Chronic hypoxia upregulates the expression and function of AT₁ receptor in rat carotid body

P S Leung, S Y Lam and M L Fung¹

Department of Physiology, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, Hong Kong

¹Department of Physiology, Faculty of Medicine, The University of Hong Kong, Hong Kong

(Requests for offprints should be addressed to P S Leung; Email: psleung@cuhk.edu.hk)

Abstract

In the present study, the effects of chronic hypoxia on the expression and localization of angiotensin II (Ang II) receptors are investigated by semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) and by immunohistochemistry. The effect of chronic hypoxia on the carotid body chemoreceptor activity was also examined by *in vitro* electrophysiology. Results from RT-PCR revealed that chronic hypoxia exhibited differential effects on the gene expression of Ang II receptors, namely AT₁ and AT₂, in the carotid body. The mRNA expression for subtypes of the AT₁ receptor, AT_{1a} and AT_{1b}, was significantly increased in the carotid body with chronic hypoxia. To further investigate the localization of the AT₁ receptor, an immunohistochemical study was performed. The results showed that AT₁ receptor

immunoreactivity was found in lobules of glomus cells in the carotid body and the immunoreactivity was more intense in chronic hypoxia than in normoxic controls. *In vitro* electrophysiological studies consistently demonstrated that chronic hypoxia enhanced the AT₁ receptor-mediated excitation of carotid body chemoreceptor activity. These data suggest that chronic hypoxia upregulates the transcriptional and post-transcriptional expression of AT₁ receptors in the rat carotid body. The upregulation of the expression also enhances AT₁ receptor-mediated excitation of the carotid body afferent activity. This might be important in the modulation of cardiorespiratory functions as well as fluid and electrolyte homeostasis during chronic hypoxia.

Journal of Endocrinology (2000) **167**, 517–524

Introduction

The carotid body is located in the bifurcation of the carotid arteries and plays a central role in cardiorespiratory responses to physiological stimuli such as changes in arterial blood gases, pH, or osmolality. In hypoxia, chemoreceptors in the carotid body increase the afferent activity of the carotid sinus nerves, which activates brainstem activities for the elevation of respiratory drive (Gonzalez *et al.* 1994). The stimulation of carotid body chemoreceptors also reflexly increases cardiac output, sympathetic flow and elevates circulating vasoactive hormones such as norepinephrine and angiotensin II (Ang II) for enhancing cardiovascular performance (Marshall 1994).

Ang II is a physiologically active component of the renin-angiotensin system (RAS), which plays an important role in regulating the blood pressure and fluid homeostasis (Peach 1977). It has been known that both systemic and tissue RAS are activated by hypoxia. In the kidney, RAS was shown to be upregulated by chronic hypoxia (Neylon *et al.* 1997, Fletcher *et al.* 1999). In addition, it has recently been demonstrated that chronic hypoxia activates the expression of local RAS component genes in the

pancreas (Chan *et al.* 2000). Interestingly, peripheral infusion of Ang II stimulates cardiorespiratory functions (Ohtake & Jennings *et al.* 1993) and the plasma Ang II level increases during hypoxia (Zakheim *et al.* 1976). Recently, Ang II has been demonstrated to increase the carotid body afferent activity, presumably via the mediation of the AT₁ receptor (Allen 1998). Thus activation of Ang II receptors in the carotid body may stimulate cardiorespiratory functions and play a role in the fluid and electrolyte homeostasis during hypoxia. In fact, carotid body functions are modulated by chronic hypoxia. For example, the carotid body releases catecholamines during hypoxia and this is believed to be an important step for the chemotransduction (Donnelly 1993). In chronic hypoxia, the production of catecholamines elevates in the carotid body and this is associated with an increase in the amount and activity of tyrosine hydroxylase, as demonstrated in rat (Gonzalez *et al.* 1979, Hanbauer *et al.* 1981, Czyzyk-Krzeska *et al.* 1992) and in humans (Lack *et al.* 1985). Moreover, carotid afferent activities are known to play a role in the natriuresis and diuresis during chronic hypoxia, although the underlying mechanism is not clear (Honig 1989).

