

Effects of AP-V and bicuculline on somatostatin-positive neurons in hypothalamus of rats subjected to acute hypobaric hypoxia¹

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KEY WORDS bicuculline; somatostatin; hypothalamus; hypoxia

ABSTRACT

AIM: To investigate the effects of 2-amino-5-phospho-*novalerate-pharmacology* (AP-V) and bicuculline on somatostatin (SST)-positive neurons in hypothalamus of rats subjected to acute hypobaric hypoxia. **METHODS:** SST-immunoreactivity (IR) and somatostatin mRNA (SS mRNA)-positive neurons were measured by immunohistochemistry and *in situ* hybridization methods. **RESULTS:** Compared with control rats, SST-IR and SS mRNA-positive neurons in hypothalamic periventricular nucleus (PeV), paraventricular nucleus (PVN), and arcuate nucleus (ARC) increased after acute hypobaric hypoxia for 6 h ($P < 0.01$), and these effects were markedly inhibited by AP-V (10 μg , icv), a highly selective *N*-methyl-*D*-aspartate (NMDA) receptor antagonist, whereas were strongly enhanced by bicuculline (1.5 $\text{mg} \cdot \text{kg}^{-1}$, ip), a gamma-aminobutyric acid (GABA_A) receptor antagonist. **CONCLUSION:** SST possibly participates in acute hypoxic reaction in hypothalamus, furthermore, glutamate and GABA can affect somatostatin release and synthesis in hypothalamus through NMDA and GABA_A receptors respectively.

INTRODUCTION

The hypothalamus is a complex brain region encompassing nuclei having different roles in the regulation of endocrine, autonomic, and behavioral activities. Moderate hypoxia can induce strong neurosecretory reaction in the hypothalamus including release of corticotropin releas-

ing factor (CRF) and vasopressin (VP)^[1]. However, the effect of hypoxia on SST in hypothalamus is not clear. Altitude medicine studies showed that hypoxia due to high altitude induced high level of SST release in plasma^[2], which indicated that it might participate in the regulation of cardiovascular activities under hypoxic condition. Recent studies have demonstrated that glutamate and GABA have neuroendocrine roles regarding SST release and synthesis from hypothalamic neurons^[3-5]. It has been reported that hypoxia can induce the release of glutamate and GABA in brain, thus altering the balance between them^[6,7]. In this study, we mainly investigated the change in SST release in rats hypothalamus under acute hypoxia and the effects of AP-V and bicuculline on SST.

MATERIALS AND METHODS

Rats Wistar rats ($n = 30$, weighing 180-200 g, Grade II, Certificate No 24301050) of either sex were provided by the Experimental Animal Center of Third Military Medical University. Rats were divided into six groups randomly as following: (1) control group ($n = 6$): 2 rats received no stimulation; 4 rats were injected with 0.9 % saline (1.0 μL , icv or 1.0 mL, ip). (2) acute hypoxia group ($n = 6$). Animals were placed in hypobaric hypoxia chamber for 6 h (altitude equivalent to 6000 m). (3) AP-V (10 μg , icv) + hypoxia group ($n = 5$). (4) AP-V (10 μg , icv) group ($n = 4$). (5) bicuculline (1.5 $\text{mg} \cdot \text{kg}^{-1}$, ip) + hypoxia group ($n = 5$). (6) Bicuculline (1.5 $\text{mg} \cdot \text{kg}^{-1}$, ip) group ($n = 4$).

Drugs and reagents AP-V and bicuculline were purchased from Sigma, SST-antiserum (1:200) from Beijing Zhongshan Biological Co, ABC reagent kit from Vector Labs, SS mRNA *in situ* hybridization kits from Department of Histology and Embryology, Third Military Medical University.

ICV injection The method of AP-V intracerebroventricular (icv) microinjection was performed as in a

¹ Project supported by the National Natural Science Foundation of China, No 39670283.

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Received 2000-01-03

Accepted 2000-10-06

reference report^[6].

