ng ml^{-1} hr^{-1}.

3. In group 1 ($n = 11$), Losartan (10 mg kg^{-1}, I.V.), the angiotensin (AII) AT_1 receptor antagonist, induced a fall in MABP (115 ± 3 to 90 ± 3 mmHg), an increase in RVC such that RBF was unchanged, and falls in glomerular filtration rate (GFR), urine flow and $U_{Na}V$. However, hypoxia induced qualitatively similar changes to those seen before Losartan treatment.

4. In group 2 ($n = 9$), we occluded the aorta distal to the renal artery to prevent basal MABP and renal perfusion pressure (RPP) from falling after addition of Losartan and to keep the hypoxia-induced fall in MABP the same as before Losartan treatment. Nevertheless, Losartan induced an increase in basal RVC, RBF, urine flow and $U_{Na}V$ whilst hypoxia induced falls in urine flow and $U_{Na}V$ that were proportionately similar to those seen prior to addition of Losartan.

5. These results indicate that in the Saffan-anaesthetized rat, AII exerts tonic, renal vasoconstrictor and consequent antidiuretic and antinatriuretic influences in normoxia, but does not contribute to the hypoxia-induced antidiuresis and antinatriuresis. We propose that renin secretion is increased by the hypoxia-induced fall in RPP rather than by an increase in renal sympathetic activity. Thus, the AII generated cannot produce antidiuresis and antinatriuresis by its known facilitatory influence on the actions of an increase in sympathetic activity on the renal tubules and is insufficient to produce these effects by direct actions. Rather, these results support the view that the antidiuresis and antinatriuresis of moderate hypoxia is predominantly due to the fall in RPP.

Some individuals who climb to high altitude show diuresis and natriuresis and tolerate the hypoxia well. Others show antidiuresis and antinatriuresis and commonly succumb to mountain sickness (Milledge & Catley, 1984; Honig, 1989). The antidiuresis and antinatriuresis has been attributed to the actions of angiotensin II (AII) upon the kidney, the AII being generated by hypoxia-induced stimulation of the renin–angiotensin system (Milledge & Catley, 1984, 1987). Although an increase in renin secretion might be explained as a reflex response to an increase in renal sympathetic activity caused by hypoxic stimulation of peripheral chemoreceptors (Marshall, 1994), this conclusion cannot be firmly drawn from high altitude studies, because those who climb to high altitude not only experience systemic hypoxia, but also undertake moderate to severe exercise and may experience cold. Experiments performed under controlled laboratory conditions have not resolved the issue. Thus, human subjects who were exposed to the equivalent of 10% O_2 in a hypobaric chamber showed a 2·5-fold increase in plasma renin activity (Tuffley, Rubinstein, Slater & Williams, 1970), while conscious dogs who breathed 7% O_2 for 20 min showed a 50-fold increase in plasma renin

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activity and a 40-fold increase in plasma AII concentration (Rose, Kimmel, Godine, Kaiser & Carey, 1983). However, in conscious rats, breathing 10% O_2 for 20 min caused no significant change in plasma renin activity, although plasma aldosterone was raised (Raff & Roarty, 1988).

Not only is the evidence of whether the renin–angiotensin system is activated in hypoxia equivocal, but very few attempts have been made to directly test whether the renin–angiotensin system is important in determining renal haemodynamics or function in systemic hypoxia, the results reported so far being far from comprehensive. Thus, Liang & Gavras (1978) found that in conscious dogs, teprotide, an angiotensin-converting enzyme (ACE) inhibitor, or saralasin, an AII receptor antagonist, reduced the increase in cardiac output, mean arterial pressure (MABP) and renal blood flow (RBF) that were induced by severe hypoxia (5% O_2 for 20 min), but not the smaller increases induced by 8% O_2. Further, in the Saffan-anaesthetized cat, another ACE inhibitor, captopril, allowed RBF to increase during systemic hypoxia induced by breathing 6% O_2 for 20 min, whereas before captopril it remained constant (Marshall & Metcalfe, 1990). These results suggest that activation of the renin–angiotensin system exerts a significant renal vasoconstrictor influence in severe hypoxia. In studies of renal function, Rose *et al.* (1983) recorded the changes in renal haemodynamics and glomerular filtration rate (GFR) induced in the conscious dog by severe hypoxia (7% O_2 for 20 min) and by hypercapnic hypoxia (8·5% CO_2 + 7% O_2) but they only tested the role of the renin–angiotensin system in the latter. The fall in GFR induced by hypercapnic hypoxia was enhanced by saralasin and Rose *et al.* (1983) concluded that AII helps to maintain GFR during this severe, but mixed, stimulus by exerting a preferential vasoconstrictor action on the efferent arterioles (see Johns, 1989). Apart from providing an incomplete picture, the results of these studies are open to question, because ACE inhibitors can lead to an increased generation of bradykinin which is vasodilatory and natriuretic (Wood, Mah & Schnell, 1990), while saralasin has agonist, as well as antagonist properties (Timmermans, Wong, Chiu & Herblin, 1991).

