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Effects of angiotensin Ⅱ on release of CRH and AVP from hypothalamus during acute hypoxia[1]

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KEY WORDS angiotensin Ⅱ; corticotropin-releasing hormone; argipressin; hypoxia

ABSTRACT

AIM: To investigate the effects of angiotensin Ⅱ (Ang Ⅱ) on corticotropin-releasing hormone (CRH) and argipressin (AVP) release from median eminence (ME) of hypothalamus during acute hypoxia in rats. **METHODS**: Simulated hypoxia was performed in a hypobaric chamber. CRH and AVP were determined by radioimmunoassay (RIA). Plasma corticosterone concentration was measured by fluorometry. **RESULTS**: Ang Ⅱ did not influence CRH release induced by hypoxia [CRH in group pretreated with Ang Ⅱ (16 ± 8) ng/ME *vs* control (15 ± 4) ng/ME, both exposed to hypoxia with simulated altitude 7 km (8.2 % O_2)]. Ang Ⅱ enhanced AVP release, from (5.7 ± 1.6) ng/ME in control decreasing to (2.6 ± 1.2) ng/ME ($P < 0.05$), meanwhile plasma corticosterone concentration was also increased markedly, from (356 ± 58) in control to (536 ± 134) μg/L plasma ($P < 0.05$), which was partly abolished by administration of AVP antiserum. **CONCLUSION**: Ang Ⅱ might stimulate hypothalamo-pituitary-adrenal axis (HPA) through activating AVP but not CRH release during acute hypoxia.

INTRODUCTION

Corticotropin-releasing hormone (CRH) is a major central regulator of ACTH secretion from anterior pituitary, argipressin (AVP) is a weak stimulator of ACTH release but CRH and AVP can synergestically potentiate ACTH release several folds[1]. There is also evidence that angiotensin Ⅱ (Ang Ⅱ) potentiates the stimulation of CRH[2] *in vitro* and *in vivo*, acting through a central mechanism[3].

Ang Ⅱ administered icv has also been shown to increase proopiomelanocortin (POMC) mRNA in pituitary and CRH mRNA levels in paraventricular nuclei (PVN)[4,5]. A significant increase in CRH levels in hypophysial portal blood has been observed 30 min after injection of 100 ng Ang Ⅱ, indicating that stimulating effect of Ang Ⅱ on ACTH secretion is mediated by central CRH[6]. It has been reported that Ang Ⅱ icv-induced pituitary ACTH secretion requires CRH mediation but not AVP's, suggesting that AVP is not necessary for the stimulating effect of Ang Ⅱ on ACTH[2,7].

Peripheral administration of Ang Ⅱ also increased plasma ACTH and this was mediated at least in part by CRH, as the effect was blocked by CRH antiserum[8]. Peripheral Ang Ⅱ-mediated CRH release might be acting through one or more circumventricular organs, which contain abundant A Ⅱ receptors[9].

The purpose of the present study was to investigate the effect of Ang Ⅱ on hypothalamo-pituitary-adrencortical axis (HPA) activity and release of CRH and AVP, and to observe whether CRH and AVP were involved in the action of Ang Ⅱ on HPA during acute hypoxia.

MATERIALS AND METHODS

Hypoxia exposure Hypoxia exposure was performed in a hypobaric chamber[10,11]. Rats were exposed to a simulated altitude of 7 km (8.2 % O_2). After being exposed to hypoxia for 1 h rats were decapitated, trunk blood was collected, and median eminence (ME) of hypothalamus was dissected for hormone assay.