Immunocytochemistry After the experiments, the rats were perfused and fixed as usual. Serial sections (35 μm) of hypothalamus were cut on a freezing microtome and processed for immunohistochemistry using the ABC technique^[8]. In the negative controls, only SST antibody was replaced by normal goat serum or PPS (0.01 mol·L⁻¹). Fixing and staining were performed as above.

In situ hybridization The hybridization procedure was followed according to the method of Bloch *et al*^[9]. The probe used was digitoxin labeled antisense cRNA (0.5 mL·L⁻¹), and the concentration of anti-Dig antibody was 1:1000. The sections were stained with NBT/BCIP (400 mg·L⁻¹ and 200 mg·L⁻¹) for 12 h at 4 °C in darkness. No signals were detected in control sections. In the present study, neurons which had grain density of at least 5 times higher than the background density were considered to show positive expression.

Statistical methods Estimation of SST-IR and SS mRNA-positive neuron was performed on serial hypothalamus sections including periventricular nucleus (PeV), paraventricular nucleus (PVN) or arcuate nucleus (ARC), and 10 sections were selected randomly from every group and objectively assessed with an image analyzer. All values were expressed as $\bar{x} \pm s$, and treated with ANOVA.

RESULTS

Effects of AP-V and bicuculline on the number of SST-IR neurons in rat hypothalamus after hypoxia There were sparse and highly variable SST-IR

neurons in hypothalamus PeV, PVN, and ARC in the control group. In acute hypobaric hypoxia group, densely and deeply stained SST-IR neurons could be seen in the above-mentioned regions, the greatest number of SST-IR neurons were in PeV (38 \pm 4). In rats given AP-V (10 μg , icv) or bicuculline (1.5 mg·kg⁻¹, ip) 30 min before the onset of hypoxia, the number of SST-IR neurons in PeV, PVN, and ARC decreased by 44.8 % ($P < 0.01$), 34.9 % ($P < 0.01$), and 30.8 % ($P < 0.01$) or increased by 27.2 % ($P < 0.01$), 39.1 % ($P < 0.01$), and 62.2 % ($P < 0.01$) respectively compared with hypoxia group. The number of SST-IR neurons had no significant change in rats given AP-V (10 μg , icv) or bicuculline (1.5 mg·kg⁻¹, ip) alone (Tab 1).

Effects of AP-V and bicuculline on the number of SS mRNA positive neurons in rat hypothalamus *In situ* hybridization, scattered SS mRNA-positive neurons were visualized in the hypothalamus of the control group. In hypoxic group, SS mRNA-positive neurons in hypothalamus PeV, PVN, and ARC were increased compared with control group ($P < 0.01$). Densely and deeply stained SS mRNA neurons were highly concentrated in the PeV (45 \pm 4). On pretreatment with AP-V (10 μg , icv) or bicuculline (1.5 mg·kg⁻¹, ip), the number of SS mRNA-positive neurons decreased by 48.0 % ($P < 0.01$), 36.2 % ($P < 0.01$), and 29.7 % ($P < 0.01$) or increased by 34.9 % ($P < 0.01$), 38.2 % ($P < 0.01$), and 55.2 % ($P < 0.01$) compared with acute hypoxia group (Fig 1, Tab 2). The number of SS mRNA positive-neurons had no significant change in rats given AP-V (10 μg , icv) or bicuculline (1.5 mg·kg⁻¹, ip) alone.

Tab 1. Effects of AP-V and bicuculline on the number of SST-IR neurons in rat hypothalamus. $\bar{x} \pm s$. ^c $P < 0.01$ vs control group. ^f $P < 0.01$ vs hypoxia group. PeV: periventricular nucleus; PVN: paraventricular nucleus; ARC: arcuate nucleus.

