

Norepinephrine attenuates hypoxia-inhibited thyrotropin-releasing hormone release in median eminence and paraventricular nucleus of rat hypothalamus

Tian-De Hou¹ & Ji-Zeng Du^{2*}

¹ Physiology Group, College of Life Sciences, Northwest Normal University, Lanzhou 730070, CHINA.

² Division of Neurobiology and Physiology, College of Life Sciences, Zhejiang University, Yuquan Campus, Hangzhou 310027, CHINA.

Correspondence to: Professor Ji-Zeng Du,
TEL: +86-571-87960630
FAX: +86-571-87960630
EMAIL: dujz@cls.zju.edu.cn

Submitted: August 19, 2004

Accepted: August 22, 2004

Key words: **adrenergic receptor; α_2 -receptor; hypoxia; hypothalamus; norepinephrine; paraventricular nucleus; thyrotropin-releasing hormone**

Neuroendocrinol Lett 2005; 26(1):43-49 PMID: 15726019 NEL260105A08 © Neuroendocrinology Letters www.nel.edu

Abstract

OBJECTIVE: We have previously found that chronic hypoxia inhibited thyrotropin-releasing hormone (TRH) mRNA expression in rat paraventricular nucleus (PVN). This study presented the effects of hypoxia on TRH secretion in rat hypothalamus, and the norepinephrine (NE) involvement in the modulation of TRH secretion during acute hypoxia exposure.

SETTING AND DESIGN: Hypoxia was simulated at altitudes of 5km (10.8% O₂) or 7km (8.2% O₂) in a ventilated hypobaric chamber, and control group was set at local altitude of 2.3km (15.8% O₂). The duration of hypoxia exposure was designed acutely and chronically for 0.5, 2, 24h, 5, 10, and 30d, respectively. TRH levels were measured by specific radioimmunoassay.

RESULTS: The results showed that hypoxia of 5km or 7km significantly enhanced TRH levels of the ME and PVN, and reduced serum T₃ levels in most hypoxia-exposed groups. Intraventricular injection (icv) of NE (4 nmol/L) induced a decrease in TRH levels in the median eminence (ME) and PVN, and an increased serum T₃ levels following hypoxia of 7km exposure for 2h, compared with icv saline control, indicating TRH release increased. The stimulating effect of NE on the TRH secretion was abolished by icv antagonist of adrenergic α_2 -receptor, yohimbine (40 nmol/L).

CONCLUSIONS: We conclude that acute and chronic hypoxia exposure produces an inhibition of hypothalamic TRH secretion from the ME and PVN. Central adrenergic system may play a stimulating role through α_2 -receptor in the acute hypoxia-modulating TRH release from rat hypothalamus.

Abbreviations:

TRH thyrotropin-releasing hormone
PVN paraventricular nucleus
NE norepinephrine
ME median eminence
HPT hypothalamo-pituitary-thyroid
TSH thyroid-stimulating hormone

GH growth hormone
SS somatostatin
PeN pariventricular nucleus
HPA hypothalamo-pituitary-adrenocortical
CRH corticotropin-releasing hormone
ACTH adrenocorticotrophic hormone