In our recent studies in the rat, we showed that moderate systemic hypoxia (breathing 12% O_2 for 20 min) induced a fall in MABP, antidiuresis and antinatriuresis. The antidiuresis and antinatriuresis still occurred when the kidney was denervated, but they were abolished when systemic arterial pressure and renal perfusion pressure (RPP) were prevented from falling during hypoxia by occluding the aorta distal to the renal artery (Neylon, Marshall & Johns, 1995). These findings clearly show that the antidiuresis and antinatriuresis can occur independently of the renal nerves, but still allow the possibility that an increase in renal nerve activity contributes to these responses when the nerves are intact. Whilst mammals larger than the rat generally show a rise in systemic arterial pressure rather than a fall, a fall does sometimes occur. Indeed, some human subjects who were exposed to hypoxia for 20 min showed a fall in arterial pressure and these were the very ones who showed antidiuresis, rather than diuresis (Heyes *et al.* 1982). Now, a fall in arterial pressure can stimulate renin release both by unloading the baroreceptors and causing a reflex increase in renal sympathetic activity and by the direct effect of a fall in RPP on the pressure-sensitive mechanisms within the kidney (Johns, 1989). Thus, we hypothesized that in the rat at least, the renin–angiotensin system is activated in acute, moderate hypoxia by peripheral chemoreceptor stimulation, baroreceptor unloading and a fall in RPP, and that the AII generated contributes to the antidiuresis and antinatriuresis. To test this hypothesis, the present study was performed in which plasma renin activity was measured in rats during air breathing and during a 20 min period of breathing 12% O_2, while renal function in normoxia and hypoxia was measured before and after administration of Losartan, which is a selective antagonist of the AT_1 receptor. The AT_1 receptor is the predominant AII receptor subtype in the vasculature and kidney of the rat (de Gasparo, Whitebread, Mele, Whitcombe, Ramjoué & Kamber, 1990) and the renal haemodynamic and tubular effects of AII are mediated primarily by AT_1 receptors (Burns, Homma & Harris, 1993).

A brief report of this work has already been made (Neylon, Johns & Marshall, 1994).

METHODS

Experiments were performed on two groups of Wistar rats: Group 1 ($n = 11$), body weight 307 ± 7 g (means ± S.E.M.) and Group 2 ($n = 9$), body weight 316 ± 6 g. In Group 2, RPP was controlled after administration of Losartan (see below). Both groups were fasted overnight prior to the experiments, but were allowed water *ad libitum*. Fasting the animals allowed us to standardize their condition and provided easier surgical access to the kidney (see below) as the digestive system was relatively empty. Their fasted state probably contributed to the background of acidosis (Table 1) against which any changes in arterial pH (pH_a) induced by hypoxia were seen.

The animals were prepared for recording respiratory, cardiovascular and renal variables as described recently (Neylon *et al.* 1995). Briefly, anaesthesia was induced with a halothane/O_2/N_2O mixture and maintained by continuous infusion of Saffan (Pitman-Moore, Uxbridge, UK) (12–20 mg kg^{-1} during surgery and 8–14 mg kg^{-1} h^{-1} during the experimental period). Respiratory frequency and tidal volume were recorded by an electrospirometer via a flowhead which was connected to the trachea cannula. The animal breathed air or a hypoxic mixture (12% O_2 in N_2) which was continuously pumped across the flow head. The femoral vein and artery were cannulated for injection of drugs or infusion of inulin in saline and for measurement of MABP, respectively. The brachial artery was cannulated for measurement of MABP and for collection of blood samples which were analysed for blood gases and pH by a Nova Stat 3 Profile analyser (V. A. Howe, Waltham, MA, USA). Renal blood flow was recorded from the left renal artery using an electromagnetic flow probe and meter. Renal

Table 1. Mean values of P_{a,O_2} (mmHg), P_{a,CO_2} (mmHg) and pH_a recorded during air breathing and during 12% O_2, before and after Losartan, for Groups 1 and 2

	Before Losartan					
	Air breathing			Hypoxia 1		
	pH	P_{a,O_2}	P_{a,CO_2}	pH	P_{a,O_2}	P_{a,CO_2}
Group 1	7·22 ± 0·01 (8)	85·59 ± 1·42 (8)	40·25 ± 1·13 (8)	7·24 ± 0·01	39·97 ± 1·46***	32·80 ± 0·98***
Group 2	7·29 ± 0·01	87·76 ± 2·02	37·64 ± 0·78	7·33 ± 0·01** (6)	45·52 ± 1·32*** (6)	30·95 ± 1·13*** (6)
	After Losartan (10 mg kg^{-1})					
	Air breathing			Hypoxia 2		
	pH	P_{a,O_2}	P_{a,CO_2}	pH	P_{a,O_2}	P_{a,CO_2}
Group 1	7·26 ± 0·01 †††	88·70 ± 1·63 ‡‡	37·27 ± 1·00 †	7·27 ± 0·01 †	43·67 ± 1·02***	29·34 ± 0·54***†
Group 2	7·34 ± 0·02 ‡‡	90·04 ± 1·98	34·84 ± 1·17 †	7·37 ± 0·02*†	47·67 ± 0·85***	29·67 ± 1·38***

Values are means ± S.E.M. * Significant difference between the value attained during hypoxia and that during air breathing. † Significant difference between the value recorded before and after Losartan. ‡ Significant difference between the hypoxic response before and after Losartan, respectively. In each case: 3 symbols, $P < 0·001$; 2 symbols, $P < 0·01$; and 1 symbol, $P < 0·05$. The number of observations in both Group 1 and Group 2 is 7 unless otherwise stated in parentheses.

vascular conductance (RVC) was calculated as RBF divided by MABP. After placement of the renal probe, a test was made of whether the renal nerves were functional: blanching of the kidney upon electrical stimulation of the coeliac ganglion (15 V, 0·2 ms duration) was taken as an indication of functional innervation. The bladder was cannulated, and allowed to drain. The left ureter was cannulated in order that urine could be collected for analysis. In addition, in Group 2, a length of thread was looped around the aorta distal to the renal artery. This was attached to a screw device and used to occlude the aorta (see below).

