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Effects of hemodynamic changes on taurine release from posterior hypothalamus of freely moving rats

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AIM: To study the effects of blood volume and vascular resistance on taurine release. **METHODS**: We used push-pull superfusion technique in the posterior hypothalamus of conscious freely moving rats. Taurine was determined in the superfusate by HPLC with fluorescence detection following automatic precolumn *o*-phthaldialdehyde (OPA) derivatization. **RESULTS**: Hypervolemia increased the release of taurine in the hypothalamus. Intravenous infusion of levarterenol (3 μg kg^{-1} min^{-1}) elicited a pronounced pressor response and an increase in the release of taurine. Conversely, a controlled hemorrhagic hypotension or iv infusion of nitroprusside (30 μg kg^{-1} min^{-1}) elicited a hypotension and a decrease in the release of taurine from the posterior hypothalamus. **CONCLUSION**: In the posterior hypothalamus, taurine might play an important role in central blood pressure regulation.

KEY WORDS taurine; posterior hypothalamus; perfusion; blood pressure; blood volume; norepinephrine; nitroprusside

Taurine, distributed widely in mammalian tissues, has various cardiovascular actions[1,2]. Centrally applied taurine lowered blood pressure accompanied by bradycardia[3]. Nevertheless, little is known about the possible function of taurine in central blood pressure regulation. We intended to investigate whether brain taurine was involved in central cardiovascular control.

MATERIALS AND METHODS

Sprague-Dawley rats (♂, $n=19$) weighing 262± s 50 g were housed in a light, temperature and humidity controlled environment, rats were anesthetized with sodium pentobabital (40 mg kg^{-1}, ip) and katamine (50 mg kg^{-1}, ip). A guide cannula was inserted into the right posterior hypothalamus (AP −3.9 mm, L 0.7 mm, V −6.4 mm)[4], and fixed with dental cement. After 2 d the rats were anesthetized again and the iliolumbar artery and jugular vein were catheterized with PE 50 and PE 20 tubings for measurement of arterial blood pressure and for infusion of drugs, respectively. Two days later, the stylet of the guide cannula was replaced by a push-pull cannula[5] (outer needle: OD 0.7 mm, ID 0.5 mm; inner needle: OD 0.2 mm, ID 0.1 mm). The posterior hypothalamus of the conscious, freely moving rat was superfused with artificial cerebrospinal fluid (ACSF) pH 7.2 at a rate of 30 μL min^{-1}. ACSF consisted (mmol L^{-1}): NaCl 140, KCl 3.0, $CaCl_2$ 2.5, $MgCl_2$ 1.0, Na_2HPO_4 1.2, NaH_2PO_4 0.3, and glucose 3.0. Levarterenol and sodium nitroprusside were dissolved in physiologic saline and infused iv at a rate of 150 μL min^{-1} to induce peripheral blood pressure changes > 70 min. The superfusate was collected continuously in 3 min periods in an ice-bath and kept at −80 ℃ until assay. Finally the brain was excised histological localization of the cannula, experiments with cannula outside the posterior hypothalamus were discarded.

Assay of taurine Taurine was determined in the superfusate by HPLC with fluorescence detection following automatic precolumn *o*-phthladialdehyde (OPA derivatization[6]. The minimal detection level was 50 fmol per sample. The retention time of taurine was 20—25 min.

Chemicals and drugs (−)-Levarterenol hy-

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drochloride and sodium nitroprusside were purchased from Sigma. 2-Mercaptoethanol, *o*-Phthaldialdehyde, and acetonitrile were obtained from Ciba-Geigy. All other reagents were of AR grade unless otherwise indicated. Water was glass-redistilled.

Instruments Pump: L-6200, autosampler: AS-4000 (Merck-Hitachi, Tokyo, Japan); analytical column: RP 18 Lichrosphere, 250 mm × 4 mm, 5 μm, and guard column: RP 18 Lichrosphere, 4 mm × 4 mm, 5 μm (Merck, Darmstadt, FRG); Integrator: D-2500 (Merck-Hitachi, Tokyo, Japan).

Statistics Results were expressed as $\bar{x} \pm s$ and compared by *t* test.

RESULTS

During control superfusion experiments, the release rate of taurine in the posterior hypothalamus remained fairly constant, and the basal release of taurine was 3.5 ± 0.5 pmol min^{-1} ($n = 7$). Physiologic saline iv infused under the same condition elicited no changes in blood pressure or in release of taurine. Infusion of levarterenol (30 μg kg^{-1} min^{-1}) for 9 min led to a pronounced rise in blood pressure, which was associated with an increase in release of taurine. Infusion of nitroprusside (30 μg kg^{-1} min^{-1}) led to a fall in blood pressure which was associated with a decrease in release of taurine (Tab 1).

Hypovolemia (a controlled hemorrhage, 0.3 mL kg^{-1}) lowered arterial blood pressure, accompanied with a decrease in release of taurine. Conversely, hypervolemia volume expansion (30 % of original blood volume) by reinjection of blood mixed with isotonic saline (1 : 1) led to an increase in blood pressure, and the release rate of taurine was increased (Tab 2).