Introduction

Hypothalamo-pituitary-thyroid (HPT) axis plays physiologically important roles, regulating body growth and development, in particular developmental brain, and energy-metabolism-balance and expenditure etc [1, 2]. The pituitary thyroid-stimulating hormone (TSH) and thyroid hormone axis were influenced by several factors, and controlled by hypothalamic thyrotropin-releasing hormone (TRH), a crucial controller of HPT axis. TRH release and biosynthesis were modulated by numbers of neurotransmitters and neuropeptides as well as hormones. The levels of TRH in the neurons of the paraventricular nucleus (PVN) and in the terminal of median eminence (ME) of the neurons in the hypothalamus showed a kinetic balance of the TRH biosynthesis and release under basal and stressed status. Examining neurons containing TRH in PVN and the terminal in ME is helpful to reveal the hypothalamic mechanisms for regulating HPT axis affected by stressors, including environmental factors of cold, hypoxia, and others, as well as autonomic nerves systems. A large bodies of evidence showed that TRH was affected by cold, psychological stress etc. Recently, TRH was reported to be involved in behavior [3], and in iron-regulatory protein binding in pituitary cells [4]. TRH was also showed to effect on GABAergic synaptic transmission of CA1 neurons of the rat hippocampal slice during hypoxia [5]. We have reported that acute simulated altitude hypoxia caused a lowered thyroid function [6]. TRH mRNA expression in rat PVN was acutely and chronically decreased by exposure to 10% O₂ [7]. However, the effects of continual hypoxia upon TRH peptides levels in PVN and ME are still unclear so far. Since acute or chronic hypoxia caused an inhibited pituitary growth hormone (GH) release and mRNA (GH mRNA) expression, and body weight gain [8], which might be correlated with hypoxia-induced somatostatin (SS) release and increased SS mRNA expression [9]. HPT axis might also be involved in hypoxia-induced inhibition of growth and development. The effects of high altitude hypoxia on healthy has long been considered important because large populations of people live at high altitude, and many others like to visit for trekking and climbing or athletic training. So investigating hypoxia-induced change of HPT axis and its acting principle are much of importance. This paper was designed to exam the continual hypoxia-caused TRH peptides alteration in hypothalamus, and the involvement of norepinephrin (NE) and adrenergic receptors in mechanisms.

Materials and Methods

Animals: Adult male Wistar rats (130–160 g, Grade II, Certificate No. 007) were purchased from the Animal Center of Northwest Plateau Institute of Biology, Chinese Academy of Sciences, China, and maintained in a 12:12 h light: dark cycle (light on 07:00–19:00 h) at 20±2°C with free access to food and water. Rats were housed in a group of six and acclimated to these conditions for one week prior to experimental manipulations.

All efforts were made to minimize animal suffering and to use small a number of animals as compatible with the accuracy of the experiment. The NIH laboratory animal care guideline was followed.

Hypoxia simulation: The rats were placed in a hypobaric chamber (1 m³ in volume). Two-altitude simulation of 5km (hypoxia, 10.8%O₂, 54.02kPa) and 7km (hypoxia, 8.2%O₂, 41.02kPa) were administrated for 0.5, 2, 24h, 5, 10, and 30 d, respectively, with the same light: dark cycle, nutrition and temperature described above. Control groups were set in the same chamber at local altitude of 2.3km altitude (15.8% O₂, 75.12kPa).

Surgery and Drug Treatment: One week prior to the experiment, the rats were implanted with a chronic brain cannula in the right lateral ventricle (AP: –1 mm; L: 1mm; H: –3.5mm; relative to the bregma [10]) of the brain for intracerebroventricular (icv) injection. The right lateral cerebral cerebroventricle was localized in accordance with the coordinates of Paxinos and Watson [11]. The cannula implantation was operated according to the method reported by Weiss [12]. Briefly, the animal was anesthetized by pentobarbital (40 mg/kg, ip) and was placed in a stereotaxic frame (SR6, Narishige, Japan) using nontraumatic ear bars. Under aseptic conditions, a small hole was drilled in the skull overlying the right lateral cerebral ventricle and a guiding cannula (22 G, C313G, PlasticOne Inc., USA) was lowered into the right lateral cerebral ventricle (the lateral coordinate for the stereotaxic surgery was 0.0). The cannula was sealed in place using cold curing dental acrylic. After the operation, the rats were nursed for 6 hours before being sent back to the animal room.

On the day of trials, the brain cannula was extended and every possible step was taken to minimize disturbance of the animal. NE (Fluka Chemie AG, Switzerland, 4 nmol/L), α₁-adrenoceptor antagonist (Prazosin, Fluka Chemie AG, Switzerland, 40 nmol/L), α₂-adrenoceptor antagonist (Yohimbine, Fluka Chemie AG, Switzerland, 40 nmol/L), and β-adrenoceptor antagonist (Propranolol, Peninsula Laboratories Inc, 40 nmol/L) or vehicle were injected, respectively, through the brain cannula 30min before stress. All injection volumes were 5μl. Controls received appropriate amounts of saline, respectively. The solution was delivered at 5μl/ min and the injection needle was left in the site for 0.5 min after completion of the infusion.

