

Enhanced reactivity to bradykinin, angiotensin I and the effect of captopril in the pulmonary vasculature of chronically hypoxic rats

P.C. Russell, C.J. Emery, Y.N. Cai, G.R. Barer, P. Howard

Enhanced reactivity to bradykinin, angiotensin I and the effect of captopril in the pulmonary vasculature of chronically hypoxic rats. P.C. Russell, C.J. Emery, Y.N. Cai, G. R. Barer, P. Howard

ABSTRACT: We compared the reactivity of pulmonary vessels to bradykinin (BK) and angiotensin I (AI) in normal and chronically hypoxic rats; the latter have pulmonary hypertension and muscularized pulmonary arterioles. These peptides are respectively inactivated and activated by the angiotensin converting-enzyme (ACE) on pulmonary endothelium. Isolated lungs were perfused at a constant flow rate when changes in pulmonary artery pressure (Ppa) reflect changes in vascular resistance. Dose-response curves to BK (1 ng-10 µg) were derived during normoxia and pre-constriction by hypoxia; BK both decreased and increased vascular resistance, *i.e.* vasodilatation and vasoconstriction. In normal rats only constriction was seen in normoxia, which reflected low basal vascular tone, whereas in chronically hypoxic rats there was only dilatation which reflected high basal vascular tone. In hypoxia in normal rats, low doses caused dilatation, high doses constriction; in chronically hypoxic rats there was again only dilatation which was larger than in controls. After the ACE-inhibitor captopril, constriction was exaggerated in control rats in both normoxia and hypoxia and took place in chronically hypoxic rats after high doses in both normoxia and hypoxia; oedema often followed. Dose-response curves to AI (1 ng-µg) in normoxia showed greatly enhanced pressor responses in chronically hypoxic compared with normal rats, probably attributable to increased sensitivity to angiotensin II (AII) rather than enhanced conversion of AI to AII. Captopril caused a proportionate reduction in responses in both groups of rats. The physiological role of BK and AI in the lung is unknown but in ACE-inhibitor therapy the consequences for the lung (and other organs) of BK persistence as well as reduced formation of AII may be important.

Eur Respir J., 1990, 3, 779-785.

Chronic hypoxia is associated with pulmonary hypertension and the growth of new muscle in normally thin-walled pulmonary arterioles. This change takes place in rats exposed to low oxygen tensions in normo- or hypobaric chambers and resembles the abnormal muscularity of pulmonary arterioles in high altitude residents and patients with hypoxic lung disease. It is associated with enhanced reactivity to several pulmonary vasoconstrictor and vasodilator stimuli, including angiotensin II (AII) [1]. Exaggerated reactivity, which might be a consequence of endothelial damage, could play a role in the progression of pulmonary vascular disease [2]. Several reports indicate that activity of angiotensin converting-enzyme (ACE), which removes the terminal two amino acids from angiotensin I (AI) and bradykinin (BK), thus activating the former and inactivating the latter, may be altered in chronic hypoxia [3, 4]. In view of the potential importance of these two peptides in the pulmonary circulation and elsewhere in hypoxic lung disease,

we have compared their activity on the pulmonary vasculature of normal and chronically hypoxic rats, together with the effect of the ACE-inhibitor captopril. In this work, reactivity has been defined as the change in pulmonary artery pressure (Ppa) at constant pulmonary blood flow after a given stimulus. Chronically hypoxic rats have both a higher Ppa and higher pulmonary vascular tone than control rats, even in normoxia, factors which influence the differences between them. An abstract of preliminary work is published [5].

Methods

Young (c.28 day old) male Wistar SPF rats were exposed to 10% O₂ in a normobaric chamber [1] for 2-3 wks. Their lungs were compared with those of littermate controls of similar age (kept in the same room in air) in an isolated perfused lung preparation previously described

University Dept of Medicine, Royal Hallamshire Hospital, Sheffield S10 2JF, UK.

Correspondence: Dr G.R. Barer, Dept of Medicine, Royal Hallamshire Hospital, Sheffield S10 2JF, UK.

Keywords: Angiotensin I; angiotensin converting-enzyme inhibition; bradykinin; chronic hypoxia.

Received: July 31, 1989; accepted after revision March 7, 1990.