All variables were displayed on an eight channel pen recorder. Data acquisition was also performed as previously described (see Neylon *et al.* 1995) using labView software and an Apple Macintosh computer. Both femoral and brachial artery pressures, RBF and air flow were digitized and from these signals, MABP, heart rate, RBF, RVC, respiratory frequency and tidal volume were finally derived as a 15 min mean of 2 s means (see Neylon *et al.* 1995). Respiratory minute volume ($\dot{V}_E$) was calculated as the product of respiratory frequency and tidal volume.

Experimental protocol

In both Groups 1 and 2, when the surgery had been completed a 2 ml inulin primer was given and inulin infusion (1·5% solution in saline at 3 ml h^{-1}) was begun. After a 2 h equilibration period, urine was collected during ten 15 min clearance periods (C1–C10). C1, C2, C4 and C5 were during air breathing while C3 was during hypoxia (breathing 12% O_2). The animals were allowed to breathe 12% O_2 for 5 min before C3 began so that initial transient changes in respiratory and cardiovascular variables were avoided. After C5, the AII receptor antagonist Losartan was given (10 mg kg^{-1}, I.V.): Losartan was generously provided by Dr R. D. Smith, Du Pont Merck Pharmaceutical Co., Wilmington, DE, USA. Then, after a 20 min period to allow recorded variables to stabilize, C6–C10 began. These were an exact replica of C1–C5, C8 being the hypoxic period, except that in Group 2, brachial artery pressure and thereby RPP was controlled by means of the occluder on the aorta (see above). Thus, brachial artery pressure during C6 and C7, i.e. after Losartan, was maintained close to the values pertaining in the basal and recovery periods C1, C2, C4 and C5 (see above); during C8 (hypoxia) further control was exerted so that brachial artery pressure fell to the same absolute level as during C3 (hypoxia) before Losartan.

Arterial samples for analysis of blood gases were taken during air breathing in C2 and C7 and in the 15th minute of the hypoxic clearance periods (C3 and C7). Plasma samples were taken during each clearance period to determine glomerular filtration from inulin clearance (see Johns, Lewis & Singer, 1976). Plasma and urinary electrolyte concentrations were determined by flame photometry.

In order to test the efficacy of the AII receptor blockade achieved by Losartan, AII was given (100 ng, I.V.; Sigma) before, 20 min after Losartan and at the end of the experiment.

Determination of plasma renin activity

Blood samples were collected from the femoral artery into cooled EDTA-coated (6% solution) syringes immediately before and during the first period of hypoxia in the fifteenth minute of C2 and C3, respectively, from a total of eighteen rats (body weight, 304 ± 6 g) of Groups 1 and 2. Plasma from these samples (~500 μl) was immediately frozen and stored at < 4 °C for later analysis. Plasma renin activity was measured by radioimmunoassay of AII using an SB-REN-2 kit (CIS Ltd).

Analysis of results

Results are expressed in absolute terms as means ± S.E.M. The mean values during C1 and C2 and during C4 and C5 were averaged to give basal and recovery periods for the first hypoxia period, respectively, while the mean values during C6 and C7 and C9 and C10 were averaged to give basal and recovery periods for the second hypoxic periods (see Figs 1 and 2). Statistical analyses

Table 2. Basal values of respiratory, cardiovascular and renal function variables during air breathing in Groups 1 and 2

	Group 1	Group 2
R_F (beats min^{-1})	97 ± 2	106 ± 6
V_T (ml)	2·3 ± 0·1	2·8 ± 0·1
$\dot{V}_E$ (ml min^{-1})	224·2 ± 10·6	299·2 ± 22·2
MABP (mmHg)	111 ± 3	112 ± 2
HR (beats min^{-1})	429 ± 12	417 ± 10
RBF (ml min^{-1} kg^{-1})	35·2 ± 1·8	35·7 ± 2·5 (8)
RVC (ml min^{-1} kg^{-1} mmHg^{-1})	0·32 ± 0·01	0·31 ± 0·02 (8)
Urine flow (μl min^{-1} kg^{-1})	91·64 ± 8·56	73·27 ± 8·23
GFR (ml min^{-1} kg^{-1})	6·8 ± 0·4	4·9 ± 0·3 (8)
$U_{Na}V$ (μmol min^{-1} kg^{-1})	20·96 ± 2·43	11·17 ± 1·90
FE_{Na} (%)	2·23 ± 0·23	1·75 ± 0·40 (8)

For each group the basal value represents the mean (± S.E.M.) of the two clearance periods before administration of Losartan (see text). R_F, respiratory frequency; V_T, tidal volume; $\dot{V}_E$, respiratory minute volume; MABP, mean arterial pressure; HR, heart rate; RBF, renal blood flow; RVC, renal vascular conductance; GFR, glomerular filtration rate; $U_{Na}V$, absolute sodium excretion; FE_{Na}, fractional sodium excretion. In Group 1, $n = 11$ and in Group 2, $n = 9$ (unless otherwise stated in parentheses).

of the respiratory, cardiovascular and renal responses to hypoxic were performed on log values because the data did not follow a normal distribution (Dixon & Massey, 1969). In both groups, the hypoxia-induced changes before and after Losartan were assessed by comparing the mean of values recorded during C1 and C2 or C6 and C7 (basal) with that recorded during C3 or C8 (hypoxia), respectively, using Student's paired t test. The hypoxia-induced changes before and after Losartan were also compared using the paired t test on the difference between the log of the basal and hypoxic values for the first and second period of hypoxia, i.e. log (hypoxic value/basal value). In other words, the changes that occurred before and after Losartan were compared as a proportion of their own basal values. In addition, assessment was made of the effect of Losartan on control values by using the paired t test. In Group 1, this was done by comparing the mean of the values recorded during C1, C2, C4 and C5 with those recorded during C6, C7, C9 and C10. By contrast, in group 2, the mean of the values recorded during C1, C2, C4 and C5 was compared with the mean of the values recorded during C6 and C7 only (see Results). Blood gas values and plasma renin activity before and during hypoxia were compared using Student's paired t test on the absolute values.