Provided that Ang II excites carotid body afferent activity, the carotid body response to Ang II may be modulated by chronic hypoxia and this could enhance the afferent activity, which potentiate the cardiorespiratory response as well as the natriuretic and diuretic response during chronic hypoxia. Accordingly, the present study is aimed at elaborating the regulation of chronic hypoxia on the expression and localization of Ang II receptors and at elucidating its effect on the chemoreceptor activity of the carotid body.

Materials and Methods

Animals and isolation of carotid body

Adult Sprague–Dawley (SD) rats of either sex weighing about 70 g at age 28 days were employed in the present study. The animals were bred in the Laboratory Animal Services Centre, the Chinese University of Hong Kong. The animals were supplied with food and water and allowed to feed *ad libitum*. Animal model and experimental procedure have been approved by the Animal Ethical Committee of the Chinese University of Hong Kong, Hong Kong. Following deep anaesthesia with halothane, SD rats were decapitated and the carotid body was dissected out from the bifurcation and the superior sympathetic ganglion in PBS, pH 7.4.

Materials and pharmacological agents

The rat Ringer solution contained (mM): NaCl 125, KCl 3.1, NaH₂PO₄ 1.25, MgSO₄ 1.3, CaCl₂ 2.4, D-Dextrose 10. Pharmacological agents or drugs were obtained from Sigma, RBI and Dupont Pharmaceutical.

Chronic hypoxic exposure

For the exposure of rats in chronic hypoxia, SD rats aged 28 days were raised inside an acrylic chamber filled with 10% ± 0.5% oxygen for isobaric hypoxia for 4 weeks, as reported previously in our laboratory (Chan *et al.* 2000). Briefly, the oxygen level was monitored continuously by an oxygen analyser and pure nitrogen was mixed with room air. The humidity and carbon dioxide level were maintained by desiccators and by soda lime placed inside the chamber. For normoxic controls, time- and age-matched rats were kept in the same housing and allowed to breathe room air.

Semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) of Ang II receptors

Total RNA was isolated from carotid body and adrenal gland which was employed as a positive tissue for the expression of Ang II receptors, according to the acid

guanidinium thiocyanate–phenol–chloroform protocol (Chomczynski & Sacchi 1987). Briefly, tissues were homogenized in 4 M guanidinium thiocyanate solution. The extracted total RNA was studied by gel electrophoresis and quantified by spectrophotometry. Total RNA (10 µg) was subjected to first strand cDNA synthesis using random hexamer primers and Superscript II transcriptase (GIBCO-BRL, Grand Island, NY, USA) in a final volume of 20 µl. After incubation at 42 °C for 1 h, the reaction mixture was treated with RNase H before proceeding to polymerase chain reaction (PCR) analysis.

The final mixture (2 µl) was directly used for PCR amplification. mRNAs of Ang II receptor subtypes, namely AT_{1a}, AT_{1b} and AT₂ were detected with primers as employed previously (Leung *et al.* 1998, 1999a). All RNA was tested to be free of DNA contamination by RT-PCR without addition of reverse transcriptase. After appropriate validation for the semi-quantitative RT-PCR as reported previously (Chan *et al.* 2000), all samples were analysed for both Ang II receptor subtype and β-actin genes in the logarithmic phase of the amplification reactions. The PCR conditions were 30 cycles of: denaturing, 94 °C, 1 min; annealing, 58 °C for AT_{1a} and AT₂ and 62 °C for AT_{1b}, 1 min and elongating, 72 °C, 2 min. The amplified mixture (10 µl) was finally separated on 2% agarose gel electrophoresis and the amplified DNA bands were detected using ethidium bromide staining. The bands were then quantified with image analyser (Molecular Dynamics Image Quant, Sunnyvale, CA, USA).

