BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1995 Jul; 16 (4): 373—375

Effect of hypoxia on angiotensin Ⅱ content and its receptor in guinea pig aorta

WANG Ju-Feng[1], DONG Hua-Jin, XIAO Wen-Bin (*Institute of Pharmacology and Toxicology, Academy of Military Medical Sciences, Beijing 100850, China*)

AIM: To study the effects of hypoxia on the vasoconstrictive response and the angiotensin Ⅱ (Ang Ⅰ) content and its receptor. **METHODS**: Hypoxia was induced in isolated aorta of guinea pig by gassing N_2. The vasoconstrictive response of aorta to Ang Ⅱ was recorded. Ang Ⅰ content and its receptor were measured by radioimmuoassay. **RESULTS**: Ang 3—3000 nmol L^{-1} increased the contractile response of aorta. Hypoxia amplified the vasoconstrictve effect of Ang Ⅰ. The concentration of Ang Ⅰ were 44±24 and 50±17 pg/g wet wt (n=10), respectively in non-hypoxic and hypoxic aortae. The receptor density in non-hypoxic aorta was 17 ±3 fmol mg^{-1}, and that in hypoxia was 33 ± 5 fmol mg^{-1} ($P<0.01$). **CONCLUSION**: The enhancement of the vasoconstrictive action of Ang Ⅰ by hypoxia is due to the increase in the angiotensin receptor density, but not associated with the changes in the Ang Ⅱ content in hypoxic aorta of guinea pig.

KEY WORDS angiotensin Ⅰ; anoxia; aorta; angiotensin receptors

Renin-angiotensin system exerts its effects via production of the octapeptide angiotensin, which acts on specific angiotensin receptors to cause vasoconstriction. Hypoxia increased the concentration of angiotensin Ⅱ (Ang Ⅰ) in rat plasma and stimulate the release of norpinphrine in cardiovascular system, and the angiotensin receptors have different characteristics in different vessels[1-3]. The purpose of the study was undertaken to investigate the changes of content of Ang Ⅱ and its receptors in guinea pig aorta after hypoxia.

MATERIALS AND METHODS

Guinea pigs of either sex weighing 400 — 500 g were provided by the Animal Center of our Academy. Ang Ⅰ was purchased from Sigma Chemical Co. [^{125}I]-Ang Assay kit was purchased from China Isotope Co (Beijing, China). [^{3}H]Ang Ⅰ was product of Amersham International PLC (UK).

Effects of hypoxia on vasoconstrictive response to Ang Ⅰ Aorta ring near the heart of guinea pig was mounted in a 5-mL bath containing the buffer: NaCl 118; KCl 4.8; $NaHCO_3$ 25; $CaCl_2$ 2.5; $MgSO_4 \cdot 7H_2O$ 0.9; NaH_2PO_4 0.2; edetic acid 0.05; glucose 11 mmol L^{-1} (pH = 7.4) at 37 C and aerated with 95 % O_2 + 5 % CO_2. A tension of 2 gram was applied. The aorta was allowed to equilibrate for 45 min and washed every 15 min with the buffer. Hypoxic aorta was made to be gassed by N_2 for 30 min and to be reoxygenated for 15 min (three times repeatedly). The buffer was changed every 15 min. Tension was measured using transducers connected to JC-2 amplifiers and ERT-882 recoders (China).

Determination of Ang Ⅰ content Aorta was homogenized in the buffer. The homogenate was centrifuged at 10 100 · g and 4 C for 10 min. The supernatant was taken to measure the content of Ang Ⅱ by radioimmuoassay.

