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Beta-endorphin suppresses release of thyrotropin-releasing hormone in rat hypothalamus during acute hypoxia exposure[1]

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KEY WORDS beta-endorphin; thyrotropin-releasing hormone; naloxone; hypoxia; median eminence; paraventricular hypothalamic nucleus; intraventricular injections

ABSTRACT

AIM: To study the influences of β-endorphin (β-EP) on the responses of thyrotropin-releasing hormone (TRH) in median eminence (ME) and paraventricular nucleus (PVN) of hypothalamus to acute hypoxia in conscious rats. **METHODS**: Brain TRH, serum T_3 and T_4 were measured by radioimmunoassay. The male Wistar rats were exposed in a simulated hypobaric chamber at 7000 m altitude (8.2 % O_2) for 2 h. β-EP was given by intraventricular injection (icv) before hypoxia. **RESULTS**: β-EP (0.1 or 1 μmol/L, icv) elevated TRH levels of ME by 12 % ($P<0.05$) and 15 % ($P<0.05$) in treated groups comparing with saline control group (4.8±0.3) μg/g protein, and enhanced TRH of PVN by 24 % ($P<0.05$) and 44 % ($P<0.01$) in treated groups comparing with control group (180±21) ng/g protein during hypoxia. Meanwhile, serum T_3 and T_4 were significantly decreased ($P<0.05$ or $P<0.01$). Naloxone 10 μmol/L abolished the effects of β-EP (0.1 μmol/L) on TRH in ME ($P<0.01$) and PVN ($P<0.01$) as well as T_3 and T_4. Naloxone (10 μmol/L, icv) alone reduced contents of TRH in ME and PVN ($P<0.05$ or $P<0.01$), but increased the levels of serum T_3 and T_4 ($P<0.01$). **CONCLUSION**: β-Endorphin was involved in the modulation of hypothalamic TRH release of rats during hypoxia, through an inhibitory mechanism of TRH release in ME and PVN of hypothalamus.

INTRODUCTION

Opiates play a significant role in modulating neuroendocrine activity. Endogenous opiate system might be activated by stress[1]. It was demonstrated that β-endorphin (β-EP) decreased plasma thyroid-stimulating hormone (TSH)[2] and morphine depressed neuronal activity in the rat paraventricular nucleus (PVN)[3]. Hypoxia, as an unspecific stress factor, altered hypothalamo-pituitary-thyroid (HPT) function. When the animal exposed to high altitude, a lowering of thyroid function was observed[4]. Hypoxia (10 % O_2) subacutely and chronically suppressed thyrotropin-releasing hormone (TRH) mRNA expression in paraventricular nucleus of rat hypothalamus[5] and acute hypoxia elevated the content of TRH in hypothalamus[6], indicating that acute hypoxia-induced increased TRH might be due to a reduced release of TRH. However, the mechanism of increased TRH has been unclear so far. This paper aimed to explore the possible role of β-EP in regulating TRH release from median eminence (ME) and PVN of the hypothalamus in

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rats during hypoxia.

MATERIALS AND METHODS

Animals and chemicals Young male adult Wistar rats (Grade II, Certificate No 007), breeding in Animal Center of Northwest Plateau Institute of Biology, Chinese Academy of Sciences, weighing 140 g±20 g, were kept for at least a week before experiment processing in ambient temperature (17 °C±2 °C) with a light cycle of 12 h day-light and 12 h darkness. β-EP was purchased from Peninsula Laboratories Inc and naloxone was the Sigma products. Radioimmunoassy (RIA) kit of TRH was purchased from Beijing North Institute of Biological Technology and RIA kits of T_3 and T_4 were from Department of Isotope, China Institute of Atomic Energy.

Hypoxia simulation Hypoxia stress was induced by placing the rats in a hypobaric chamber. Simulated altitudes were set at 7000 m (8.2 % O_2, 41.04 kPa). The local altitude of our lab (Xining, Qinghai province) is 2300 m altitude, setting as control (15.8 % O_2, 75.12 kPa). The duration of exposure in chamber was 2 h.

Surgical procedure For icv injection, permanent stainless steel cannula was implanted in right lateral ventricle (AP: -1 mm; L: 1 mm; H: -3.5 mm; relative to the bregma[7]) 7 d before the experiments. Stereotaxic operations were performed under pentobarbital sodium anesthesia (40 mg/kg, ip). β-EP and naloxone were injected at 20 min before hypoxia exposure. β-EP and naloxone were dissolved in saline and injection volume was 5 μL. Control was given equal volume of saline.