Experimental animals Wistar rats (♂), weighing 195 g ± 20 g, were purchased from Experimental Animal Center, Northwest Plateau Institute of Biology, Chinese Academy of Sciences (Grade Ⅱ, Certificate № 007). Rats were raised under standard conditions with a 12-h light/dark cycle (light on at 8:00 AM), and

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diet and water were available *ad lib*. To study the effect of central administration of Ang Ⅱ, rats were anesthetized with sodium pentobarbital (40 mg/kg, ip) and injected Ang Ⅱ 2 μL (saline as vehicle, 0.9 % NaCl) with or without Ang Ⅱ antiserum using microsyringe into the third ventricle: 2 mm posterior to the bregma on the midline and 4 mm below skull surface. Injection of 2 μL was delivered in 1 min. All reagents were dissolved in 0.9 % NaCl (saline). Control group received only 2 μL saline. Rats were randomized into 4 groups. Group 1: normal control group in 2 km, 15 % O_2 (icv saline 2 μL). Group 2: exposed to hypoxia with a simulated altitude of 7 km (8.2 % O_2) in a hypobaric chamber for 1 h (icv saline 2 μL). Group 3: hypoxia + Ang Ⅱ (rats, receiving icv Ang Ⅱ 0.1 nmol/L in saline 2 μL, were exposed to hypoxia with a simulated altitude of 7 km in a hypobaric chamber for 1 h). Group 4: hypoxia + Ang Ⅱ + Ang Ⅱ anti-serum (rats, injected icv with Ang Ⅱ 0.1 nmol/L + Ang Ⅱ anti-serum 2 μL, were exposed to the same altitude as group 3).

Hormone measurement CRH and AVP were determined by specific radioimmunoassay (RIA). Plasma corticosterone level was estimated by fluorometric method.

Statistical analysis Data were expressed as $\bar{x} \pm s$ and compared with t-test.

RESULTS

Effect of Ang Ⅱ on plasma corticosterone Rats, icv saline, 0.1 nmol/L Ang Ⅱ, Ang Ⅱ 0.1 nmol/L + Ang Ⅱ antiserum 2 μL, were exposed to hypoxia for 1 h. Plasma corticosterone levels in hypoxia group [icv saline, exposed to hypoxia, (356 ± 58) μg/L plasma] significantly increased compared to control group (icv saline, not exposed to hypoxia, (213 ± 89) μg/L plasma) and was lower ($P < 0.05$) than the Ang Ⅱ group (536 ± 134) μg/L plasma ($P < 0.05$). Ang Ⅱ antiserum decreased the Ang Ⅱ-induced increase in plasma corticosterone levels [plasma corticosterone in group Ang Ⅱ + Ang Ⅱ antiserum: (414 ± 126) μg/L plasma]. This indicated that Ang Ⅱ stimulated the increase in plasma corticosterone levels and the effect of Ang Ⅱ on plasma corticosterone was partly blocked by Ang Ⅱ antiserum (Fig 1).

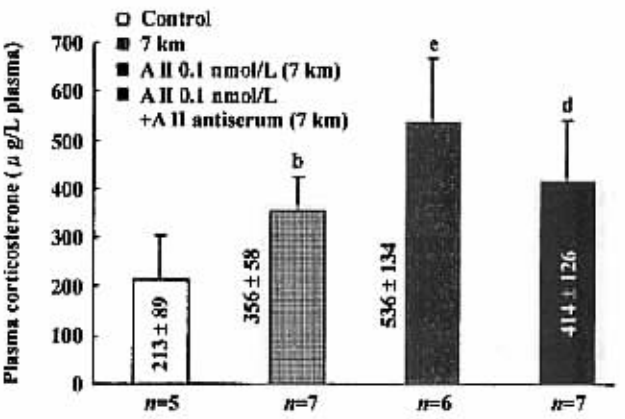

Fig 1. Effect of icv A Ⅱ on plasma corticosterone concentration during hypoxia. n = the number of rats. $^bP < 0.05$ *vs* control group. $^dP > 0.05$, $^cP < 0.05$ *vs* 7 km group.