RESULTS

The absolute values for the arterial partial pressures of oxygen and carbon dioxide (P_{a,O_2} and P_{a,CO_2}, respectively) and pH_a in Groups 1 and 2 are shown in Table 1, while the basal values for respiratory, cardiovascular and renal function variables for both groups are shown in Table 2.

Group 1

During the first period of hypoxia, there was an increase in respiration and concomitant falls in both the partial pressures of oxygen and carbon dioxide, but no significant increase in pH_a (Table 1 and Fig. 1). This was accompanied by a fall in MABP, but no significant change in heart rate (HR). There was an increase in RVC which allowed RBF to remain constant. Meanwhile, there were falls in urine flow, absolute and fractional sodium excretion ($U_{Na}V$ and FE_{Na}, respectively), but no significant change in GFR.

After Losartan, the control level of respiratory frequency (R_F) was increased, but there was no change in tidal volume (V_T) or minute volume ($\dot{V}_E$) (Fig. 1). P_{a,O_2} and pH_a during air breathing were also increased while P_{a,CO_2} was decreased (Table 1). There was also a significant fall in the control level of MABP after Losartan treatment (Fig. 1). This was associated with a rise in RVC, no change in RBF, but falls in urine flow, GFR and $U_{Na}V$ (Fig. 1).

The hypoxia-induced increase in R_F was smaller after Losartan when considered as a proportion of the new basal value (see Methods) but the increase in $\dot{V}_E$ was similar to that which occurred before addition of Losartan (Fig. 1). In accord with the effects on basal values (see above), the pH_a and P_{a,CO_2} values attained during hypoxia were higher and lower, respectively, than before Losartan, but the changes in P_{a,O_2}, P_{a,CO_2} and pH induced by hypoxia after Losartan were fully comparable with those induced before (Table 1). The systemic cardiovascular, renal haemodynamic changes and the changes in renal function induced by hypoxia after Losartan were qualitatively similar to those induced before Losartan. However, statistical analyses performed on the log (basal/hypoxic values) as described above showed that the fall in MABP and increase in RVC were smaller after Losartan as a proportion of the new basal values. On the other hand, the decreases in urine flow, $U_{Na}V$ and FE_{Na} were proportionally greater after Losartan; this result reflecting the fact that basal values were lower after