Immunohistochemical localization of AT₁ receptor

The carotid bifurcation from normoxic and hypoxic SD rats (*n*=8) were removed bilaterally from the head, rinsed in PBS and frozen in iso-pentane. Consecutive cryosections (8–10 µm) were fixed with freshly prepared paraformaldehyde (4%) for 30 min. The sections were then processed for indirect immunoperoxidase staining as described previously (Leung *et al.* 1999a,b). Briefly, sections were incubated overnight at 4 °C with anti-AT₁ receptor serum (Santa Cruz Biotech. Inc, Santa Cruz, CA, USA), diluted to 0.2 µg/ml, or with anti-tyrosine hydroxylase serum (Chemicon International Inc., CA, USA), diluted to 1:2000 overnight at 4 °C. The primary antibody was detected using an avidin–biotin conjugate kit (Vectastain Elite ABC kit; Vector Laboratories, Burlingame, CA, USA) according to the manufacturer's instructions. Positive immunoreactivity was visualized by reacting sections with Vector VIP substrate kit (Vector Laboratories). Sections were slightly counterstained with haematoxylin, dehydrated and mounted. The following controls were used: (i) substitution of primary antibodies with buffer; (ii) incubation with rabbit non-immune serum; (iii) liquid phase pre-adsorption of AT₁ receptor with excess blocking peptide (Santa Cruz Biotech. Inc).

In vitro electrophysiological study of AT₁ receptors in chronically hypoxic carotid body

The isolated carotid body was incubated in collagenase and protease for 30 min at 35 °C (Kholwadwala & Donnelly 1992). It was then held in the recording chamber at 35 ± 1 °C, and perfused (2–3 ml/min) with oxygenated Ringer and the sinus nerve was recorded for single- or pauci-fiber activities with suction electrodes. The signal was amplified, filtered, monitored and digitized for off-line analysis. Ang II (0.1–100 nM) was perfused for 3 min for the dose-dependent response. For the blockade of the AT₁ receptor losartan (10 µM), was perfused for 10 min before the Ang II (100 nM) treatment. Acute hypoxia was induced by perfusion of rat Ringer gassed with (95% N₂ and 5% CO₂) to confirm the chemosensitivity of the afferent fibers.

Data analysis and statistics

For RT-PCR, the Ang II receptor expression is normalized in % control of the β-actin expression. Results were expressed as the means ± s.e.m.s for the normoxic controls and the chronically hypoxic group. Statistical comparisons were made by ANOVA and differences between individual mean values were compared using unpaired Student's *t*-test. Values of *P* < 0.05 were considered statistically significant. For *in vitro* electrophysiology, we compared the discharge rate (spike/s, average over 1–3 min) before, during and after the drug treatment. Values were normalized in % control of resting activity and were presented as means ± s.e.m.s. Statistical comparisons between the response to Ang II in normoxic and chronically hypoxic group were made by ANOVA with *post hoc* tests. The same analysis was used for comparison in drugs studies among groups. Differences were considered significant at *P* < 0.05.

Results

Effect of chronic hypoxia on the expression of AT₁ receptors

The mRNA expression of AT₁ receptors at both AT_{1a} and AT_{1b} subtypes in the carotid body was investigated using RT-PCR in conjunction with specific primers corresponding to their respective non-coding sequences. A marked effect of chronic hypoxia on the expression of AT_{1a} and AT_{1b} subtypes was observed in the carotid body, whereas the expression levels of β-actin remained unchanged in chronic hypoxia when compared with that in controls (Figs 1 and 2). There were significant increases in the mRNA expression for the AT_{1a} subtype (Fig. 1A) and for the AT_{1b} subtype (Fig. 2A) in the chronic hypoxia group compared with their respective controls. The relative expression changes of AT_{1a} subtype (Fig. 1B) and