Group	n	SST-IR neurons		
		PeV	PVN	ARC
Control	6	15.2 \pm 1.7	10.1 \pm 0.9	9.0 \pm 2.6
Hypoxia	6	38 \pm 4 ^c	21 \pm 3 ^c	18 \pm 5 ^c
Hypoxia + AP-V	5	20.7 \pm 2.5 ^f	14 \pm 3 ^f	12.8 \pm 2.7 ^f
AP-V	4	16.7 \pm 2.1	12.1 \pm 2.4	8.7 \pm 1.6
Hypoxia + bicuculline	5	48 \pm 3 ^f	29.5 \pm 2.3 ^f	30 \pm 4 ^f
Bicuculline	4	17.1 \pm 1.5	9.5 \pm 1.3	11.0 \pm 1.3

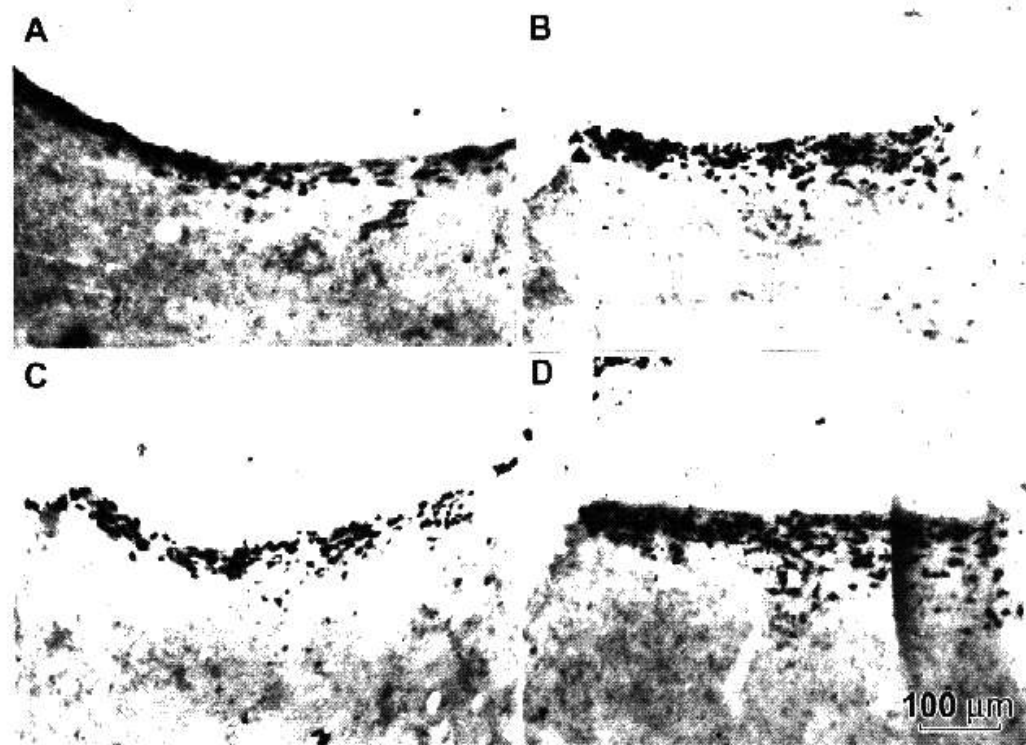


Fig 1. Photomicrographs of SS mRNA-positive neurons in the hypothalamus PeV. (NBT/BCIP stain, $\times 130$). A) Control; B) Hypoxia group; C) Hypoxia + AP-V; D) Hypoxia + bicuculline.

Tab 2. Effect of acute hypoxia on the number of SS mRNA-positive neurons in rat hypothalamus. $\bar{x} \pm s$. $^*P < 0.01$ vs control group. $^{\dagger}P < 0.01$ vs hypoxia group.