Extraction and assay of hormone After hypoxia exposure, animals were sacrificed between 9:00-11:00 am and trunk blood was collected for thyroid hormone determination. The ME tissue was immediately removed from the hypothalamus[8] and stored in liquid nitrogen. Brains were quickly removed, frozen and placed at -20 °C. Brain slices of 100 μm thickness were cut with a cryostat-microtome (Microm GmbH HM505E, Germany) in temperature -17 °C. The PVN tissue was punched from slices and PVN was checked by light microscopy according to atlas of rat brain in stereotaxic coordinates[9]. ME and PVN tissues were homogenized later in a glass homogenizer in buffer solution (phosphate 0.01 mol/L, NaCl 0.15 mol/L, pH 7.5) and extracted with methanol. After centrifugation for 30 min at 10 000×*g* (4 °C), the supernatants were dried at 60 °C. The dried supernatants were suspended in buffer solution containing 0.25 % bovine serum albumin and stored at -40 °C[10] until analysis. An aliqot of homogenized liquid was taken for the protein determination according to Lowry's method.

The contents of TRH, T_3, and T_4 were measured by using the kits of radioimmunoassay. All samples were measured in duplicate. The intra- and inter-assay coefficients of variation were less than 5 % and 10 %, respectively.

Statistical analysis One-way analysis of variance with Duncan's test was used for calculating a significant difference. Values were shown as mean±SD.

RESULTS

Icv administration of β-EP (0.1, 1 μmol/L) caused increases of TRH levels in ME from (4.8±0.3) μg/g protein in control to (5.36±0.29) μg/g protein ($P<0.05$) and (5.5±0.5) μg/g protein of rats ($P<0.05$), respectively in hypoxia of simulated 7000 m altitude for 2 h (Fig 1A). The increased effect of β-EP (0.1 μmol/L) on TRH was abolished by icv of naloxone (naloxone 10 μmol/L+β-EP 0.1 μmol/L), showing that the TRH level was (4.3±0.3) μg/g protein. Naloxone injection alone (10 μmol/L) decreased the levels of TRH [(3.7±0.5) μg/g protein ($P<0.01$)]. The content of TRH in PVN was (180±21) ng/g protein in control and β-EP (0.1, 1 μmol/L) produced increased TRH of 24 % ($P<0.05$) and 44 % ($P<0.01$). Icv β-EP +naloxone and naloxone alone reduced TRH in PVN by 23 % ($P<0.05$) and 26 % ($P<0.05$), respectively, compared with control during hypoxia (Fig 1B). The levels of T_3 and T_4 in serum were significantly lowered in β-EP administered groups thanthose of saline groups (Fig 2A, B). The reduced effects of β-EP (0.1 μmol/L) on T_3 and T_4 were abolished by icv naloxone+β-EP 0.1 μmol/L and naloxone alone.

DISCUSSION

It has been understood that hypothalamic TRH neurons morphologically projected to ME of hypothalamus, in which TRH neuro-hormone released and transported into the anterior pituitary through pituitary portal blood, as well as regulated TSH activity. Therefore, the level of TRH in ME would be a mark of TRH neuron behavior in PVN of hypothalamus. In general, lowered level of TRH in ME indicated an increased release of TRH from ME and/or a reduced biosynthesis of TRH in the neurons and/or in both, depending on the physiological state tested. For example, the decreased level

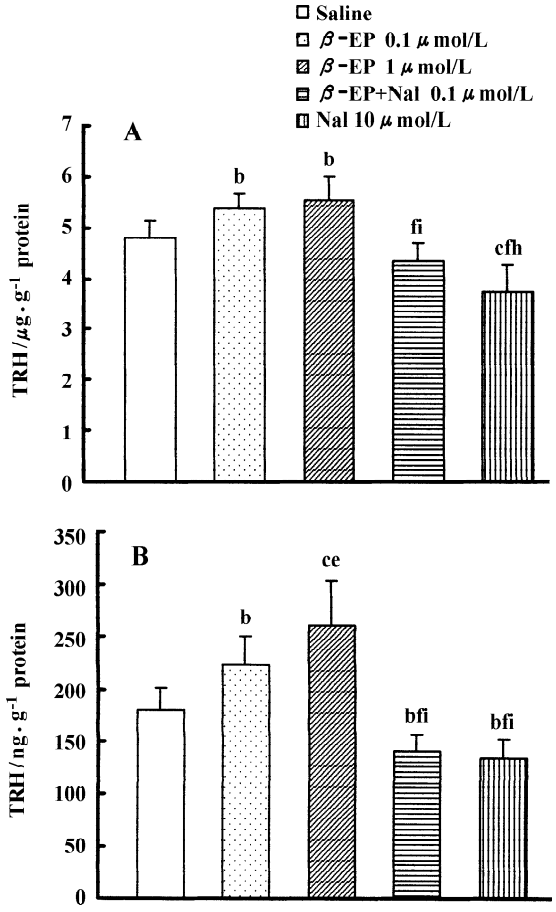

Fig 1. b-EP (icv) increased TRH levels in ME (A) and PVN (B) of conscious male rats at simulated hypoxia 7000 m (8.2 % O_2) for 2 h. The effects of b-EP were abolished by icv Naloxone. The naloxone (Nal) injection alone reduced TRH level. n=5. Mean±SD. ^{b}P<0.05, ^{c}P<0.01 *vs* saline control group. ^{e}P<0.05, ^{f}P<0.01 *vs* b-EP 0.1 mmol/L group. ^{h}P<0.05, ^{i}P<0.01 *vs* b-EP 1 mmol/L group.