[1]. After pentobarbitone anaesthesia (60 mg·kg⁻¹, *ip.*), the lungs were perfused *in situ* with blood of normal pH and haematocrit at 38°C at a constant flow rate of 20 ml·min⁻¹. Blood entered the lung through a cannula in the pulmonary artery and returned to a heated reservoir from a cannula in the left atrium. At constant flow, changes in Ppa represent changes in vascular resistance. A similar flow rate was used for normal and chronically hypoxic rats because we found that the latter had a pulmonary vasculature of similar volume to controls despite reduced weight and skeletal growth [1]; we have evidence that the lungs grow fast relative to other body parts in chronic hypoxia [6]. The lungs were ventilated with 95% air + 5% CO₂ (normoxia) or 2% O₂ + 5% CO₂ + 93% N₂ (hypoxia), and end-expiratory pressure was held at 0.26–0.39 kPa (2–3 mmHg).

Dose-response curves to AI (Sigma, 1, 10, 100 and 500 ng, 1 and 10 µg) and BK (Sigma, 1, 10 and 100 ng, 1 and 10 µg), were measured during normoxia and hypoxia (BK only) before and after captopril (20 mg·kg⁻¹). They were dissolved in 0.15 M NaCl solution and given in 0.1 ml amounts (captopril slightly more) into the pulmonary arterial line; equal volumes of saline were given as a control and caused injection artefacts only. The order of doses was from the lowest upwards and smaller doses were frequently tested again later in the experiment: no evidence for tachyphylaxis was seen.

Means±SEM are calculated and given in text and figures. Differences between means in chronically hypoxic (CH) and control (C) rats were compared by analysis of variance as described in the text and were considered significant at $p < 0.05$.

Results

Baseline Ppa and pressor responses to hypoxia in control and chronically hypoxic rats

As in previous experiments, at constant blood flow CH rats had a higher Ppa than C rats during normoxia 4.59±0.16 and 2.55±0.16 kPa (34.5±1.2 and 19.2±1.2 mmHg) although the vascular volume is similar [1]. The pressure difference resembles that measured *in vivo* in anaesthetized rats breathing air [7]. The mean increase in Ppa caused by hypoxia was 2.22±0.33 (16.7±2.5) in CH and 1.78±0.25 kPa (13.4±1.9 mmHg) in C rats.

Effect of single doses of BK during normoxia and hypoxia

BK had mixed dilator and constrictor effects. Figure 1 shows traces of Ppa in a C rat above and a CH rat below. BK (1 µg) had no effect from the baseline in the C rat but caused dilatation in the CH rat; during hypoxia there was dilatation in both rats but this was greater in the CH rat. Figure 2 similarly shows the effect of 10 µg BK in a C rat above and a CH rat below. There was constriction during normoxia in the C rat but dilatation in the CH rat; during hypoxia this dose caused dilatation in both rats but in the C rat dilatation was preceded by constriction. In both C and CH rats in hypoxia, 10 µg BK led to a slow delayed dilatation; in the C rat comparison with a preceding hypoxic test showed that this delayed dilatation differed from the slow decay of hypoxic vasoconstriction sometimes observed, as in this rat.