Radioligand binding assay of Ang Ⅱ The aorta membranes were prepared[4]. Binding of Ang Ⅱ to aorta receptors was studied over a concentration range of [^{3}H]Ang Ⅰ (specific activity 1410 g L^{-1} TBq L^{-1}; 20 — 160 nmol L^{-1}. Prepared aorta (2 g L^{-1})

[1] To whom correspondence should be addressed and now in: *Department of Pharmacology, University of Limburg, PO Box 616, 6200 MD Maastricht, The Netherlands.*

Received 1993-12-06 Accepted 1994-11-23

was incubated in a medium containing [^{3}H]Ang Ⅰ, Tris-HCl 10 mmol L^{-1}, $MgCl_2$ 5 mmol L^{-1} and bovine serum albumin (BSA) 2 g L^{-1} at pH 7.4 and at 37 C for 1 h in duplicate. Non-specific binding was determined in the presence of excess unlabeled Ang Ⅰ (16 μmol L^{-1}). The reaction was stopped by filtration under reduced pressure through GF/B filters, which were presoaked in washing buffer with 0.1 % BSA and washed immediately 4 times with 3 mL of ice-cold Tris-HCl 10 mmol L^{-1} containing NaCl 0.15 mol L^{-1}. The radioactivity trapped on the filter was counted using a scintillation counter (Wallac 1409, Germany).

RESULTS

Effects of hypoxia on vasoconstrictor response to Ang Ⅱ Ang Ⅱ 3—300 nmol L^{-1} increased the contraction of the isolated aorta in a concentration-dependent manner. After hypoxia, Ang Ⅱ also amplified the contraction of aorta ($P<0.05$ *vs* non-hypoxic group) (Fig 1).

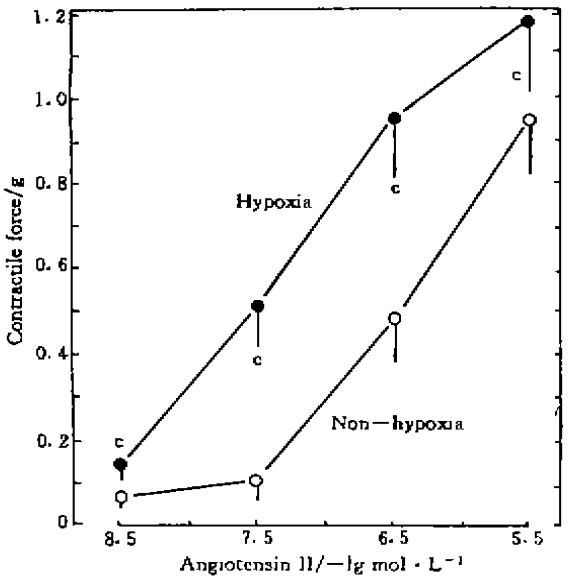

Fig 1. Effect of angiotensin Ⅱ on contraction of aorta of guinea pig. $n=8$, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* **non-hypoxia group.**

Ang Ⅱ content and its receptor Non-hypoxic and hypoxic aortae had similar concentrations of Ang Ⅰ: 44±24 and 50±17 pg/g wet wt ($n=10$), respectively ($P>0.05$). There was a difference in the binding characteristics of Ang Ⅱ receptor between the non-hypoxic and hypoxic aorta (Tab 1).

Tab 1. Angiotensin Ⅱ content ($n=10$) and its receptors ($n=7$). $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, *vs* **Normal.**

Aorta	Ang Ⅱ content, pg/g wet wt	Receptor density, fmol mg^{-1}	Affinity, μmol L^{-1}
Normal	44±24	17±3	0.15±0.08
Hypoxia	50±16	33—5^b	0.19±0.02

DISCUSSION

Our experiment showed that Ang Ⅱ increased the contraction of the aorta. After hypoxia, Ang Ⅱ markedly amplified the vessel contraction ($P<0.05$ compared with non-hypoxic aorta at an equal concentration). The data showed that hypoxia enhanced the vasoconstrictive action of Ang Ⅱ.

In angiotensin system, we considered that this enhanced sensitivity to Ang Ⅱ might reflect two mechanisms: 1. hypoxia activated ACE at local vessel, and then the production of Ang Ⅰ from Ang Ⅱ was accelerated by ACE; 2. Ang Ⅱ receptor was changed.