Effect of A Ⅱ on CRH levels in ME Fig 2 shows the effect of icv Ang Ⅱ on CRH content in ME. In hypoxia group CRH contents were lower than control group (icv saline) which was not exposed to hypoxia [(15 ± 4) ng/ME *vs* (19.6 ± 1.9) ng/ME, $P < 0.05$]. Ang Ⅱ icv did not significantly influence hypoxia-induced change in CRH content [icv Ang Ⅱ group: (16 ± 8) ng/ME]. The results of CRH levels (Fig 2) and plasma corticosterone changes (Fig 1) indicated that hypoxia increased CRH release from ME and Ang Ⅱ had no effect on hypoxia-induced CRH release from ME.

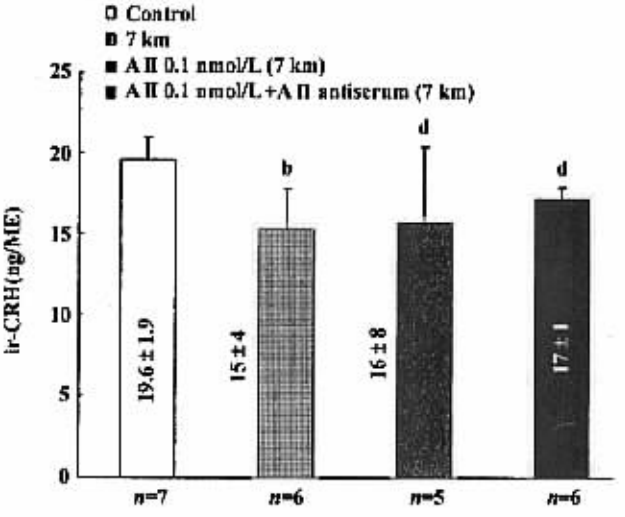

Fig 2. Effect of icv A Ⅱ on CRH content in ME during hypoxia. n = the number of rats. $^bP < 0.05$ *vs* control group. $^dP > 0.05$ *vs* 7 km group.

Effect of A Ⅱ on AVP levels in ME As shown in Fig 3, hypoxia had no effect on AVP content in ME compared with control [(4.5 ± 2.2) ng/ME in control, (5.7 ± 1.6) ng/ME in hypoxia, $P > 0.05$]. Ang Ⅱ icv group showed a decrease in AVP levels in ME [(2.6 ± 1.2) ng/ME] compared to hypoxia control

group, indicating that icv Ang Ⅱ may induce an increased release of AVP from ME during acute hypoxia exposure. Ang Ⅱ antiserum administration icv partly abolished the effect of Ang Ⅱ on AVP during hypoxia exposure.

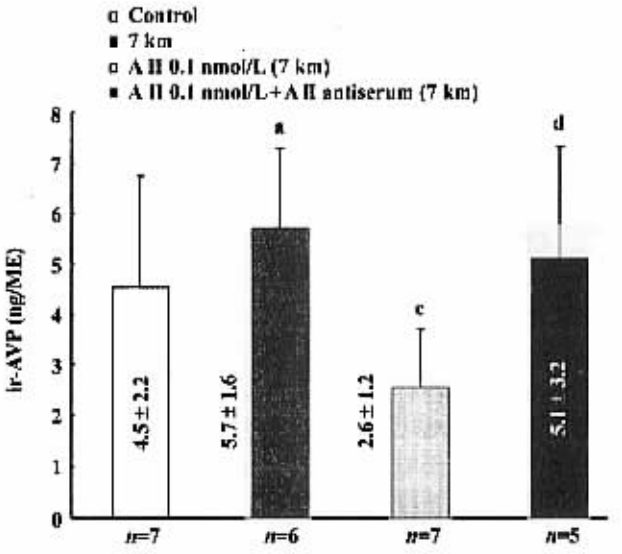

Fig 3. Effect of icv A Ⅱ on content of AVP in ME during hypoxia. n = the number of rats. $^aP > 0.05$ *vs* control group. $^bP > 0.05$, $^cP < 0.05$ *vs* 7 km group.