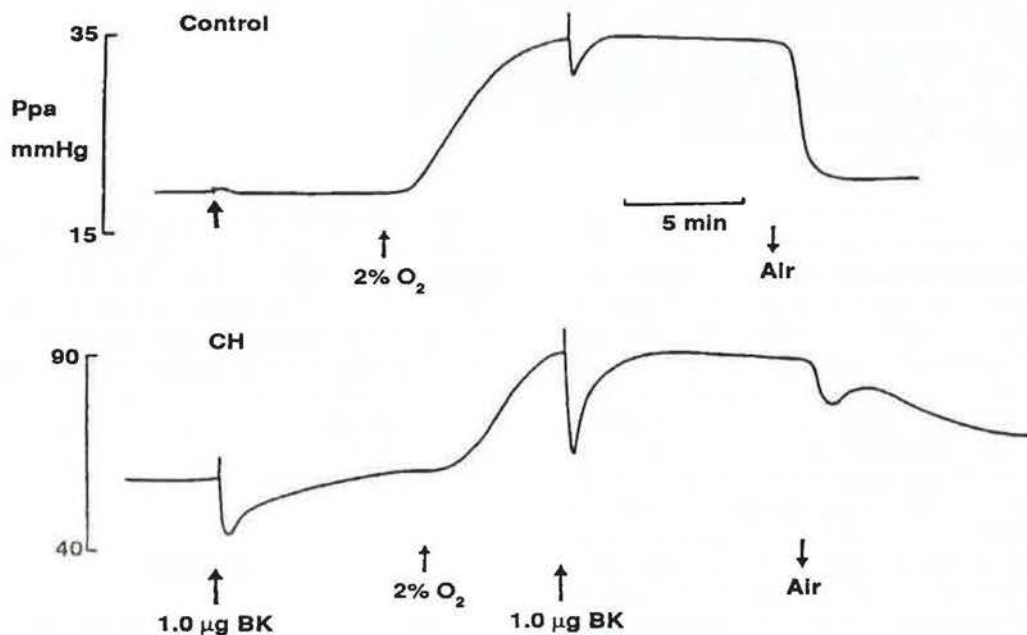


Fig. 1. — Traces of pulmonary artery pressure (Ppa) from a control (C) rat above and a chronically hypoxic (CH) rat below. Effect of 1 µg bradykinin (BK) during normoxia causes dilatation in CH rat and a small artefact only in C rat (at arrow vertically above that for CH rat). 2% O₂ causes an increase in Ppa to a plateau; at maximum Ppa, 1 µg BK causes a small dilatation in C rat and a larger dilatation in CH rat. Note that on return to air ventilation the fall in Ppa is rapid in C rat but slower and biphasic in CH rat.

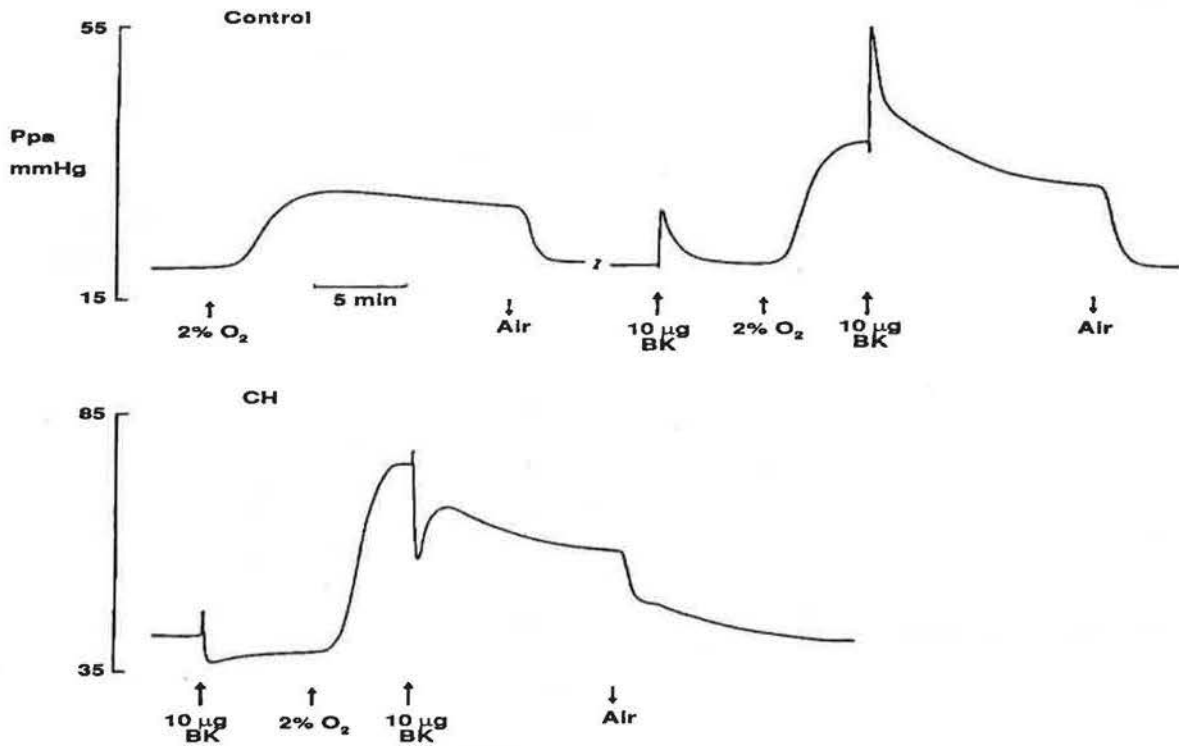


Fig. 2. - Traces of pulmonary artery pressure (Ppa) in a control (C) rat above and a chronically hypoxic (CH) rat below showing that 10 µg bradykinin (BK) causes mainly constriction in the C rat and dilatation in the CH rat. Above in C rat from left to right, 2% O₂ causes a rise in Ppa which slowly declines, 10 µg BK causes a rise in Ppa from the baseline and during a second hypoxic test 10 µg BK causes a further rise in Ppa followed by delayed fall. Below, CH rat, 10 µg BK causes a possible brief rise (or injection artefact) followed by a prolonged fall in Ppa. During an hypoxic test, 10 µg BK caused a fall in Ppa, some recovery and then a delayed further fall.