Figure 1 (A) RT-PCR analysis of the mRNA expression of AT_{1a} receptor in the rat carotid body. Lane M, DNA marker; Lane 1, AT_{1a} receptor expression in normoxic (control) carotid bodies; Lane 2, AT_{1a} receptor expression in hypoxic carotid bodies; Lane 3, β-actin expression in the control carotid bodies; Lane 4, β-actin expression in hypoxic carotid bodies; Lane 5, AT_{1a} expression in adrenal glands. The arrows indicate the expected size of amplified products from AT_{1a} receptor (385 bp) and β-actin (240 bp). (B) The relative expression of AT_{1a} receptor/β-actin mRNA (% of control) in the normoxic (control) and chronically hypoxic group of carotid bodies. The data are expressed as means ± s.e.m. (*n* = 5 for each group). **P* < 0.05.

AT_{1b} subtype (Fig. 2B) were about twofold in both subtypes, as demonstrated by RT-PCR and image analysis.

Effect of chronic hypoxia on the expression on AT₂ receptor subtype

The mRNA expression of AT₂ receptor was also studied in the carotid body. Chronic hypoxia had a less prominent effect on the AT₂ receptor expression (Fig. 3A). There was a slight but consistent increase of about 15% of the relative expression of AT₂ receptor in the chronically hypoxic group (Fig. 3B).

Figure 2 (A) RT-PCR analysis of the mRNA expression of AT_{1b} receptor in the rat carotid body. Lane M, DNA marker; Lane 1, AT_{1b} receptor expression in control carotid bodies; Lane 2, AT_{1b} receptor expression in hypoxic carotid bodies; Lane 3, β -actin expression in control carotid bodies; Lane 4, β -actin expression in the hypoxic carotid bodies; Lane 5, AT_{1b} expression in adrenal glands. The arrows indicate the expected size of amplified products from AT_{1b} receptor (204 bp) and β -actin (240 bp). (B) The relative expression of AT_{1b} receptor/ β -actin mRNA. The data are expressed as means $\pm$ S.E.M. ($n=5$ for each group). * $P<0.05$.

Cellular localization of the AT_1 receptor by chronic hypoxia

To further elucidate the precise localization of AT_1 receptor expression and its upregulation during chronic hypoxia, immunohistochemistry was employed. Results from immunohistochemistry showed that there was an increase in the intensity of the immunoreactivity for the AT_1 receptor in chronically hypoxic carotid body compared with control (Fig. 4). Intense immunoreactivity for tyrosine hydroxylase was observed in the glomus cells, which was employed as a positive immunohistochemical marker for parenchymal type I glomus cells (Kameda *et al.* 1990). Tyrosine hydroxylase immunoreactivity was more intense in the hypoxic group (Fig. 4B) than in normoxic controls (Fig. 4A), as described previously (Wang *et al.* 1998). Detailed examination of consecutive sections of carotid body showed that distinct but less intense immunoreactivity for AT_1 receptor was predominantly localized to glomus cells in normoxic control (Fig. 4C,D) when compared with that in chronically hypoxic carotid body,

Figure 3 (A) RT-PCR analysis of the mRNA expression of AT_2 receptor in the rat carotid body. Lane M, DNA marker; Lane 1, AT_2 receptor expression in control carotid bodies; Lane 2, AT_2 receptor expression in hypoxic carotid bodies; Lane 3, β -actin expression in control carotid bodies; Lane 4, β -actin expression in hypoxic carotid bodies; Lane 5, AT_2 expression in adrenal glands. The arrows indicate the expected size of amplified products from AT_2 receptor (511 bp) and β -actin (240 bp). (B) The relative expression of AT_2 receptor/ β -actin mRNA. The data are expressed as means $\pm$ S.E.M. ($n=5$ for each group). * $P<0.05$.

which exhibited very intense immunostaining of AT_1 receptor (Fig. 4E and F). Intense immunostaining of AT_1 receptor was consistently observed in glomus cells of chronically hypoxic carotid body with sections counterstained with haematoxylin (Fig. 4G). Specificity of the immunostaining was validated by the negative control experiments when specific antibody was pre-absorbed in excess with its AT_1 receptor antigen either with counterstaining (Fig. 4H) or without counterstaining (Fig. 4I).