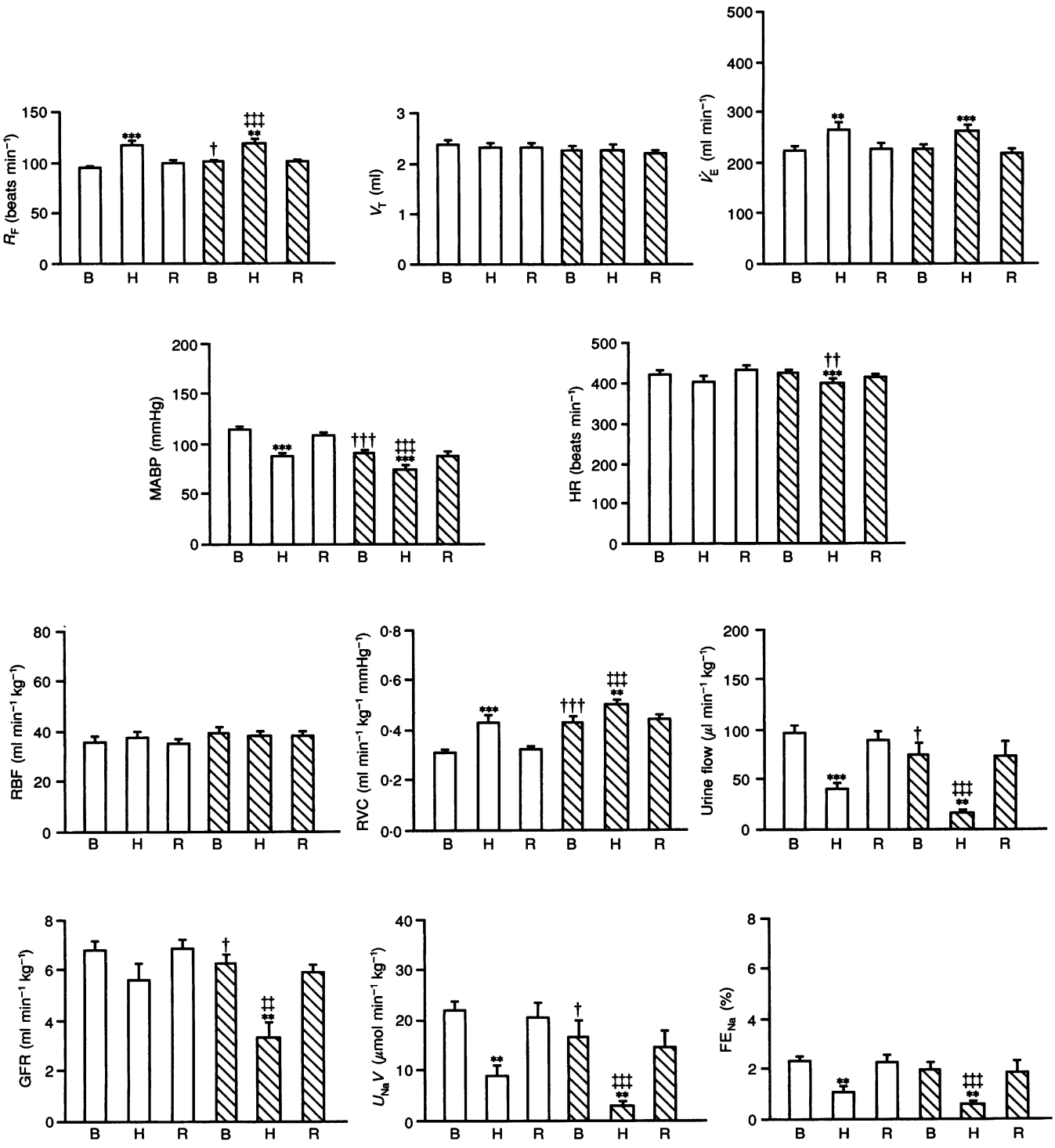

Figure 1. Effect of Losartan upon respiratory, cardiovascular and renal function changes induced by 12% O_2 in Group 1

The columns represent the mean (± S.E.M.) of the basal values (B), the hypoxic values (H) and the recovery values (R); see text for further explanation. □, before and ▨, after Losartan. The number of observations for each variable is 11. * Significant difference between basal and 12% O_2 value, the comparison being made on log values, see text. † Significant difference between the control values (mean of the basal and recovery periods) recorded before and after Losartan. ‡ Significant difference between the hypoxic response before and after Losartan, respectively. In each case: 3 symbols, $P < 0{\cdot}001$; 2 symbols, $P < 0{\cdot}01$; and 1 symbol, $P < 0{\cdot}05$. All abbreviations for variables as in Table 2.

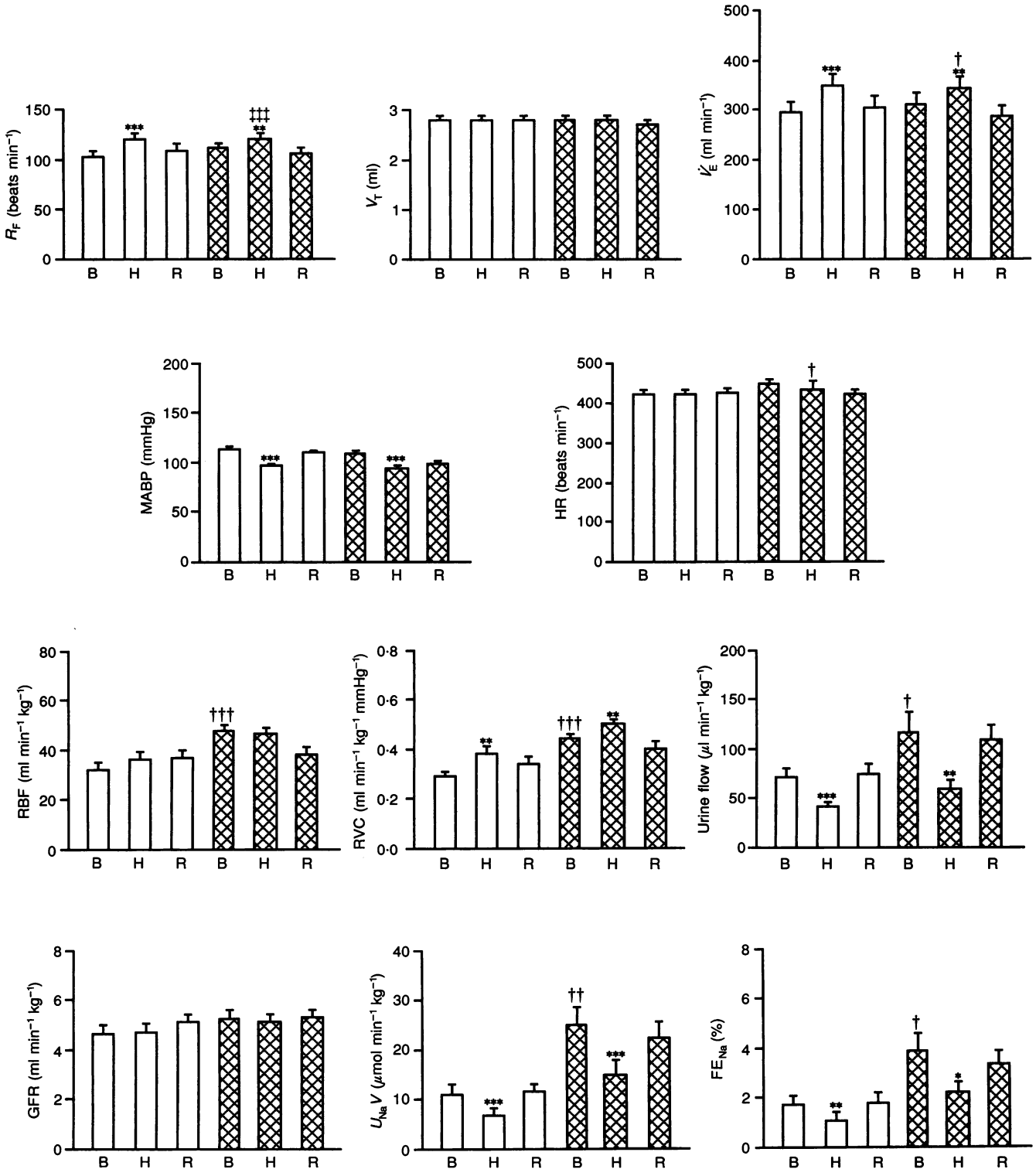

Figure 2. Effect of Losartan upon respiratory, cardiovascular and renal function changes induced by 12% O_2 in Group 2, when RPP was controlled after Losartan

All abbreviations and symbols as in Table 2 and Fig. 1. □, before and ▩, after Losartan. The number of observations for each variable is 9 except for RBF, RVC, GFR and FE_{Na}, when the number is 8.