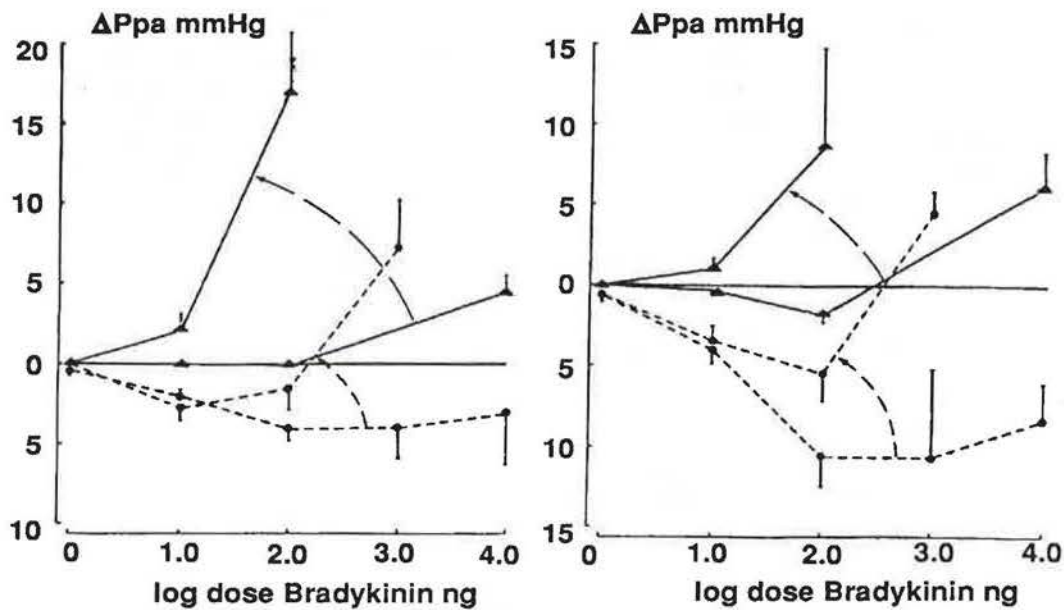


Fig. 3. - Log dose-response curves to bradykinin (BK) in seven control (C) and eight chronically hypoxic (CH) rats before and after captopril, 20 mg·kg⁻¹; actual doses 1, 10, 100 ng, 1 and 10 µg (not every rat received all the doses). The curves are rotated to the left after captopril as shown by the arrows. Means ± SEM. Points above the line represent rises and below the line falls in pulmonary artery pressure (Ppa). On the left tests were during normoxia; on the right during prior constriction by hypoxia. ▲—▲: control; ●---●: chronically hypoxic.