ME and PVN Tissue Preparation: After stress, the animals were sacrificed between 09:00 to 11:00 h to keep the same daily rhythmic hormone release. Blood was collected for the T₃ (triiodothyronine) assay. Brains were quickly removed, frozen in liquid nitrogen and stored at –80°C. Serial sections of 300 μm (Cryostat Microtome HE 505, Germany) were cut and different anatomical structures were micropunched under a dissecting microscope according to the methods [13]. The bilateral PVN (bregma 1.3–2.12) and ME (2.12–4.0), corresponding to those in plates 25 and 26 [11] were sampled. They were later homogenized in glass homogenizers (0.01 M phosphate, 0.15 M NaCl, pH7.5). An aliquot (0.1 ml) was taken for protein determination according to the Lowry's methods and the remainder was for extraction

with methanol. After centrifugation for 30 minutes at 10000×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 -80 °C for TRH assay [14, 15].

TRH and T₃ assay: The TRH and T₃ content were measured by sensitive and specific radioimmunoassay (RIA), respectively [16]. RIA kit of TRH was purchased from Beijing north institute of biological technology and RIA kits of T₃ was from department of isotope, China institute of atomic energy, China. The sensitivity of the TRH assay was 2 pg/tube. The intra- and interassay coefficients of variation were 5.0% and 9.0%, respectively. T₃ assay sensitivity was 2 pg/tube; intra- and interassay variations were 5.0% and 9.0%, respectively.

Statistical Analysis: Data were calculated as mean±SD. The significance of differences was assessed with Student's t-test and one-way analysis of variance with Duncan's test. The SPSS statistical package (Version 10.0) was utilized for the analysis, and the level of significance was set at 0.05.

Results

Responses of TRH in ME and PVN of hypothalamus to acute and chronic-continual hypoxia

Effects of acute and chronic hypoxia on TRH in ME and in PVN were showed in Fig. 1 and Fig. 2. Acute hypoxia of altitude 5km for 2h or 24h significantly elevated the levels of TRH in both ME and PVN (vs. control, $P < 0.05$), and the peak of TRH occurred 24h after the hypoxia exposure. Severe acute hypoxia of 7km altitude for 2h elevated TRH in ME and PVN of hypothalamus (vs. control, $P < 0.01$), however the hypoxia for 24h significantly caused an increased TRH in PVN but the level of TRH in ME did not exceed control, the peak of TRH occurred 2h after the hypoxia exposure rather than 24h (7km hypoxia vs. 5km hypoxia).

Prolonged exposure of the hypoxia, either 5km or 7km, for 5, 10, and 30d significantly increased the TRH in ME (both altitude vs. control, $P < 0.05$ or $P < 0.01$), but increased the TRH in PVN only for some of exposed day.

The responses of TRH in PVN and ME to the acute continual hypoxia of 5km or 7km seemed to be dependent with hypoxia severity and time duration. (Fig. 1 and Fig. 2).

Responses of circulating T₃ to acute and chronic-continual hypoxia

Acute and chronic-continual hypoxia of 5km or 7km for 0.5, 2, 24h, 5, 10, and 30d, markedly reduced the rat serum T₃ (except for the hypoxia 5km for 30d), compared with control ($p < 0.05$ or $P < 0.01$). The reduced serum T₃ with hypoxia-severity and time course dependent manner fast (through 0.5, 2, and 24h) went down to the lowest point at 24h of the hypoxia exposure (vs. control, $P < 0.01$, Fig.3.A). The reduced serum T₃ was chronically lasted through 5, 10, and 30d except for the hypoxia of 5km for 30d (end of examined day) (Fig. 3. B). The 5km hypoxia-induced decreased circulating

T₃ were initially from at 0.5h, and were reached lowest point at 24h, then gradually returned to control level on the day 30. However, 7km hypoxia-induced lowed circulating T₃ level was similar with 5km hypoxia, but seemed to be unrecoverable. The lowered circulating T₃ level was due to a reduced TRH release in PVN and ME of the hypothalamus.