Increase of afferent nerve activities of the carotid body in vitro by Ang II

Ang II increased the resting activity of carotid bodies in normoxic and chronically hypoxic (CH) rats. Figure 5 shows that Ang II stimulated the afferent discharge of

Figure 4 Immunohistochemical localization of AT_1 receptor and tyrosine hydroxylase proteins in normoxic and chronically hypoxic carotid body of the rats. Distinct immunoreactivity for tyrosine hydroxylase was localized to the perinuclear cytoplasm of glomus cells in the carotid body from normoxia (A) and chronic hypoxia (B). Less intense immunoreactivity for the AT_1 receptor was predominantly localized to glomus cells from normoxic controls in lower magnification (C) and in high magnification (D). Intense immunostaining was consistently localized to glomus cells from chronic hypoxia in low magnification (E) and in high magnification (F). Distinct immunostaining in glomus cells from chronically hypoxic carotid body was shown in serial sections counterstained with haematoxylin (G). No immunostaining was observed in pre-adsorption of primary antibody with excess of AT_1 receptor antigen either with (H) or without (I) counterstaining. The bars represent 100 μ m.

Figure 5 Ang II increases afferent nerve activities of the carotid body *in vitro*. At rest (A), the carotid body had a low basal activity and, in (B), the activity was increased following the perfusion of Ang II (10 nM, for 3 min). The single unit activity is shown in (C) by overlapping plots of 40 spikes and the discharge rate (Hz) is shown in (D). Note that the bar in D presents the perfusion of Ang II. Calibration bars for A and B are 1.5 s; for C, 0.5 ms; for D, 3 min. (E) The dose-dependent curves of the stimulatory effect of Ang II on the nerve discharge in the normoxic (N, ●, $n=8$) and chronic hypoxia (CH, ■, $n=8$) groups. Note that the concentration of Ang II is plotted on a logarithmic scale and that the sensitivity for the Ang II response was enhanced in the CH groups compared with the normoxic control. The data are expressed as means $\pm$ S.E.M. *Statistical significance when compared with corresponding dosage in the normoxic group ($P<0.05$).

carotid body isolated from a CH rat. The perfusion of Ang II increased the basal discharge rate of a single unit (Fig. 5A–D). Ang II (0.1–100 nM) dose-dependently increased afferent discharge of the carotid body. The average dosage response to Ang II (100 nM) is 430% in the CH group compared with 220% in the normoxic group (Fig. 5E). Thus, the response to Ang II was enhanced in the CH group and the dosage–response curve for the CH group was shifted to the left, suggesting that maximal binding capacity for the Ang II was increased in the CH carotid body.

Inhibition of Ang II-mediated response in chronically hypoxic carotid body by losartan

To determine whether AT₁ receptors mediate the enhanced response to Ang II, the CH carotid body was pretreated with specific antagonist for AT₁ receptor, losartan (10 μ M, 10 min), before the perfusion of Ang II (100 nM). The response to Ang II was significantly attenuated by the pretreatment of losartan. Figure 6 shows the afferent activities before (A), during (B) and following wash (C) in a carotid body. In all CH carotid bodies tested ($n=9$) losartan reduced the response to Ang II (Fig. 6E). The response to Ang II was partially recovery following wash-out for 60 min (Fig. 6E). Results suggested that AT₁ receptors mediate the enhanced response to Ang II in the CH carotid body.

Discussion

The present study has clearly demonstrated the upregulation of the expression and function of Ang II receptor by chronic hypoxia in rat carotid body. Chronic hypoxia caused a marked increase in the expression of both AT₁ and AT₂ receptors, notably AT₁ receptor as evidenced by RT-PCR analysis. Results from RT-PCR showed that chronic hypoxia significantly upregulates both mRNA expression for AT_{1a} and AT_{1b} subtypes. In parallel with the transcriptional level, the AT₁ receptor protein was enhanced in expression, and the expression was specifically localized to the glomus cells of the carotid body, as demonstrated by immunohistochemistry. In addition, *in vitro* electrophysiological study coupled with specific antagonist for AT₁ receptor further demonstrated that the upregulation of AT₁ receptor expression was associated with the increased excitatory response to Ang II during chronic hypoxia. Results strongly suggest the increase in functional AT₁ receptors in the carotid body during chronic hypoxia.