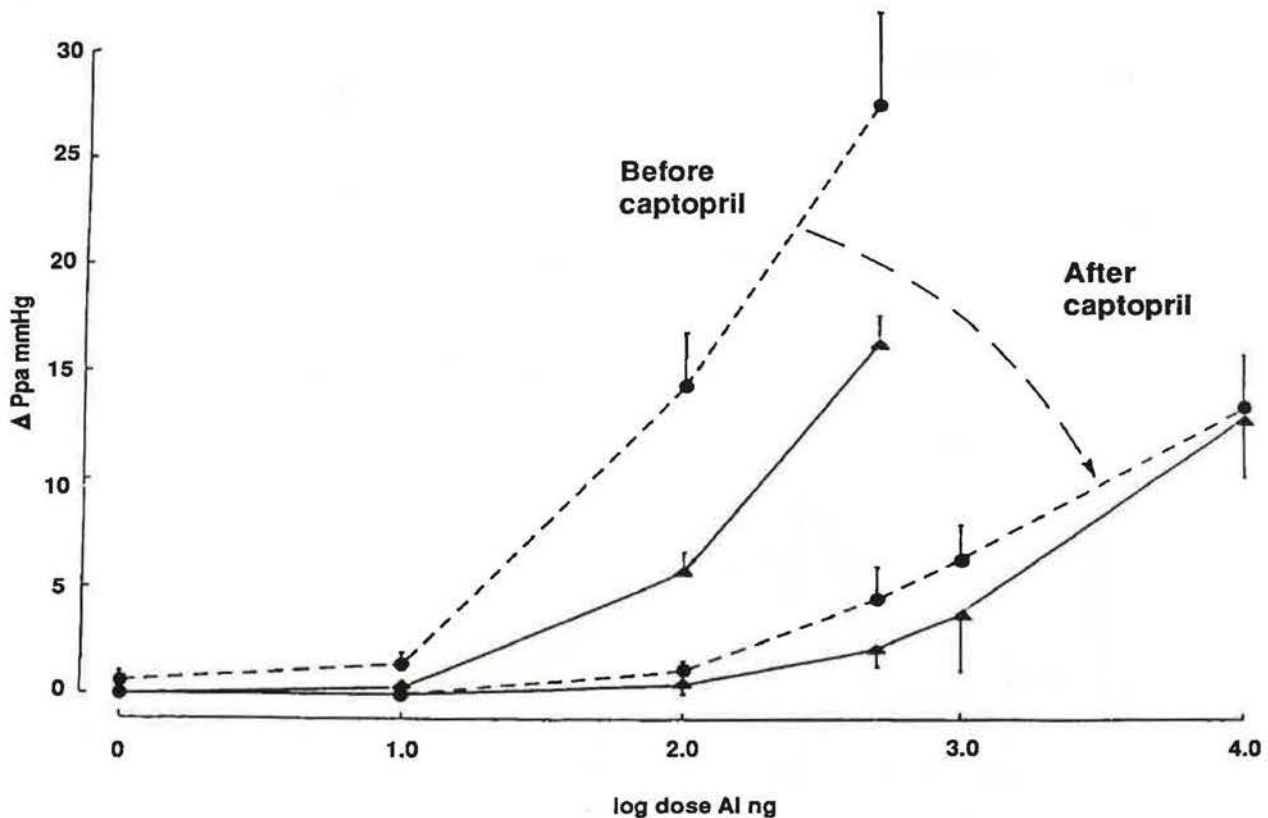


Fig. 4. - Log dose-response curves to angiotensin I (AI) in seven control (C) and nine chronically hypoxic (CH) rats before and after captopril, 20 mg·kg⁻¹; actual doses 1, 10, 100, 500 ng, 1 and 10 µg (not every rat received all the doses). After captopril the curves rotate to the right as indicated (for CH rats) by arrow. ▲—▲: control; ●—●: chronically hypoxic.

Dose-response curves for BK during normoxia and hypoxia

Figure 3 shows dose-response relations in seven C and eight CH rats during normoxia (left) and hypoxia (right). During normoxia C rats showed constriction only at the highest dose. CH rats showed dilatation at all doses, albeit slightly reduced at high doses but no constriction took place; in a small earlier study [8] some constriction was observed at the highest dose in CH rats in normoxia. During hypoxic vasoconstriction, dilatation was observed in C rats at low doses although this was less than that seen in CH rats and the threshold dose was higher. Constriction was again seen in C rats at the highest dose. In CH rats during hypoxia, there was profound dilatation which lessened at higher doses. At high doses in both groups there was a balance between dilatation and constriction such that two phase effects were sometimes seen (fig. 2). Constriction preceded dilatation. In figure 3 the larger phase is included in the mean. In some rats during hypoxia, there was a constrictor effect and several minutes later a dilator effect or a second dilator effect (fig. 2, CH rat); this delayed dilatation might have been caused by secondary release of another substance. The larger doses of BK were associated with small increases in tracheal inflation pressure; since the upper airway has no blood supply in this preparation, we assume that constriction of peripheral airways supplied by pulmonary vessels took place.

Actions of BK after the cyclooxygenase inhibitor meclufenamate

Meclofenamate (106 µg) was given to five C and four CH rats; it caused a rise in Ppa in CH but negligible change in C rats (CH rats, Ppa rose from 6.21±0.73 to 7.28±0.92 Kpa (46.7±5.5 to 54.7±6.9 mmHg); C rats, Ppa rose from 2.70±0.12 to 2.83±0.20 Kpa (20.3±0.9 to 21.3±1.5 mmHg)). In three CH rats the dilator action of 10 µg BK was not significantly changed compared to C rats during both normoxia (changes in Ppa, 0.51±0.26 and 0.25±0.25 Kpa (-3.8±1.4 to -1.9±1.9 mmHg), though in one CH rat the effect disappeared) and hypoxia (1.56±0.48 and 1.54±0.49 Kpa (-11.7±3.6 *CF* -11.6±3.7 mmHg)). In these CH and C rats the constrictor effects were unchanged after meclufenamate.