Losartan (Fig. 1). HR and GFR fell significantly after Losartan but not before (Fig. 1).

In all animals of Group 1, AII (100 mg, I.V.) evoked a sharp increase in MABP and falls in RBF and RVC which were abolished when re-tested 20 min after Losartan and at the end of the experiment.

Group 2

The animals included in the analysis were ones in which the basal level of brachial artery pressure after Losartan (i.e. during C6 and C7) was successfully controlled to within 10% of the mean of the basal and recovery values for the first hypoxic period (C1, C2 and C4 and C5): we assumed that RPP was similarly controlled. This seemed to be a reasonable basis on which to make comparisons because MABP during the recovery period after the first hypoxic period (C4 and C5) was generally lower than that recorded during C1 and C2 (see Fig. 1), even with maximal occlusion of the aorta, and the extent to which MABP 'recovered' was not correlated with the extent to which it could be successfully controlled before and during the second period of hypoxia.

The absolute values recorded during air breathing at the beginning of the experiment were similar to those recorded in Group 1 except for renal function (see Tables 1 and 2); urine flow, GFR, $U_{Na}V$ and FE_{Na} tended to be lower in Group 2. The pattern of respiratory, cardiovascular and renal response induced by the first period of hypoxia was also comparable with that recorded in Group 1 (see Table 1, compare Figs 1 and 2), except that GFR showed a greater tendency to fall during hypoxia in Group 1.

After Losartan and with MABP in the brachial artery, and thereby RPP, artificially maintained at the control level recorded before Losartan (see above), there were no significant changes in the control levels of the respiratory variables (Fig. 2), but pH_a during air breathing was increased while P_{a,CO_2} was decreased as in Group 1 (see Table 1). The control value of RVC was increased as in Group 1, but in contrast to Group 1, control RBF was also raised. Moreover, in direct contrast to Group 1, when RPP was maintained after Losartan in Group 2, control values of urine flow, $U_{Na}V$ and FE_{Na} were greatly increased (Fig. 2 cf. Fig. 1).

After Losartan and with RPP during hypoxia artificially controlled so that it fell to the same level as during hypoxia before Losartan (Fig. 2), the accompanying changes in respiration and blood gases were qualitatively similar to those recorded during hypoxia before Losartan (Fig. 2): the increases in R_F and $\dot{V}_E$ were smaller after Losartan, but the changes in P_{a,O_2}, P_{a,CO_2} and pH were fully comparable (Table 1). The changes induced in the renal haemodynamic variables and in renal function by hypoxia after Losartan were also qualitatively comparable to those induced before Losartan (Fig. 2). When analysed as a proportion of the new basal values, the falls in urine flow, $U_{Na}V$ and FE_{Na} were not significantly different before and after Losartan, this result reflecting the fact that the increases in the basal values of those variables were accompanied by increases in the absolute magnitude of the changes induced by hypoxia (see Fig. 2).

As in Group I, AII (100 ng, I.V.) evoked a pressor response which was abolished 20 min after giving Losartan and at the end of the experiment.

Plasma renin activity

Plasma samples taken from animals of Group 1 and 2 showed that the first period of hypoxia evoked a significant increase in plasma renin activity: from $3{\cdot}02 \pm 0{\cdot}68$ ng $ml^{-1}\,h^{-1}$ during air breathing to $8{\cdot}36 \pm 1{\cdot}8$ ng $ml^{-1}\,h^{-1}$ during hypoxia ($P < 0{\cdot}01$).

DISCUSSION

The respiratory, cardiovascular and renal responses induced in the rat by moderate hypoxia in the present study were comparable with those we reported recently (Neylon *et al.* 1995). Thus, in Groups 1 and 2, P_{a,O_2} fell to ~40 mmHg and stimulation of peripheral chemoreceptors led to an increase in respiration and a fall in P_{a,CO_2} that was accompanied by a modest fall in MABP and a renal vasodilatation whilst RBF remained constant. For reasons that are not clear, given that the animals of Groups 1 and 2 came from the same breeding station and were treated in the same way before and during the experiment, Group 1 show greater renal function during air breathing than Group 2, i.e. higher mean GFR, urine flow and sodium excretion. Nevertheless, Groups 1 and 2 showed comparable changes in renal function in response to the first period of hypoxia: an antidiuresis and antinatriuresis. The antidiuresis and antinatriuresis were comparable with those recorded in conscious rats when they breathed 12% O_2 for 30 min (Behm, Mewes, DeMuinck Keizer, Unger & Rettig, 1993).