By the determination of Ang Ⅱ content, we found that the concentration of Ang Ⅱ was not significantly different between non-hypoxic and hypoxic aorta. The experiments indicated that the amplified vasoconstrictive response to Ang Ⅱ by hypoxia might be of no relationship with the changes in Ang Ⅱ concentration. However, many investigators found that angiotensin receptor changes were known to occur in responses to some conditions[5—7]. In our study, significant difference was seen in receptor density after hypoxia. Therefore, we suggest that hypoxia enhanced

vasoconstrictive response to Ang Ⅱ in guinea pig is due to the increase in angiotensin receptor, but is unrelated to the changes in the concentration of Ang Ⅱ.

ACKNOWLEDGMENT To Prof GU Du-Xin for his careful reading of the manuscript.

REFERENCES

1 Cai QQ, Yang YF, Cai YN, Deng XX, Zhou XM, Zhang YF, *et al.* Changes of plasma level of ANP, renin-angiotensin and hemodynamics in spontaneously hypertensive rats during chronic hypoxia. Chin J Appl Physiol 1992; **8**: 116—9.

2 Clark KL, Robertson MJ, Drew GM. A comparison of the charactristics of angiotensin receptors in the renal and mesenteric vascular beds of the anesthetized cat. J Cardiovasc Pharmacol 1992, **19**: 515—24.

3 Carlsson L, Abrahamson T, Almgren U. Release of noradrenaline in myocardial ischemia-importance of local inactivation by neuronal and extraneuronal mechanisms. J Cardiovasc Pharmacol 1986; **8**: 545—53.

4 Chang RSL, Siegl PKS, Clineschmidt BV, Mantlo NB, Chakravarty PK, Greenlee WJ, *et al.* *In vitro* pharmacology of L-158,809, a new highly potent and selective angiotensin Ⅱ receptor antagonist. J Pharmacol Exp Ther 1992; **262**: 133—8.

5 Vaughan DL, Rassell GI. Influence of dietary protein on glomerular angiotensin Ⅱ—receptor binding in normatensive and spontaneously hypertensive rats. Clin Sci 1992; **83**: 23—8.

6 Kimura B, Sumners C, Phillips MI. Changes in skin angiotensin Ⅱ receptors in rats during wound healing. Biochem Biophys Res Comm 1992; **187**: 1083—90.

7 Graves SW, Moore TJ, Seely EW. Increased platelet angiotensin Ⅱ receptor number in pregnancy-induced hypertension. Hypertension 1992; **20**: 627—32.

缺氧对豚鼠主动脉中血管紧张素Ⅱ含量和受体的影响

汪巨峰，董华进，肖文彬（军事医学科学院毒物药物研究所，北京100850，中国）

目的：观察缺氧对血管紧张素Ⅱ (angiotensin Ⅱ，Ang Ⅱ)收缩血管，Ang Ⅱ含量及受体的影响。**方法**：冲氮气诱导离体豚鼠主动脉缺氧记录加 Ang Ⅱ后，主动脉的收缩变化。放射免疫方法测定 Ang Ⅱ含量，并进行受体结合实验。**结果**：Ang Ⅱ 3—3000 nmol L^{-1}加强主动脉收缩。缺氧明显加强 Ang Ⅱ的血管收缩作用，缺氧和不缺氧主动脉中 Ang Ⅱ含量分别为50±17和44±24 (pg/g wet wt，n=10)，而受体密度则明显不同，前者为33±5，后者17±3 fmol mg^{-1}。**结论**：缺氧加强 Ang Ⅱ的血管收缩作用与血管紧张素受体有关。

关键词 血管紧张素Ⅱ；缺氧；主动脉；血管紧张素受体

Information for authors

Acta Pharamcol Sin 1995 Jan; **16** (1): 3—16.

Br Med J 1991 Feb 9; **302** (6772): 338—41.

N Engl J Med 1991 Feb 7; **324** (6): 424—8.