Effect of captopril on the dose-response to BK

Figure 3 shows that after captopril there was an anti-clockwise rotation of the dose-response curve to BK; captopril had no consistent effect on baseline Ppa in either group, although occasional small changes in either direction took place. In C rats in normoxia constriction occurred at lower doses than before captopril and was larger and greatly prolonged; oedema sometimes followed large doses. During hypoxia in C rats there was no longer

dilatation; all doses caused constriction. In CH rats during normoxia dilatation was reduced after captopril and the highest dose, 1 µg, caused constriction. During hypoxia a similar anticlockwise movement of the curve took place; there was also frequent oedema after captopril. Comparison of the difference in responses to 100 ng BK in C and CH rats before and after captopril indicated that during normoxia the absolute difference in Ppa was much greater after captopril ($p < 0.01$) though it was unchanged during hypoxia ($p > 0.05$).

Dose-response curves to AI before and after captopril

Figure 4 shows a dose-response to AI during normoxia before and after captopril in seven C and nine CH rats. In CH rats the constrictor responses to 100 and 500 ng AI were much greater than in C rats. In both groups after captopril, the curve was rotated clockwise; constriction was abolished at the lower doses. Comparison of the difference in pressor responses to 100 ng and 0.5 µg AI in C and CH rats before and after captopril showed that, although the absolute difference was much greater before captopril ($p < 0.01$), the proportionate difference was unchanged ($p > 0.05$).

Discussion

Possible causes of increased reactivity to BK and AI in chronic hypoxia

The enhanced actions of BK and AI in chronically hypoxic rats could be related to the increased muscularity and narrowed lumen of small arterioles, which have tone even in normoxia [1, 9]. A similar degree of muscle shortening from an initially smaller circumference will have a greater effect on resistance [1, 10]. Alternatively, the new muscle might have different properties or a different population of receptors. The pulmonary endothelium may be damaged or its properties altered after exposure to severe hypoxia [11]. The vascular changes in these rats resemble those seen in hypoxic cor pulmonale, although the patients have additional features which suggest severe intimal and endothelial damage [12].

Dual action of BK on lung vessels

We confirmed that BK has a dual action on lung vessels, dilatation and constriction; the result depends on pre-existing vascular tone and dose. We previously showed that it increased flow through atelectatic lungs *in vivo*, a fact which might prove important in disease [13]. Others showed in isolated pulmonary arteries that BK has two dilator mechanisms, one endothelium-dependent, one due to prostacyclin release [14]. Our tests with meclofenamate make it unlikely that prostacyclin was released in our experiments; also our attempts to damage endothelium and prevent release of endothelium-derived relaxing factor (EDRF) in isolated perfused lungs proved unsuccessful and caused lung oedema. Large doses of

BK, especially after captopril, caused oedema probably through microvascular leakage.

The vascular sites at which BK exerts its dilator and constrictor actions are not known. Neither do we know the circumstances and sites of BK release. However, its liberation as a "secondary mediator" in inflammatory and allergic reactions suggests that its pulmonary vascular actions are important in pathological situations. Dilatation combined with bronchoconstriction might worsen ventilation/perfusion mismatch in poorly ventilated lung. The increased activity of BK in chronic hypoxia and especially after captopril should be taken into account. After captopril, the actions of BK were not only enhanced but much prolonged. The relation of BK to cough in ACE inhibitor therapy has been discussed [15].

Enhanced reaction to AI in chronic hypoxia

We showed that reactivity to AII is increased in chronically hypoxic rat lungs [1]. Although results from earlier workers are conflicting, JACKSON *et al.* [3] showed that ACE activity was impaired in chronic hypoxia in rats; conversion of AI to AII in a single pass through the pulmonary circulation was impaired. Diminished conversion might reflect an enzyme change or a reduced capillary surface area in chronic hypoxia [4]. A reduction in ACE activity is not incompatible with our results. The greater effect of AI which we observed may result from increased sensitivity to AII as suggested by our earlier work [1]. JIN *et al.* [16] examined the systemic and pulmonary pressor responses to AI and AII in normal and chronically hypoxic rats. Systemic responses to both AI and AII were reduced after hypoxic exposure but, after 28 days exposure, pulmonary pressor responses to AII were enhanced.