The present study provided the additional information that moderate hypoxia was accompanied by a 2·8-fold increase in plasma renin activity as might be explained by the reflex response to peripheral chemoreceptor stimulation, baroreceptor unloading and the fall in RPP (see the introduction, and below). Such an increase in plasma renin activity would be expected to lead to a similar increase in AII in plasma, assuming ACE activity is not affected by hypoxia. A few years ago, Milledge & Catley (1984) suggested that although renin activity is increased in systemic hypoxia, ACE activity is inhibited, so allowing a marked disparity in the extent to which renin and AII levels are increased in different individuals: this was used to explain the differences between individuals in their susceptibility to

acute mountain sickness. However, after performing further series of experiments, Milledge & Catley (1987) completely withdrew their claim that hypoxia inhibits ACE activity. Thus, it is reasonable to assume that raised AII levels had the potential to influence cardiovascular and renal function in hypoxia in the present study.

Since the dose of Losartan we used blocked the vasoconstrictor responses evoked by a supramaximal dose of AII, we conclude that it was fully effective in blocking the AT_1 subtype of AII receptor (see the introduction). Thus, the fact that Losartan caused a fall in the control level of MABP and an increase in the control level of RVC in Group I indicates that during air breathing, AII exerted a tonic vasoconstrictor influence on the kidney and probably on other vascular beds. Since RVC still increased in Group 2 when MABP and thereby RPP were prevented from falling after Losartan treatment by aortic occlusion, it is clear that the renal vasodilatation reflected a primary effect on the kidney, rather than a secondary, myogenic dilatation to the fall in MABP. This accords with our recent observation that captopril, the ACE inhibitor, caused a decrease in MABP and vasodilatation in kidney and limb muscle in Saffan-anaesthetized rats (Louwerse & Marshall, 1992).

Angiotensin II could exert a tonic vasoconstrictor influence by acting directly on vascular smooth muscle, or by facilitating sympathetic transmission onto vascular smooth muscle (Johns, 1989). However, AII can also exert a tonic influence on arterial pressure and body fluid homoeostasis by acting on AT_1 receptors within the central nervous system (Bunneman, Iwai, Metzger, Fuxe, Inagami & Ganten, 1992); and Losartan, at a dose of 10 mg kg^{-1} h^{-1}, has been shown to cross the blood–brain barrier and antagonize central AT_1 receptors (Song, Zhug & Mendelsohn, 1991). The falls in the control level of urine flow and sodium excretion seen in Group 1 after Losartan can be attributed to the fall in MABP and thereby in RPP. Although AII can help to maintain GFR by exerting a vasoconstrictor action on the renal efferent arterioles (Johns, 1989), there is no need to invoke this as an important site of action for Losartan during air breathing, for in Group 2 the control level of GFR did not fall when the control level of MABP was prevented from falling after Losartan, by aortic occlusion.

The effects of Losartan on the hypoxia-induced changes in renal haemodynamics and function in Group 1 can also be attributed to the effects of Losartan on systemic MABP, rather than to any effects of a hypoxia-induced increase in AII upon AT_1 receptors in the kidney. Thus, the increase in RVC induced by hypoxia after Losartan was sufficient to prevent RBF from falling with the hypoxia-induced fall in MABP, just as it had been before Losartan. That GFR fell significantly during hypoxia after Losartan but not before, even though RBF remained constant, suggests that hypoxia caused dilatation of the efferent as well as the afferent arterioles, as we proposed before (Neylon *et al.* 1995) and raises the possibility that the fall in GFR during hypoxia was facilitated because Losartan removed a vasoconstrictor influence of AII upon the efferent arterioles. However, the absolute level of MABP reached during hypoxia after Losartan (~75 mmHg) was substantially lower than that attained before Losartan (~87 mmHg) and below the autoregulatory range for GFR in the rat (~80 mmHg; Ofstad & Aukland, 1985). Thus, the fall in GFR in hypoxia after Losartan could have been the secondary consequence of MABP reaching a lower level.

When considered as a proportion of the basal values, the hypoxia-induced falls in urine flow and sodium excretion were greater after Losartan (urine flow: 50% before *vs.* 77% after Losartan; total sodium excretion: 58 *vs.* 83%), but this simply reflected the fall in the basal value; in absolute terms they were fully comparable before and after Losartan. Thus, there was no evidence that Losartan blocked an antinatriuretic and antidiuretic effect of AII upon the renal tubules (Johns, 1989). Rather, because of the fall in basal values, renal function showed greater impairment in hypoxia after Losartan than before.

The fact that RVC showed a similar increase in hypoxia in the animals of Group 2 both before and after Losartan treatment, and the fact that GFR did not fall during hypoxia in these animals after Losartan when the hypoxia-induced fall in MABP was controlled, may support the idea that GFR fell during hypoxia after Losartan in Group 1 because MABP fell below the autoregulatory range (see above). However, it must be noted that GFR showed far less tendency to fall during hypoxia *before* Losartan treatment in the animals of Group 2 than in those of Group 1 (Fig. 1 cf. Fig. 2).

The higher basal values of urine flow and sodium excretion seen in Group 2 after Losartan can be attributed to the increase in basal RVC and RBF. But, from these new base lines, hypoxia induced similar reductions in urine flow and sodium excretion as it had done before Losartan, when these changes were expressed as proportional changes (41 *vs.* 43%, 42 *vs.* 42%, respectively) and in absolute terms the reductions were even greater after Losartan. Thus, as in Group 1, there is no evidence that a hypoxia-induced increase in AII contributed to the antinatriuresis and antidiuresis. However, in these animals, it was the case that with RPP maintained after Losartan, renal function was less impaired during hypoxia than before, in that urine flow and sodium excretion were maintained at much higher values.