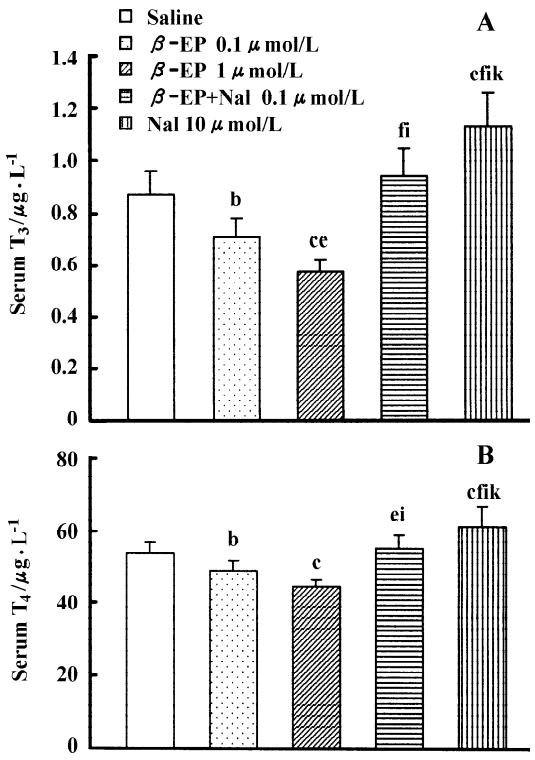

Fig 2. b-EP (icv) reduced the levels of serum T_3 (A) and T_4 (B) of conscious male rats at simulated hypoxia 7000 m for 2 h. Icv naloxone (Nal) abolished the effects of b-EP. Nal injection alone increased the level of serum T_3 and T_4. n=5. Mean±SD. ^{b}P<0.05, ^{c}P<0.01 *vs* saline control group. ^{e}P<0.05, ^{f}P<0.01 *vs* b-EP 0.1 mmol/L group. ^{i}P<0.01 *vs* b-EP 1 mmol/L group. ^{k}P<0.05 *vs* b-EP+Nal group.

of TRH in ME by thyroidectomy was due to an increased TRH release[11]. Hypothyroidism pathophysiologically reduced TRH level *in vivo* and increased release of TRH from ME[12]. Immunocytochemical study showed that TRH neurons essential for TSH secretion projected to ME were exclusively distributed in the most medial parts and the rostral parts of the PVN[13].

The variety of stress led to the changes of TRH in ME. We previously reported that acute hypoxia (7000 m, 2 h) elevated the levels of TRH in ME and PVN, and reduced serum T_3 and T_4 in rats[14], but what a mechanism would be involved has been unclear so far. In the present study, it was showed that β-EP treated icv induced a further enhancement of the level of TRH in ME during hypoxia (7000 m, for 2 h), indicating a decreased secretion of TRH from ME and PVN but not an increased biosynthesis of TRH in TRH neurons. We had also demonstrated that chronic hypoxia exposure (10 % O_2, exposed from 2 d to 30 d) suppressed TRH mRNA expression in PVN but acute hypoxia (2 h) did not significantly altered TRH mRNA level in PVN[5], which might be due to relatively short time for changing TRH biosynthesis or might be relatively lower TRH mRNA production, which was undetectable. Therefore, β-EP-induced further increased TRH level in ME possibly was due to an inhibitory effect on TRH release from ME, thus decreased circulating T_3 and T_4 levels were consequently followed. During hypoxia, β-EP-induced decreased release of TRH from ME was indirectly supported by similar reports, such that a microinjection of β-EP into the third ventricle resulted in a fall in plasma TSH[2]. Opioids and morphine depressed neuronal activity in the rat PVN slices[3]. Morphological observation demonstrated that pro-opiomelanocortin (POMC)-derived peptide neurons send fiber to the PVN, providing an evidence for the presence of endogenous

opioid synaptic relations between POMC and PVN neurons[15].

Considering that β-EP-induced inhibited action on TRH release was blocked by icv administration of naloxone, we proposed that endogenous opioid receptor involved in such suppressed mechanisms during hypoxia. However, which type of receptor was mediated remained unknown.

In conclusion, β-EP icv injection led to reducing TRH release from ME and PVN of the hypothalamus during acute hypoxia and this suppressed mechanism of TRH secretion from ME was acted through an endogenous opioid receptor.

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