The profound effects of AI and AII which we and others [17] have seen in isolated rat lungs, where large doses can be given, does not necessarily imply an important function *in vivo*. We detected no vasoconstrictor action of AII in isolated or *in vivo* ferret lungs and in cats and dogs minimal constriction was observed *in vivo*; a rise in Ppa was seen only after doses which caused extreme systemic hypertension and elevation of left atrial pressure [18]. Conversion of AI to AII during passage through the lung is very efficient [19] and ACE has been identified on alveolar capillary endothelium [20]; however, we do not know if conversion of AI to AII occurs sufficiently soon during passage to affect pulmonary vascular smooth muscle, or whether both veins and arteries are responsive. It may be that AII formed in the lung has its actions wholly downstream. Recent work suggests that local formation of AI and AII is functionally important and that AI, AII and renin activity in blood may represent a "spill-over" from events in specific organs [21]. Exactly where lung AII fits into this hypothesis is unclear. Because of its huge surface area, pulmonary capillary endothelium is a major site of AII formation and there is little destruction of AII in the lung compared with other organs [20]. A growth function for this peptide has also been described [22].

Conclusions

Our results suggest that, in using ACE inhibitors, we should consider consequences for BK as well as AII. This applies not only to effects in the lung but to actions in other vascular beds and in the kidney. KENTERA *et al.* [23] made the important observation that captopril treatment significantly reduced the pulmonary hypertension and right ventricular hypertrophy of chronically hypoxic rats. The basis of this attenuation remains unsolved.

Acknowledgement: The authors thank C. Wright for excellent technical assistance.

References

- Emery CJ, Bee D, Barer GR. – Mechanical properties and reactivity of vessels in isolated lungs of chronically hypoxic rats. *Clin Sci*, 1981, 61, 569–580.
- Barer GR, Emery CJ, Bee D, Wach RA. – Mechanisms of pulmonary hypertension, an overview. *In: The pulmonary circulation in health and disease*. J.A. Will, C.A. Dawson, E.K. Weir, C.K. Bruckner eds, Academic Press, Orlando, 1987, pp. 409–422.
- Jackson RM, Nakartes AJ, Oparil S. – Impaired pulmonary conversion of angiotensin I to angiotensin II in rats exposed to chronic hypoxia. *J Appl Physiol*, 1986, 60, 1121–1127.
- Rickaby DA, Bougara RD, Tristani MJ, Linehan JA, Dawson CA. – Effects of gas composition and pH on kinetics of lung angiotensin-converting enzyme. *J Appl Physiol*, 1987, 62, 1216–1221.
- Russell PC, Emery CJ, Cai YN, Barer GR. – Enhanced reactivity of pulmonary vessels to angiotensin I and bradykinin in chronically hypoxic rats. *Clin Sci*, 1988, 21P.
- Hunter C, Barer GR, Shaw JW, Clegg EJ. – Growth of the heart and lungs in hypoxic rodents. A model of human hypoxic disease. *Clin Sci*, 1974, 46, 375–391.
- Herget J, Suggett AJ, Leach E, Barer GR. – Resolution of pulmonary hypertension and other features induced by chronic hypoxia in rats during complete and intermittent normoxia. *Thorax*, 1978, 33, 468–473.
- Barer GR, Cai YN, Russell PC, Emery CJ. – Reactivity and site of vasomotion in pulmonary vessels of chronically hypoxic rats: relation to structural changes. *Am Rev Respir Dis*, 1989, in press.
- Finlay M, Barer GR, Suggett A. – Quantitative changes in the rat pulmonary vasculature in chronic hypoxia—relation to haemodynamic changes. *Q J Exp Physiol*, 1986, 71, 151–163.
- Folkow B. – The haemodynamic consequences of adaptive structural changes of the resistance vessels in hypertension. *Clin Sci*, 1971, 41, 1–12.
- Scott KW, Barer GR, Leach E, Mungall IPF. – Pulmonary ultrastructural changes in hypoxic rats. *J Pathol*, 1978, 126, 27–33.
- Wilkinson M, Langhorne CA, Heath D, Barer GR, Howard P. – A pathophysiological study of 10 cases of hypoxic cor pulmonale. *Q J Med*, 1988, 66, 65–85.
- Howard P, Barer GR, Thompson B, Warren PM, Abbott CJ, Mungall IPF. – Factors causing and reversing vasoconstriction in unventilated lung. *Respir Physiol*, 1975, 24, 325–345.
- Chand N, Diamantes W, Sofia RD. – Relationship of pharmacological agents, pulmonary endothelial cells and pulmonary circulation. *In: The pulmonary circulation in health and disease*. J.A. Will, C.A. Dawson, E.K. Weir, C.K. Buckner eds, Academic Press, Orlando, 1987, pp. 147–165.
- Berkin KE. – Respiratory effects of angiotensin-converting enzyme inhibition. *Eur Respir J*, 1989, 2, 198–201.
- Jin H, Oparil S, Ann HS, Yanf R, Jackson RM. – Hypoxia-induced inhibition of converting-enzyme: role in vascular regulation. *J Appl Physiol*, 1987, 63, 1012–1018.
- McMurtry IF, Davidson AB, Reeves JT, Grover RF. – Inhibition of hypoxic vasoconstriction by calcium antagonists in rat lungs. *Circ Res*, 1976, 38, 99–104.
- Suggett AJ, Mohammed FH, Barer GR. – Angiotensin, hypoxia, verapamil, and pulmonary vessels. *Clin Exp Pharmacol Physiol*, 1980, 7, 263–274.
- Vane JR. – The fate of angiotensin I. *In: Handbook of experimental Pharmacology*, Vol. 37. I.H. Page, F.M. Bumpus eds, Springer Verlag, New York, 1974, pp. 17–39.
- Ryan US, Ryan JW. – Relevance of endothelial surface structure to the activity of vasoactive substances. *Chest*, 1986, Suppl., 203S–207S.
- Campbell DJ. – Site of angiotensin production. *J Hypertension*, 1985, 3, 199–207.
- Fernandez LA, Twickler J, Mead A. – Neovascularization produced by angiotensin II. *J Lab Clin Med*, 1985, 105, 141–145.
- Kentera D, Susic D, Cvetkovic A, Djordjevic G. – Effects of SQ 14225 an orally active inhibitor of angiotensin-converting enzyme on pulmonary hypertension and right ventricular hypertrophy on rats. *Basic Res Cardiol*, 1981, 76, 344–351.