The present study is the first to present data on the upregulation of the expression and function of AT₁ receptors by chronic hypoxia in carotid body by means of molecular biological, immunohistochemical and *in vitro* electrophysiological studies. Chronic hypoxia regulates the expression of RAS in a number of tissues, including the kidney (Neylon *et al.* 1996), the lung (Zhao *et al.* 1996),

Figure 6 (A)–(C) present the perfusion of Ang II. In (A), perfusion of Ang II (100 nM, 3 min) increased the afferent discharging rate (Hz) of a CH carotid body. (B) The response to Ang II was attenuated by pretreatment with losartan (10 μM, 10 min). (C) Following wash for 60 min, the response to Ang II was partially recovered. The unitary activity is shown in (D) by overlapping plots of 40 spikes. Calibration bars for A–C are 3 min; for D, 0.4 ms. (E) Summary of the response to Ang II in CH carotid bodies before the treatment of losartan (ANG), after the treatment of losartan (LOS+ANG) and following wash-out (WASH+ANG). The data are expressed as means ± S.E.M. *Statistical significance when compared with ANG+LOS ($P < 0.05$).

the heart (Morrell *et al.* 1997) and the pancreas (Chan *et al.* 2000). In the carotid body, Ang II-binding sites have been demonstrated by *in vitro* autoradiography (Allen 1998). The present study provides evidence for the gene expression of both AT₁ and AT₂ receptors in the carotid body during normoxic condition. More importantly, our data showed that chronic hypoxia upregulates the expression of the AT₁ receptors at both the gene and protein levels. The upregulation of the expression of Ang II receptors during chronic hypoxia appears to be subtype- and tissue-specific. Hence the upregulation in the carotid body was prominent in the AT₁ but not AT₂ subtype, whereas the major upregulation in the pancreas is the AT₂ subtype (Chan *et al.* 2000). Moreover, it is more intriguing to demonstrate that chronic hypoxia can activate the expression of both AT_{1a} and AT_{1b} subtypes. Yet the relative importance of the subtypes for the AT₁ receptor is currently unknown (Matsubara *et al.* 1994) and the functional properties of the two subtypes are different in the signalling mechanisms (Tian *et al.* 1996). Their functional roles and upregulation in the carotid body remain to be elucidated.

The present study confirmed that the localization of the expression of AT₁ receptors in the carotid body is mainly in the glomus cells. In addition, it is known that AT₁ receptor may also express in the pre-synaptic terminals of sympathetic innervation (Castren *et al.* 1987). Consistent with previous finding that sympathetic denervation does not change the Ang II binding in the carotid body (Allen 1998), our results also suggests the sympathetic AT₁ receptors if any would be in small amount in the carotid body. Glomus cells are believed to be the chemoreceptors and are the catecholamine-containing cells for the

secretion of catecholamines during hypoxia (Fidone *et al.* 1982). In chronic hypoxia, the enzyme activity and the gene expression of the tyrosine hydroxylase increase, which in turn leads to increased production of catecholamine in the carotid body (Donnelly 1993). Indeed, our results also showed the enhanced tyrosine hydroxylase-immunoreactivity in the glomus cells with chronic hypoxia. Consistently, the upregulation of gene expression of the AT₁ receptor was supported by the present observation that the intensity of AT₁-immunoreactivity was higher in the chronically hypoxic group than in the normoxic control. This finding suggests that chemoreceptors increase the density of functional AT₁ receptors in chronic hypoxia.