NE-induced stimulating response of TRH release in PVN and ME to acute hypoxia of 7km

The influence of NE on TRH was showed in Fig. 4 and Fig. 5. Intraventricular administration of NE (5μl, 4 nmol/L) caused a significant decrease of TRH level in ME from 5.36 ± 0.28 μg/g protein in control to 4.54 ± 0.46 μg/g protein ($P < 0.05$) of rats in hypoxia group of 7km for 2h (Fig. 4). The decreased effect of NE (4 nmol/L) on TRH in ME was abolished by an icv injection of yohimbine (40 nmol/L yohimbine + 4 nmol/L NE) during the hypoxia, showing that the TRH level was increased to 6.15 ± 0.54 μg/g protein in ME (Yohimbine + NE vs. NE, $P < 0.01$). The content of TRH in PVN was 225.31 ± 14.78 ng/g protein in control, icv NE (4 nmol/L) decreased TRH by 18.64% (Fig. 5, $P < 0.05$). Icv NE + yohimbine abolished NE-induced TRH decrease ($P < 0.01$) during the hypoxia. Propranolol failed to abolish the NE-caused TRH decrease in ME and in PVN (icv prop + NE vs. icv NE group). Prazosine (icv) significantly abolished the NE-induced TRH decrease in PVN (icv Praz + NE vs. icv NE group, $P < 0.01$), but not in ME during the hypoxia exposure.

NE-induced response of serum T₃ enhancement to acute hypoxia of 7km

Icv NE produced a significant serum T₃ enhancement (vs. icv saline, 0.75 ± 0.07 μg/L, $P < 0.01$, Fig. 6). The NE-induced serum T₃ enhancement was significantly abolished by icv yohimbine + NE ($P < 0.01$, vs. icv NE group), but neither by icv prazosine nor propranolol during the hypoxia exposure.

The reduced ratio of serum / blood during chronic continual hypoxia

Acute hypoxia of 5km or 7km for 0.5h caused no significant alteration of the ratio of serum / blood, but chronic continual hypoxia of 5km or 7km for 5, 10, and 30d caused very significant reduction of the ratio of serum / blood (vs. control, $P < 0.01$, Table 1.)

Discussion

The data from this study showed that acute or chronic continual hypoxia exposure significantly affected the TRH levels in PVN and ME of rat hypothalamus, where TRH neuron located and TRH neuronal terminal projected, respectively. Acute or chronic continual hypoxia of altitudes of 5km or 7km for various tested duration significantly increased TRH levels in PVN and ME (only some exception), indicating that reduced release of the TRH occurred. A number of investigations have been conducted physiologically and morphologically to delineate the area of the PVN

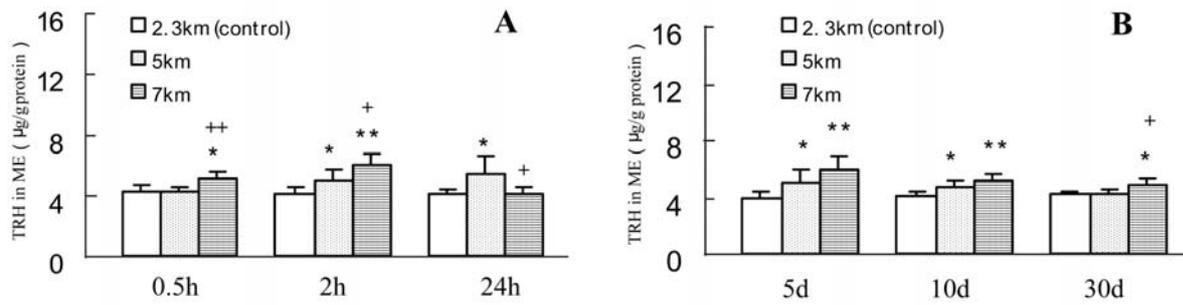


Figure 1. The TRH levels of ME (A and B) in rats were influenced by hypoxia. TRH contents in ME were increased in most hypoxia-exposure groups at 5km and 7km altitude. Values were given as the mean ± SD. n=6. *P<0.05, **P<0.01 vs. control (2.3km). +P<0.05, ++P<0.01 vs. 5km. 2.3km altitude ≈15.8% O₂, 5km altitude ≈10.8% O₂, 7km altitude ≈8.2% O₂. Data were analyzed by T-test.