Accentuation de la réactivité à la bradykinine et à l'angiotensine I, et effet du Captopril sur la vascularisation pulmonaire de rats chroniquement hypoxiques. P.C. Russell, C.J. Emery, Y.N. Cai, G.R. Barer, P. Howard.

RÉSUMÉ: Nous avons comparé la réactivité des vaisseaux pulmonaires à l'égard de la bradykinine (BK) et de l'angiotensine I (AI) chez des rats normaux et chroniquement hypoxiques. Ces derniers ont une hypertension pulmonaire et des artérioles pulmonaires muscularisées. Ces peptides sont respectivement inactivés et activés par l'angiotensine convertase (ACE) de l'endothélium pulmonaire. Les poumons isolés ont été perfusés à un débit constant, quand les modifications de la pression artérielle pulmonaire reflétaient des modifications de la résistance vasculaire. Les courbes dose-réponse à l'égard de la bradykinine (1 ng à 10 µg) ont été enregistrées au cours de la normoxie et au cours de la préconstriction par hypoxie; la bradykinine provoque à la fois une diminution et une augmentation de la résistance vasculaire, c'est-à-dire une vasodilatation et une vasoconstriction. Chez les rats normaux, seule la constiction est observée en normoxie, ce qui reflète un tonus vasculaire basal bas. Par contre, chez les rats chroniquement hypoxiques, l'on n'a observé qu'une dilatation, ce qui reflète un tonus vasculaire basal élevé. Chez les rats hypoxiques normaux, les petites doses provoquent une dilatation et les fortes doses une constriction. Chez les rats chroniquement hypoxiques, l'on observe à nouveau seulement une dilatation, celle-ci étant plus importante que chez les contrôles. Après administration de Captopril (inhibiteur de l'ACE), la constriction est accentuée chez les rats contrôle, à la fois en normoxie et en hypoxie; elle se développe chez les rats chroniquement hypoxiques après de fortes doses, à la fois en normoxie et en hypoxie; un oedème survient fréquemment. Les courbes dose-réponse à l'égard de l'angiotensine I (1 ng à 10 µg) en normoxie, montrent des réponses de pression fortement accentuées chez les rats chroniquement hypoxiques par rapport aux rats normaux; ceci est probablement attribuable à une augmentation de la

sensibilité à l'égard de l'angiotensine II (AII) plutôt qu'à une conversion accentuée de AI en AII. Le Captopril a provoqué une réduction proportionnelle des réponses dans les deux groupes de rats. Le rôle physiologique de la bradykinine et de l'angiotensine I au niveau pulmonaire est inconnu, mais en cas

de traitement par les inhibiteurs de l'angiotensine convertase, les conséquences pour le poumon et d'autres organes de la persistance de la bradykinine et d'une réduction de la formation d'angiotensine II pourraient être importantes.
Eur Respir J., 1990, 3, 779-785.