Group	n	SST-IR neurons		
		PeV	PVN	ARC
Control	6	18.3 \pm 1.5	11.0 \pm 2.7	12.1 \pm 0.9
Hypoxia	6	45 \pm 4 [*]	23.5 \pm 2.2 [*]	19 \pm 3 [*]
Hypoxia + AP-V	5	24.5 \pm 2.5 [†]	15.0 \pm 1.9 [†]	14 \pm 5 [†]
AP-V	4	20.5 \pm 2.0	10.7 \pm 1.8	10.9 \pm 1.7
Hypoxia + bicuculline	5	61 \pm 3 [*]	32 \pm 3 [*]	30 \pm 4 [*]
Bicuculline	4	19.8 \pm 2.5	10.7 \pm 2.1	13.0 \pm 1.4

DISCUSSION

In this study, we observed that acute hypoxia increased the number of SST-IR and SS mRNA-positive neurons in hypothalamus PeV, PVN, and ARC. Using somatostatin immunohistochemistry, somatostatin positive neurons were mainly seen in periventricular nucleus. Cell bodies immunoreactive for somatostatin were most

abundant in the preoptic and anterior periventricular hypothalamic areas. These neurons were shown to project to the median eminence which contained the highest concentration of somatostatin in the hypothalamus. In addition, by using *in situ* hybridization, somatostatin synthesizing neurons were highly concentrated in periventricular nucleus of the hypothalamus. We propose that hypoxia evoked release of somatostatin from hypothalamus mainly

came from enhanced somatostatin neurons synthesis, Rage and coworkers have drawn this conclusion consistently in cultured hypothalamic neurons^[4].

In rats treated with bicuculline before exposure to hypobaric hypoxia chamber, we demonstrated that SST-IR neurons and SS mRNA-positive neurons in the hypothalamus PeV, PVN, and ARC increased greatly. Our results suggest that GABA inhibit somatostatin release and synthesis from hypothalamus in the rats subjected to acute hypoxia. Moreover, GABA exerts inhibitory effect on somatostatin neurons mainly through GABA_A receptor, which is in keeping with the morphological evidence demonstrating somatostatin and alpha 2 sub-unit of GABA_A coexpression in PeV utilizing a dual labeling *in situ* hybridization technique^[10]. On the other hand, AP-V inhibited the increase of SST-IR neurons and SS mRNA-positive neurons in the hypothalamus induced by hypoxia. We can infer that acute hypoxia stimulated somatostatin release and synthesis in hypothalamus, which was affected by amino acids; glutamate facilitated somatostatin release and synthesize through NMDA receptor, while GABA inhibited this process through GABA_A receptor.

The findings suggest that somatostatin may participate in acute hypoxic reaction. Glutamate and GABA through NMDA and GABA_A receptors respectively modulate somatostatin level in rat hypothalamus following acute hypoxia. This also indicates that the balance between glutamate and GABA may mediate the change in somatostatin levels in systematic hypoxic environment.

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AP-V 和荷包牡丹碱对急性低压性低氧大鼠下丘脑内生长抑素阳性神经元的影响¹

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关键词 荷包牡丹碱; 生长抑素; 下丘脑; 低氧

目的: 研究 2-Amino-5-phosphonovalerate-pharmacology (AP-V) 和荷包牡丹碱对急性低压性低氧大鼠下丘脑内生长抑素(SST)阳性神经元的影响. 方法: 用免疫组织化学和原位杂交技术检测大鼠下丘脑内 SST-IR 和 SS mRNA 阳性神经元数. 结果: 急性低氧引起下丘脑室周核 (PeV)、室旁核 (PVN)、弓状核 (ARC) SST-IR 和 SS mRNA 阳性神经元数明显增多, 该效应可被 NMDA 受体拮抗剂 AP-V (10 μg, icv) 明显抑制; 而 GABA_A 受体拮抗剂荷包牡丹碱(1.5 mg·kg⁻¹, ip) 则使其增强. 结论: 这些结果表明下丘脑内的生长抑素参与了急性低氧反应, 且谷氨酸和 GABA 分别通过 NMDA 受体与 GABA_A 受体影响下丘脑内生长抑素的释放与合成.

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