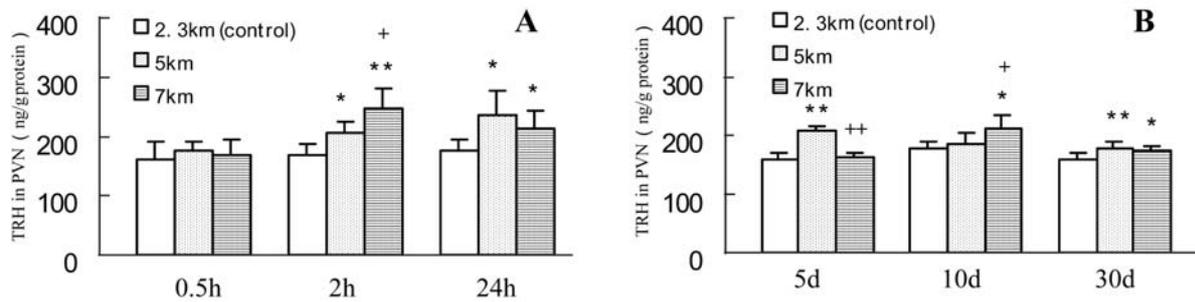


Figure 2. The TRH levels of PVN (A and B) in rats were influenced by hypoxia. TRH contents in PVN were increased in most hypoxia-exposure groups at 5km and 7km altitude. Values were given as the mean ± SD. n=6. *P<0.05, **P<0.01 vs. control (2.3km). +P<0.05, ++P<0.01 vs. 5km. Data were analyzed by T-test.

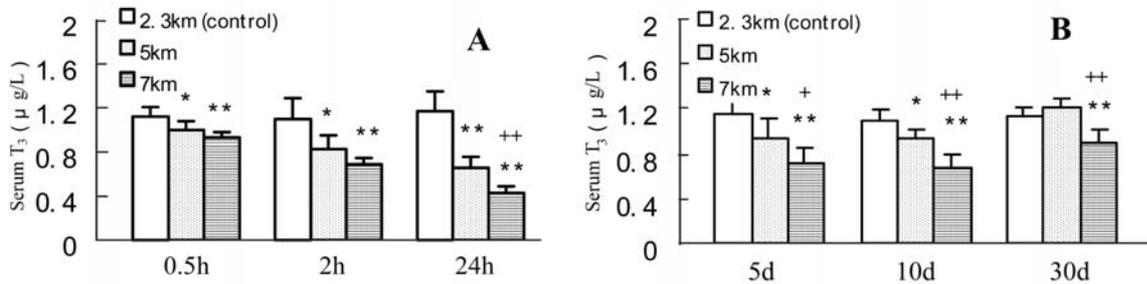


Figure 3. The alteration of serum T₃ (A and B) induced by hypoxia. Hypoxia at 5km and 7km altitude decreased circulating total T₃ in most hypoxia-exposure groups. Values were given as the mean ± SD. n=6. *P<0.05, **P<0.01 vs. control (2.3km). +P<0.05, ++P<0.01 vs. 5km. Data were analyzed by T-test.

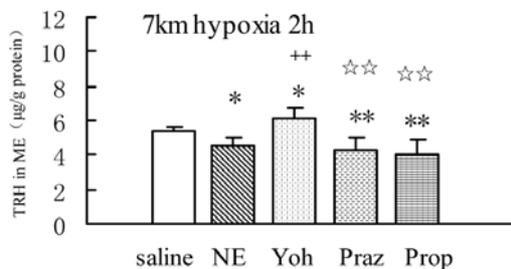


Figure 4. NE modulated the response of TRH in ME. NE (icv) decreased TRH levels in hypoxia-exposure groups. Values were given as the mean ± SD. n=6. *P<0.05, **P<0.01 vs. icv saline control group of hypoxia. ++P<0.01 vs. icv NE group. **P<0.01 vs. icv yohimbine (Yoh) +NE group. Data were analyzed by Duncan's test.

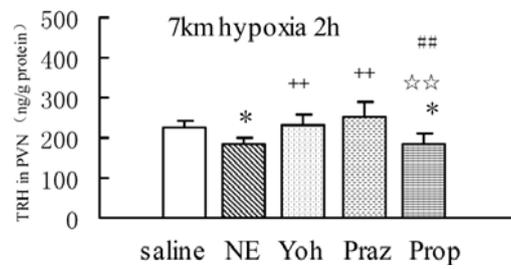


Figure 5. NE modulated the response of TRH in PVN. NE (icv) decreased TRH levels in hypoxia-exposure groups. Values were given as the mean ± SD. n=6. *P<0.05 vs. icv saline control group of hypoxia. ++P<0.01 vs. icv NE group. **P<0.01 vs. icv Yoh +NE group. ##P<0.01 vs. icv Prazosine (Praz) +NE group. Propranolol (Prop) did not alter TRH levels compared to icv NE. Data were analyzed by Duncan's test.