DISCUSSION

The results of the present experiments provided a new evidence for dual effects of Ang Ⅱ on the HPA functioning at hypothalamus levels, that is besides stimulating CRH release from ME under normal physiological conditions and perhaps stimulating AVP release from ME after hypoxia exposure.

Stress may influence CRH release from hypothalamus as the psychological stress[12]. Content of peptide in ME reflects the balance between the rate of supply of newly formed and the rate of release of the peptide. We have previously reported that decreased CRH content in ME may represent its secretion in ME during acute stress[13]. In the present study, hypoxia stress also decreased CRH content in ME and increased plasma corticosterone levels suggesting that hypoxia induced CRH secretion from ME and consequently activated HPA activity. AVP secretion, however, was not statistically affected by this acute hypoxia exposure. In rats, administered icv Ang Ⅱ, and exposed to hypoxia the AVP content in ME decreased and plasma corticosterone level significantly increased compared with control (icv saline) and the secretion of CRH induced by hypoxia was not significantly changed. This suggests that increased AVP secretion from ME might contribute to augmentation of plasma corticosterone levels. A possible reason for this might be related to Ang Ⅱ receptor involvement which may be differently activated under different physiological conditions. The distribution of Ang Ⅱ receptor mRNA in PVN, and the double staining data provide evidence, that Ang Ⅱ receptors are co-expressed with CRH in CRH cells of parvicellular division of the PVN. The location and morphological characteristics of the vasopressinergic cells containing Ang Ⅱ receptor mRNA are consistent with those of parvicellular CRH neurons which co-express with VP, but not with magnocellular VP cells[14]. These data might be the basis for explaining the Ang Ⅱ effects demonstrated above. We propose, therefore, that during acute hypoxia exposure, Ang Ⅱ may centrally modulate hypoxia-activated HPA function through stimulating AVP release from ME by activated Ang Ⅱ receptors located in parvicellular CRH neurons of expressing both CRH and AVP neuropeptides in PVN of rats (it seems not through stimulating CRH release), and AVP activated consequently improves ACTH and corticosterone secretion. This suggests that Ang Ⅱ regulates activity of HPA by a combination of receptors in different subpopulations of CRH neuron under different physiological condition. In conclusion, Ang Ⅱ stimulates HPA by increasing CRH secretion under normal physiological conditions, but might increase AVP secretion to regulate HPA in hypoxia-stressed rat, suggesting that different sub-populations of parvicellular CRH neurons of PVN or different Ang Ⅱ receptors may be involved in regulation of HPA under different physiological conditions.

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急性低氧条件下血管紧张素Ⅱ对下丘脑 CRH 和 AVP 分泌的作用[1]

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关键词 血管紧张素Ⅱ；促肾上腺皮质素释放素；精氨酸加压素；低氧

目的：研究低氧条件下血管紧张素Ⅱ对下丘脑促肾上腺皮质素释放素(CRH)和精氨酸加压素(AVP)分泌的作用. **方法**：低氧暴露采用人工模拟低压低氧. CRH 和 AVP 的测定采用放射免疫测定法. 血浆皮质酮测定采用荧光法. **结果**：7 km (8.2 % O_2) 急性低氧暴露 1 h，Ang Ⅱ不影响低氧引起的下丘脑 CRH 的分泌[低氧对照组：(15 ± 4) ng/ME，低氧、脑室给 Ang Ⅱ组：(160 ± 8) ng/ME]但可增加下丘脑 AVP 的分泌[ME 中 AVP 水平从低氧对照组(5.7 ± 1.6) ng/ME 下降到低氧、脑室给 A Ⅱ组(2.6 ± 1.2) ng/ME]，同时血浆皮质酮水平也升高[从(356 ± 58) μg/L plasma 升高到(536 ± 134) μg/L plasma]，此作用可被 AVP 抗体部分反转. **结论**：急性低氧条件下，Ang Ⅱ对 CRH 的分泌无明显直接作用，它可能通过增加下丘脑 AVP 的分泌进而刺激下丘脑-垂体-肾上腺皮质轴的功能.

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