In vitro electrophysiological studies further elucidated that Ang II increased afferent discharge of the carotid body. This finding is consistent with a previous report that Ang II stimulated carotid chemoreceptor activity (Allen 1998). Following chronic hypoxia, the response to Ang II was elevated in the carotid body and the dose–response curve shifted leftward. This suggests that the affinity to Ang II binding or the receptor density is enhanced in the chronic hypoxic group. The latter possibility is supported by the fact that the gene expression of the AT₁ receptor in the carotid body was increased in chronic hypoxia. A previous report has shown that the AT₁ receptor is the major subtype, which mediates the carotid body response to Ang II in normoxic rats (Allen 1998). In chronic hypoxia, the response to Ang II is inhibited by losartan, a specific antagonist for AT₁ receptor. This suggests that the AT₁ receptor mediates the response to Ang II and that the receptor is upregulated in the carotid body during chronic hypoxia. Hence, the upregulation of AT₁ receptors

elevates the sensitivity and physiological response of the carotid body to Ang II stimulation. However, the mechanism underlying the upregulation of the AT₁ receptor in chronic hypoxia remains undefined and merits further investigation.

In summary, the present study has provided evidence that chronic hypoxia could upregulate the expression of Ang II receptors, predominantly the AT₁ receptor, which might result in enhancing the carotid afferent nerve activity to Ang II stimulation. This could, in turn, increase the cardiorespiratory response and regulate the salt and water homeostasis during the stress of chronic hypoxia.

Acknowledgements

The work was supported by the Research Grants Council of Hong Kong and the Chinese University of Hong Kong. Support for MLF was provided by grants from the Research Grants Council and the University of Hong Kong. We would like to thank Dr E Y P Cho for his technical advice. Losartan was kindly provided as a gift from Merck & Co., Inc., New Jersey.