Figure 6. NE modulated response of circulating T₃ to acute hypoxia (2h, 7km altitude).

NE (icv) increased level of total T₃ of serum.
 *P<0.05, **P<0.01 vs. icv saline control group of hypoxia.
 ++P<0.01 vs. icv NE group.
 *P<0.05, **P<0.01 vs. icv Yoh +NE group.
 Data were analyzed by Duncan's test.

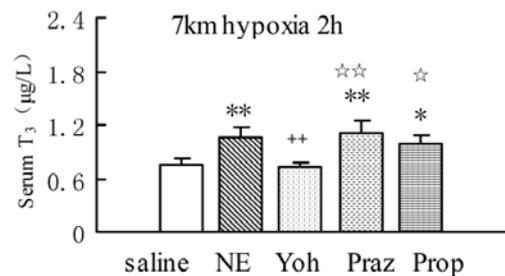


Table 1. Variation of rat serum at hypoxia

Time of hypoxia exposure	Serum/blood (%)		
	control	5km	7km
0.5 h	46.50 (±4.24)	43.05 (±3.17)	42.66 (±3.40)
5 days	44.84 (±3.63)	35.59 (±3.00)**	32.53 (±2.87)** +
10 days	44.61 (±2.41)	32.28 (±2.57)**	20.41 (±3.22)** ++
30 days	43.10 (±3.03)	23.95(±2.54)**	14.25 (±2.31)** ++

*P<0.05, **P<0.01 vs. control (2.3km). +P<0.05, ++P<0.01 vs. 5km.
 Data were analyzed by T-test. Blood was centrifuged at 3000 rpm for 20 min at 4°C.

of the hypothalamus, which was involved in control of TSH secretion from the anterior pituitary [17]. The level of TRH in ME would be a mark of TRH neuron behavior in PVN of the hypothalamus for its release [18, 19, 20]. In general, lowered levels of TRH in ME indicate a possible increase of TRH release from ME and/or a reduced biosynthesis of TRH in the neurons of PVN and/or in both, depending on the physiological states tested. We have previously demonstrated that hypoxia (10% O₂) chronically suppressed TRH mRNA expression in PVN of rats, but did not acutely the hypoxia for 2h. Therefore, the enhanced TRH neuropeptides in PVN and ME of rats seemed to be an inhibited release of TRH neuropeptides from the PVN and ME. Acute hypoxia might have an intensively inhibitory/reduced effect on TRH secretion and induced a TRH accumulation in the neuronal perikarya and terminal. Thus, due to the decreased TRH release, a decreased circulating T₃ level was consequently followed. Besides, it was hard to say clearly why the TRH level in ME in response to hypoxia of 7km for 24h was not kept in a higher level than control? Assumedly, it might be a lowered TRH biosynthesis in TRH neurons during the hypoxia exposure. Having compared the inhibited TRH release in hypothalamus and reduced circulating T₃ levels during the different acute hypoxia of altitude of 5km and 7km for 0.5 and 2h. It showed that the responses of TRH in hypothalamus and circulating T₃ levels to the acute hypoxia are of hypoxia-severity and time course dependent manner. Prolonged continual hypoxia exposure of 5km and 7km for 5, 10, and 30d basically induced a similar, as acutely, reduced TRH secretion, and may also inhibited TRH biosynthesis. Because, the study of TRH biosynthesis showed that hypoxia of 10% O₂ exposure declined TRH mRNA expression by 43.6%, 32.2%, 65.0%, 44.9% for 2, 5, 10, and 30 d, respectively [7]. Therefore, hypoxia-induced the higher TRH level in PVN and ME might be the result from a reduced release in ME and in PVN, not

from an increased TRH biosynthesis in TRH cell bodies of PVN of the hypothalamus.

