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慢性给予左旋千金藤立定对纹状体多巴胺 D_1 和 D_2 受体密度和更新率的影响[1]

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关键词 左旋千金藤立定；纹状体；SCH-23390；放射配位体测定；多巴胺 D_1 受体；多巴胺 D_2 受体

目的：观察慢性给予左旋千金藤立定(SPD)对纹状体多巴胺(DA)受体密度和更新率的影响，推论SPD的药理性质 **方法**：应用EEDQ失活DA受体，用放射受体结合分析法测定受体的密度，计算有关的动力学参数. **结果**：慢性给予SPD 21 d使纹状体 D_1 与 D_2 受体密度分别增加41 5 %和43.7 %. 并且慢性给予SPD改变DA受体的更新率，使 D_1 受体的生成速度由对照组的1 77 pmol·h^{-1}/g protein增加至3.08 pmol·h^{-1}/g protein，D_2 受体的生成速度和降解速率分别由对照组的1 10 pmol·h^{-1}/g protein和0 0049 h^{-1}增加至2 65 pmol·h^{-1}/g protein和0 0082 h^{-1} D_1 和 D_2 受体更新一半所需的时间分别由144.4 h和141 4 h缩短至117 5 h和84.5 h **结论**：SPD增加DA受体的密度并加快受体更新，呈现 D_1 与 D_2 混合型阻滞剂的作用特性.

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Hypoxia effects on hypothalamic corticotropin-releasing hormone and anterior pituitary cAMP[1]

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KEY WORDS Sprague-Dawley rats; pikas; anoxia; cyclic AMP; corticosterone; anterior pituitary gland; corticotropin-releasing hormone; argipressin; norepinephrine

AIM: To study the effects of acute and chronic hypoxia on hypothalamus-anterior pituitary-adrenocortex axis. **METHODS**: Rats and pikas were exposed to different altitude and periods. Animals were injected with CRH, Arg and NE in the third ventricle of the brain of rats. **RESULTS**: Anterior pituitary cAMP and plasma corticosterone levels of rats obviously increased during 1 h of hypoxia. cAMP was increased from 2.23 ± 0.13 of control group to 7.7 ± 0 7 of 5 km and 13.4 ± 1.9 nmol /g wet tissue of 8 km, respectively. icv CRH, Arg and NE all activated HPA axis. The effects of CRH were most potent. CRH 2 μL 0.75 nmol icv increased anterior pituitary of cAMP from 3.5 ±0 4 of control to 22.4 ± 2.2 nmol/kg wet tissue. Stimulating altitude of 5000 m resulted in a 16.9 % decrease in corticosterone level ($P < 0.05$), 8000 m resulted in a 47.5 % decrease ($P < 0.01$) after hypoxia for 25 d. Hypoxia did not activate HPA axis in pikas. **CONCLUTION**: 1) Hypoxia stress activates the secretion of corticotrophin (ACTH) via cAMP; 2) Adrenocotical function of rats decays during chronic hypoxia; 3) Arg and NE regulate the secretion of plasma corticosterone and synthesis of pituitary cAMP at the hypothalamus level; 4) Hypoxia tolerance of the pika was high.

Corticotropin-releasing hormone (CRH), argipressin (Arg) and norepinephrine (NE) regulate the secretion of corticotrophin in anterior pituitary

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gland[1-4]. cAMP acts as a second messenger[5]. Arg and NE modulate the secretion of corticotrophin through CRH in hypothalamus, too. NE stimulates the release of both Arg and CRH from the rat hypothalamus *in vivo*[6]. Endogenous Arg inhibites the secretion of CRH[7]. Hypoxia causes a marked activation of pituitary-adrenal cortex. Hypoxia stimulates the secretion of corticotrophin and adrenal cortical hormones[8].

The aim of the present study was to investigate the effects of acute and chronic hypoxia on pituitary-adrenal system and compare the ability of tolerating the hypoxia state of two species, and demonstrate that the secretions of CRH were modulated by Arg and NE at the hypothalamic level, in order to understand the effects of CRH, Arg, and NE under hypoxia.

MATERIALS AND METHODS

Synthetic rat CRH was purchased from Peninsula Lab, Inc. Arg was donated by Prof DU Yu-Chang. cAMP kits were from Beijing Atomic Energy Institute. All reagents were of analytical grade. Experiments were performed on Sprague-Dawley rats (♂, $n=168$, 173 ± s 5 g) and plateau adult pika (*Ochotona curzoniae*) ($n=84$, 118 ± s 7 g) of either sex, which captured from Haibei, Qinhai-Xizang (Tibet) plateau, with an average altitude of 3200 m (69.1 kPa), raised in laboratory for 4 wk. SD rats and pikas were kept in our laboratory (altitude 2300 m) for several days before use. Rats were fed chow and water *ad lib*. Pikas were fed fresh grass and carrot.