References

- Allen AM 1998 Angiotensin AT₁ receptor-mediated excitation of rat carotid body chemoreceptor afferent activity. *Journal of Physiology* **510** 773–781.
- Castren E, Kurihara M, Gutkind JS & Saavedre JM 1987 Specific angiotensin binding sites in the rat stellate and superior cervical ganglion. *Brain Research* **422** 347–351.
- Chan WP, Fung ML, Nobiling R & Leung PS 2000 Activation of local renin–angiotensin system by chronic hypoxia. *Molecular and Cellular Endocrinology* **160** 107–114.
- Chomczynski P & Sacchi N 1987 Single-step method of RNA isolation by acid guanidium thiocyanate–phenol–chloroform extraction. *Analytical Biochemistry* **162** 156–159.
- Czyzyk-Krzeska MF, Bayliss DA, Lawson EE & Millhorn DE 1992 Regulation of tyrosine hydroxylase gene expression in the rat carotid body by hypoxia. *Journal of Neurochemistry* **58** 1538–1546.
- Donnelly DF 1993 Electrochemical detection of catecholamine release from rat carotid body *in vitro*. *Journal of Applied Physiology* **74** 2330–2337.
- Fidone S, Gonzalez C & Yoshizaki K 1982 Effects of low oxygen on the release of dopamine from the rabbit carotid body *in vitro*. *Journal of Physiology* **333** 93–110.
- Fletcher EC, Bao G & Li R 1999 Renin activity and blood pressure in response to chronic episodic hypoxia. *Hypertension* **34** 309–314.
- Gonzalez C, Kwok Y, Gibb J & Fidone S 1979 Effects of hypoxia on tyrosine hydroxylase activity in rat carotid body. *Journal of Neurochemistry* **33** 713–719.
- Gonzalez C, Almaraz L, Obeso A & Rigual R 1994 Carotid body chemoreceptors: from natural stimuli to sensory discharge. *Physiological Review* **74** 829–898.
- Hanbauer I, Karoum F, Hellstrom S & Lahiri S 1981 Effects of hypoxia lasting up to one month on the catecholamine content in rat carotid body. *Neuroscience* **6** 81–86.
- Honig A 1989 Salt and water metabolism in acute high-altitude hypoxia: role of peripheral arterial chemoreceptors. *News of Physiology Sciences* **4** 109–111.
- Kameda Y, Amano T & Tagawa T 1990 Distribution and ontogeny of chromogranin A and tyrosine hydroxylase in the carotid body and glomus cells located in the wall of the common carotid artery and its branches in the chicken. *Histochemistry* **94** 609–616.
- Kholwadwala D & Donnelly DF 1992 Maturation of carotid body chemoreceptor sensitivity to hypoxia: *in vitro* studies in the newborn rat. *Journal of Physiology* **453** 461–473.
- Lack EE, Perez-Atayde AR & Young JB 1985 Carotid body hyperplasia in cystic fibrosis and cyanotic heart disease: a combined morphometric, ultrastructural, and biochemical study. *American Journal of Pathology* **119** 301–34.
- Leung PS, Yao XQ, Chan HC, Fu LXM & Wong PYD 1998 Differential gene expression of angiotensin II receptor subtypes in the epididymides of mature and immature rats. *Life Sciences* **62** 461–468.
- Leung PS, Chan WP, Wong TP & Sernia C 1999a Expression and localization of renin–angiotensin system in the rat pancreas. *Journal of Endocrinology* **160** 13–19.
- Leung PS, Wong TP & Sernia C 1999b Angiotensinogen expression by rat epididymis: evidence for an intrinsic, angiotensin-generating system. *Molecular and Cellular Endocrinology* **155** 115–122.
- Marshall JM 1994 Peripheral chemoreceptors and cardiovascular regulation. *Physiological Review* **74** 543–594.
- Matsubara H, Kanasaki M, Murasawa S, Tsukaguchi Y, Nio Y & Inada M 1994 Differential gene expression and regulation of angiotensin II receptor subtypes in rat cardiac fibroblasts and cardiomyocytes in culture. *Journal of Clinical Investigation* **93** 1592–1601.
- Morrell NW, Danilov SM, Satyan KB, Morris KG & Stenmark KR 1997 Right ventricular angiotensin converting enzyme activity and expression is increased during hypoxic pulmonary hypertension. *Cardiovascular Research* **34** 393–403.
- Neylon M, Marshall J & Johns EJ 1996 The role of the renin–angiotensin system in the renal response to moderate hypoxia in the rat. *Journal of Physiology* **491** 479–488.
- Neylon M, Marshall JM & Johns EJ 1997 The effects of chronic hypoxia on renal function in the rat. *Journal of Physiology* **501** 243–250.
- Ohtake PJ & Jennings DB 1993 Angiotensin II stimulates respiration in awake dogs and antagonizes baroreceptor inhibition. *Respiratory Physiology* **91** 335–351.
- Peach MJ 1977 Renin–angiotensin system: biochemistry and mechanisms of action. *Physiological Review* **57** 313–370.
- Tian Y, Baukal AJ, Sandberg K, Bernstein KE, Balla T & Catt KJ 1996 Properties of AT_{1a} and AT_{1b} angiotensin receptors expressed in adrenocortical Y-1 cells. *American Journal of Physiology* **270** E831–E939.
- Wang ZZ, Dinger B, Fidone SJ & Stensaa L 1998 Changes in tyrosine hydroxylase and substance P immunoreactivity in the cat carotid body following chronic hypoxia and denervation. *Neuroscience* **83** 173–1281.
- Zakheim RM, Molteni A, Mattioli L & Park M 1976 Plasma angiotensin II levels in hypoxic and hypovolemic stress in unanesthetized rabbits. *Journal of Applied Physiology* **41** 462–465.
- Zhao L, Al-Tubuly R, Sebkh A, Owji A, Nunez DJR & Wilkins MR 1996 Angiotensin II receptor expression and inhibition in the chronically hypoxic rat lung. *British Journal of Pharmacology* **119** 1217–1222.

Received 4 May 2000

Accepted 2 August 2000