There was evidence showing that hypothalamic neuropeptides were affected by hypoxia, and the hypoxia-affected neuropeptides might be involved in the modulation of hypoxia-reduced TRH releases. Hypoxia of 5km for 2 and 24h acutely caused the somatostatin release in ME and SS mRNA expression in paraventricular nucleus (PeN) of rats [9]. Moreover, The somatostatin suppressed TRH release [21, 22]. Intraventricular injection of β-endorphin inhibited TRH release from PVN and ME of rats [23]. Acute hypoxia (5%O₂, 0.5h) activated β-endorphin release and elevated β-endorphin level of systemic circulation in lamb [24]. Therefore, the somatostatin and opiate peptides may play important roles in inhibitory effects on TRH release during acute hypoxia. It was previously documented that exposure of hypoxia (10.8% O₂ and 8.2% O₂) for various time periods stimulated hypothalamo-pituitary-adrenocortical (HPA) axis in rats; acute and sub-acute hypoxia increased corticotropin-releasing hormone (CRF) release (much more release by 8.2% O₂ than by 10.8% O₂) from ME, and enhanced both plasma adrenocorticotrophic hormone (ACTH) and corticosterone [7]. Considering the anatomical connection between CRF and TRH neurons [25, 26], the possibility that CRF inhibiting TRH release might not be ruled out. It was observed that fornix took part in the decreased TRH release of PVN but not in ME during 2h hypoxia exposure (unpublished observation). The inhibitory fibers of fornix from hippocampus [27] might be involved in the modulation of TRH release during acute and chronic hypoxia. The TRH contents of PVN on the day 5 were not remained in higher levels following 7km altitude hypoxia exposure. It was assumed that it might be associated with highly inhibitory role in TRH biosynthesis, and might be due to highly expressed CRF mRNA in PVN during hypoxia. Because we have

reported that CRF mRNA expression in PVN of rats underwent a "Peak-point" on the day 5 following 10% O₂ exposure [7].

We have previously demonstrated that hypoxia acutely stimulated NE in PVN and in amygdala of rats, which was responsible for CRF release in ME and in PVN [28]. As hypothesized, NE was a stimulatory mediator on the HPT axis activity [29]. The α_1 - and α_2 -adrenergic receptor were involved in mediating an increased TSH release in basal rats [30]. Perfusion with artificial cerebrospinal fluid containing NE elicited a rapid rise in TRH release of the rat hypothalamic fragments though a α_1 -adrenergic receptor mechanism and an injection of NE into ME induced an increased TRH release [31]. The report that NE mediated TRH release in the stress animals has not so much been seen, however. Icv NE decreased TRH level in ME and in PVN of conscious rat during 2h hypoxia exposure at 7km altitude in the present study. The results showed that NE might play a role in the modulation of TRH secretion, which enhanced TRH release in hypoxia-exposed rats. NE injection also led to the circulating T₃ increase. The NE-induced release of TRH of ME and PVN was completely abolished by icv yohimbine, suggesting that adrenergic α_2 -receptor mediated NE-induced increased TRH release, but α_1 -adrenergic and β -adrenergic receptor did not produce significant influence on modulating ME TRH release, as α_1 -adrenergic receptor antagonist prazosine and β -adrenergic receptor antagonist propranolol could not abolish the NE-induced effects on TRH release of ME during the hypoxia.

Since icv yohimbine reversed NE-induced TRH release in PVN and ME through α_2 -receptor mechanism, consequently reversed NE-induced enhanced circulating T₃ level. As chronic hypoxia improved physiological adaptation by increasing red blood cells and hemoglobin contents, which led to increasing blood cells, but decreasing plasma or serum volumes (Table 1.), in some degree, this may influence T₃ concentration in serum as comparing to normal stage.

In conclusion, hypoxia acutely and chronically induced an inhibition of hypothalamic TRH secretion from the median eminence and the paraventricular nucleus of hypothalamus that consequently lowered thyroid function. Central adrenergic system might have a stimulating effect on the modulation of the TRH release from the hypothalamus of acute hypoxia-exposed rat through α_2 -receptor that consequently result in activating thyroid function.

Acknowledgements

This work was carried out in the Prof. Du's Lab of Northwest Plateau Institute of Biology, Chinese Academy of Sciences. The authors thank the Institute for the assistance.

This work was supported by the Science Technology Innovation Project of Northwest Normal University (No.02), and supported by the grant from NSFC (Major Project No. 30393134 and Project No. 30070289; 30270232).

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