Groups of 7 animals were exposed to 3 different chambers of stimulating atomospheric pressures 77.3 kPa (equivalent to altitude 2300 m), 55.1 kPa (equivalent to altitude 5000 m), and 37.9 kPa (equivalent to altitude 8000 m). Air inflow 10 L · min^{-1}, temperature 18 ~ 20 ℃, light-dark cycle 12:12 h. Animals were placed in these chambers for periods ranging from 1 h to 25 d. Animals were killed between 9:00 and 10:00 am to minimize circadian effects.

Groups of 6 animals were injected with CRH, Arg, and NE into the third ventricle of the brain. All reagents dissolved in 0.9 % saline and 2 μL were injected stereotaxically over 4 min with microinjector. Injection coordinates were 2 mm behind bregma, on midline and 4 mm below dura. After 1 h, the animals were decapitated.

Trunk blood and anterior pituitary gland were sampled. Plasma corticosterones were measured[9]. Anterior pituitary glands were fixed in liquid nitrogen. After weighed the gland were placed in ice-cold 1 mL $HClO_4$ (1 mol · L^{-1}), homogenized in a polytron homogenizer 10 s, and centrifuged at 2000 × *g* for 15 min. The supernatant was neutralized with 20 % KOH and centrifuged at 2000 × *g* for 15 min again. The supernatant was dried at 60 ℃ and 72 kPa. cAMP was measured by radioassay.

ANOVA was used to test for significance.

RESULTS

Anterior pituitary cAMP and plasma corticosterone levels of rats obviously increased during 1 h of hypoxia. The increase correlated with increasing altitude ($P<0.01$). There were no changes in anterior pituitary cAMP levels during hypoxia for 1 h, 1, 7, and 25 d (Tab 1), but the contents of plasma corticosterone gradually attenuated. Stimulating altitude of 5000 m resulted in a 16.9 % decrease in corticosterone level ($P<0.05$), 8000 m resulted in a 47.5 % decrease ($P<0.01$) after hypoxia for 25 d (Tab 2).

There were no changes in the levels of anterior pituitary cAMP and plasma corticosterone of pikas during hypoxia from acute to chronic (Tab 1, 2).

The levels of anterior pituitary cAMP and plasma corticosterone markedly increased after injection of CRH, Arg, and NE into the third ventricle of the brain (Tab 3). The effects of CRH were most potent ($P<0.01$).

Tab 1. Anterior pituitary cAMP (μmol/g wet wt) of rat and pika during hypoxia. $n=7$, $\bar{x} \pm s$. $^{c}P<0.01$ *vs* control (2300 m).

Hypoxia kPa		1 h	1 d	7 d	25 d
77.3	Rat	2.23 ± 0.13	2.45 ± 0.18	2.5 ± 0.3	2.4 ± 0.4
(2300 m)	Pika	2.0 ± 0.3	2.2 ± 0.3	2.6 ± 0.4	1.87 ± 0.14
55.1	Rat	7.7 ± 0.7^{c}	2.1 ± 0.3	2.3 ± 0.3	2.4 ± 0.3
(5000 m)	Pika	2.1 ± 0.4	2.0 ± 0.4	2.6 ± 0.4	1.95 ± 0.22
37.9	Rat	13.4 ± 1.9^{c}	2.6 ± 0.3	2.4 ± 0.3	2.3 ± 0.3
(8000 m)	Pika	2.5 ± 0.7	2.1 ± 0.5	2.5 ± 0.3	2.0 ± 0.5

Tab 2. Plasma corticosterone ($\mu g \cdot L^{-1}$) of rat and pika during hypoxia. $n = 7$, $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* control (2300 m).

Hypoxia kPa		1 h	1 d	7 d	25 d
77.3	Rat	58 ± 9	61 ± 10	58 ± 7	59 ± 5
(2300 m)	Pika	40 ± 8	47 ± 10	52 ± 10	35 ± 13
55.1	Rat	121 ± 14^c	101 ± 18^b	61 ± 10	49 ± 9^b
(5000 m)	Pika	46 ± 10	57 ± 5	51 ± 7	34 ± 11
37.9	Rat	184 ± 12^c	108 ± 15^c	61 ± 11	31 ± 6^c
(8000 m)	Pika	47 ± 7	53 ± 7	47 ± 6	42 ± 13