The present results may seem at variance with those of previous studies. Thus, stimulation of the brachial nerves in anaesthetized rats produced a 20% increase in renal sympathetic nerve activity which was associated with a 2-fold increase in plasma renin activity, and reductions in

urine flow and sodium excretion of 44 and 49%, respectively (Handa & Johns, 1987). These changes were similar to those induced by hypoxia in the present study and yet the changes in renal function induced by brachial nerve stimulation were greatly reduced when AII receptors were antagonized with saralasin, or when ACE activity was blocked with captopril. In that study, it was proposed that AII generated from the increase in renin activity acted presynaptically to facilitate the antinatriuretic and antidiuretic actions of the renal nerves upon the proximal tubule, rather than directly on the proximal tubule (Handa & Johns, 1987). This was based on previous findings that, although low-frequency stimulation of the renal nerves produced a 2-fold increase in renin activity as well as an increase in tubular reabsorption of sodium, the latter was completely blocked by an α-adrenoreceptor antagonist; there was no indication that a moderate increase in AII exerted a direct action on the renal tubules (Osborn, Holdaas, Thames & DiBona, 1983; Hesse & Johns, 1985). Thus, a possible explanation for the apparent disparity between the present results and those of Handa & Johns (1987) is that during moderate hypoxia there was little or no activation of the renal sympathetic nerves.

Certainly, the renal vasoconstriction that occurs when strong, short-lasting stimuli are selectively applied to the carotid chemoreceptors can be attributed to an increase in renal sympathetic activity (see Marshall, 1994 for references) while an increase in renal sympathetic activity has been recorded in the rat during severe systemic hypoxia (breathing 6% O_2) lasting just 45 s (Fukuda, Sato, Suzuki & Trzebski, 1989). Moreover, under Saffan anaesthesia (which does not disrupt transmission through the brainstem defence areas like other anaesthetics), sympathetically mediated renal vasoconstriction can be evoked by selective chemoreceptor stimulation and thereby by acute systemic hypoxia as a component of the characteristic pattern of the alerting or defence response which is generally evoked by noxious stimuli (see Marshall, 1994 for references). But, in the present and our previous study we purposely avoided the renal vasoconstriction of the alerting response 'contaminating' the response to systemic hypoxia by excluding from our analysis the first 5 min of the hypoxic period when the components of the alerting response most commonly occur (see Neylon & *et al.* 1995). Indeed, it is reasonable to propose that moderate hypoxia *per se* does not lead to a sustained increase in renal sympathetic activity. A lack of effect on renal sympathetic activity would in fact be consistent with our previous observation that against the background of the diuresis and natriuresis caused by acute renal denervation, moderate hypoxia still produced an antidiuresis and antinatriuresis that were proportionately similar to those induced in the innervated kidney (Neylon *et al.* 1995).

If there was no significant increase in renal sympathetic activity in moderate hypoxia, then the increase in renin activity we recorded must have been due to the effect of the fall in RPP acting at the afferent arteriole, rather than to an increase in renal sympathetic activity caused by baroreceptor unloading, or peripheral chemoreceptor stimulation. We can then further interpret the results of our previous study. Thus, the observation that the antidiuresis and antinatriuresis induced by hypoxia were abolished when RPP was prevented from falling, left open the possibilities that the antidiuresis and antinatriuresis were caused by the direct effects of a decrease in RPP upon renal function, or by the renal actions of AII generated as a secondary consequence of the fall in RPP (Neylon *et al.* 1995). We can now conclude that the direct effect of the fall in RPP is the dominant factor: if AII generated by the fall in RPP were important then we should have been able to reduce the antidiuresis and antinatriuresis in Group 2 of the present study when we blocked the AT_1 receptors with Losartan but controlled the basal RPP and the hypoxia-induced fall in it so that they were the same as before Losartan.

We can also attempt further interpretation of the results of other studies (see the introduction). Thus, the finding that conscious rats did not show a rise in plasma renin activity when they breathed a more severe hypoxic mixture than used in the present study (10% O_2 for 20 min; Raff & Roarty, 1988) may reflect the fact that the hypoxia-induced fall in MABP is not as great in the conscious rat as in the Saffan-anaesthetized rat (see Neylon *et al.* 1995). On the other hand, observations that conscious dogs showed an increase in plasma renin activity when breathing 7% O_2 even though they showed a concomitant rise in MABP (Rose *et al.* 1983) and that a renal vasoconstrictor influence for AII could be demonstrated during severe hypoxia (5% O_2), but not during moderate hypoxia (8% O_2; Liang & Gavras, 1978) indicates that activation of the renin–angiotensin system *can* occur in systemic hypoxia by a reflex increase in renal sympathetic activity resulting from hypoxic stimulation of the peripheral chemoreceptors, but it requires that a very strong stimulus be applied to the chemoreceptors. Under these circumstances, AII would be expected to facilitate the antidiuretic and antinatriuretic influences of increased renal sympathetic activity upon tubular function (see above).

In summary, the results of the present study have shown that the antidiuresis and antinatriuresis induced in the Saffan-anaesthetized rat by moderate systemic hypoxia are associated with a 2·8-fold increase in plasma renin activity, which is probably caused by a fall in, rather than by an increase in, renal sympathetic activity. They also suggest that AII, by acting on AT_1 receptors, exerts an important tonic, vasoconstrictor influence in the kidney and elsewhere which limits the fall in RPP and thereby the decrease in renal function produced by moderate hypoxia. They provide no evidence that AII acts within the kidney to contribute to the antidiuresis and antinatriuresis of

moderate hypoxia by acting on the renal tubules. Finally, the present results strengthen the view that in the rat, the fall in RPP *per se* plays the dominant role in determining renal function in moderate hypoxia.

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