Tab 3. Anterior pituitary cAMP and plasma corticosterone of rat after injection of CRH, Arg, and NE into 3rd ventricle of brain. $n = 6$, $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* control (2300 m).

	nmol	cAMP, nmol/g wet wt	Corticosterone, $\mu g \cdot L^{-1}$
CRH	0.00	3.5 ± 0.4	86 ± 9
	0.05	6.3 ± 1.5^c	89 ± 16
	0.10	8.6 ± 2.3^c	126 ± 6^c
	0.40	16.0 ± 2.3^c	170 ± 25^c
	0.75	22.4 ± 2.2^c	188 ± 19^c
Arg	0.00	3.0 ± 0.3	100 ± 20
	1.00	4.1 ± 1.1	181 ± 37^b
	4.00	4.9 ± 1.1^c	196 ± 47^c
	10.00	5.3 ± 1.3^c	251 ± 56^c
	100.00	5.9 ± 1.2^c	243 ± 71^c
NE	0.00	3.1 ± 0.1	94 ± 9
	1.00	3.9 ± 0.6^b	102 ± 16
	2.00	4.9 ± 0.8^c	124 ± 28^b
	8.00	8.2 ± 1.0^c	160 ± 30^c

DISCUSSION

Acute hypoxia stimulated the concentration of cAMP in anterior pituitary and plasma corticosterone of rats markedly increased. It suggests that hypoxia activated hypothalamic CRH-anterior pituitary cAMP. Hypoxia stimulated the secretion of CRH, then CRH exerted its actions on the corticotrophin through cyclic AMP-dependent mechanism.

Median eminence secretes a large amount of CRH under acute hypoxia. It resulted in the secretion of CRH decreased after rats exposure to hypoxia for a long term. Arg might be synergically potent of the function of CRH to stimulate the secretion of corticotrophin, when the secretion of CRH decreased.

Hypoxia resulted in the attenuation of adrenal cortex function. The reason might be that 1) under hypoxia, the low oxygen content of artery blood resulted in the decrease of adrenal cortical hormones synthesis; 2) the decreased sensitivity of corticotrophin receptor in adrenal cortex cell to corticotrophin, which decreased sensitive resulted in the decrease of corticosterone synthesis and secretion.

Corticotropin-releasing factor is potentiated by Arg[10]. CRH immunoreactive neurons of the paraventricular nucleus become Arg positive after adrenalectomy[11]. Injected Arg stimulates and potentiates the secretion of corticotrophin in anterior pituitary. It indicates that the effects of Arg regulating the secretion of corticotrophin act at pituitary and hypothalamic level. Arg regulating the secretion of corticotrophin is through Arg stimulating CRH. Injected NE activates CRH neurons. NE stimulates the secretion of CRH. NE is an exciting neurotransmiter.

Hypoxia did not activate the HPA axis of pikas. It suggests that hypothalamo-pituitary adrenal axis of pika has high tolerance to hypoxia.

It suggested that 1) acute hypoxia stress activated the synthesis of anterior pituitary cAMP and elevated plasma corticosterone, the formation and secretion of corticotrophin mediated through cAMP, the function of adrenal cortex would decay during chronic hypoxia; 2) CRH stimulated the synthesis of pituitary cAMP, which was regulated by Arg and NE in the hypothalamus; 3) hypoxia tolerance of the plateau pika was extremely high.

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低氧对下丘脑促肾上腺皮质素-释放激素和前垂体 cAMP 的作用

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关键词 近交系大鼠; 高原鼠兔; 缺氧症; 环腺苷一磷酸; 皮质酮; 前叶垂体; 促肾上腺皮质素释放素; 精加压素; 去甲肾上腺素 cAMP

A **目的**: 探讨急、慢性低氧对下丘脑-前垂体-皮质轴的作用机制. **方法**: 大鼠及高原鼠兔暴露于不同海拔高度及时间, 动物 icv CRH、Arg 和 NE **结果**: 低氧 1 小时, 大鼠前垂体 cAMP 显著上升 cAMP 分别由对照组的 2.23±0 13 增至 5 km 的 7 7±0 7 和 8 km 的 13 4±1 9 nmol/g 湿组织. icv 2 μL 0 75 nmol 的 CRH 使前垂体 cAMP 由对照组 3 5±0.4 升至 22 4±2 2 nmol/g 湿组织. **结论**: 1) 低氧激活 ACTH 分泌是以 cAMP 为中介物; 2) 大鼠肾上腺皮质功能在慢性低氧中可能衰退; 3) Arg 和 NE 在下丘脑水平调节前垂体 cAMP 及血浆皮质酮; 4) 高原鼠兔对低氧耐受性强.

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