Tab 1. Effects of levarterenol (Lev, 3 μg kg^{-1} min^{-1}) and nitroprusside (30 μg kg^{-1}/min) infusion on release of taurine and mean blood pressure. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$ *vs* 0 min.

		Control 0 min	Infusion 3 min	Infusion 6 min	Infusion 9 min	After infusion 12 min	After infusion 15 min
Levarterenol $n=6$							
Tau	pmol	4.1±0.8	5.7±1.0^a	7.0±1.3^b	8.1±1.4^b	7.6±1.5^b	5.0±1.2^a
BP	kPa	12.5±0.9	19.7±1.1^b	20.1±1.8^b	19.9±1.8^b	13.4±1.7^a	12.5±1.4^a
Nitroprusside $n=7$							
Tau	pmol	3.9±1.0	3.2±1.0^b	3.4±0.8^a	3.3±0.9^a	3.5±0.8^a	3.8±0.7^a
BP	kPa	14.3±0.7	9.9±0.6^b	9.0±0.6^b	9.6±0.6^b	16.6±0.7^a	17.2±0.7^b

Tab 2. Effects of hemorrhage and iv injection diluted blood on release of taurine and mean blood pressure. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ compared with 0 min.

		Control 0 min	During 3 min	After 6 min	9 min	12 min
hemorrhage (0.3 mL kg^{-1}, $n=6$)						
BP	kPa	13.4±0.8	9.4±0.5^b	10.7±1.0^b	12.1±1.2^a	12.2±1.0^a
Tau	pmol	3.8±1.1	2.7±0.9^b	3.9±0.9^a	4.6±1.0^a	4.1±1.0^a
iv blood (of original blood, $n=6$)						
BP	kPa	12.6±0.6	15.2±0.8^b	14.4±1.0^b	13.8±1.2^a	13.7±1.1^a
Tau	pmol	3.9±1.0	13.7±1.2^c	7.4±0.9^c	6.2±0.9^b	5.5±0.9^b

DISCUSSION

In the present study, experimentally induced hemodynamics changes influences release rate of taurine in the posterior hypothalamus: rising in blood pressure elicited by levarterenol and hypervolemia enhanced the release rate of taurine; nitroprusside or hypovolemia lowered blood pressure and diminished taurine release. It seems that taurine released from hypothalamic neurons possesses a counteracting action thus contributing to homeostasis of arterial blood pressure. Since a pressor and a depressor response decreased and increased the release rate of taurine, respectively. The endogenously released taurine might possess a hypotensive function. To our knowledge, this is the first demonstration that taurine release is altered in response to variations in blood pressure. The finding suggests that an interplay of taurine together with several neurotransmitters is required to elicit an adequate regulatory effect on the cardiovascular system to counteract changes in blood pressure.

Although the precise mechanism of hypotensive effect of taurine is still unknown, several evidences suggest that it is mainly due to the suppression of sympathetic activity[7,8,9]. Intraventricularly injected taurine alters the metabolism of hypothalamic noradrenaline and dopamine, which are known to be involved in central cardiovascular control[10]. In conclusion, our present results indicate that taurine in this brain areas may act as a neurotransmitter or neuromodulator in the central blood pressure control.

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血流动力学改变对自由活动大鼠下丘脑后部牛磺酸释放的影响

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目的: 研究实验性引起血容量和血管阻力的改变, 对大鼠下丘脑后部牛磺酸释放的影响.

方法: 应用推-挽灌流技术, 定位灌流清醒大鼠下丘脑后部, 然后用高效液相色谱荧光检测测定流出液中牛磺酸的含量.

结果: 血容量增加, 或静脉注射去甲肾上腺素 (3 $\mu g\ kg^{-1}\ min^{-1}$) 引起加压反应的同时, 下丘

脑后部牛磺酸的释放增加；相反，通过失血使血容量减少或静脉注射硝普钠引起血压下降时，下丘脑后部牛磺酸的释放减少.

结论：下丘脑后部牛磺酸对血压的中枢调节起着很重要的作用.

关键词 牛磺酸；下丘脑后部；灌注法；血压；血容量；去甲肾上腺素；硝普钠

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Relationship between muscarinic receptor subtypes and cyclic nucleotides in pons-medulla oblongata[1]

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AIM: To study the relationship between muscarinic receptor (M-R) subtypes and cyclic nucleotides in pons-medulla oblongata (MeOb). **METHODS**: The contents of cGMP and cAMP in Sprague-Dawley rat pons-MeOb, cerebellum and cerebral cortex were assayed by radioimmunoassay and competitive protein-binding assay, respectively, after ip injections of drugs. Control rats were given ip normal saline. **RESULTS**: M_1-R agonist pilocarpine (6, 15 mg kg^{-1}, ip) increased the content of cGMP in the pons-MeOb and cerebral cortex, but did not bring about any noticeable change in the cAMP content. The increase of cGMP was antagonized by ip pirenzepine or scopolamine. On the other hand, ip M_2-R agonist 6β-acetoxy nortropane (6β-AN) 25 μg kg^{-1} reduced not only cAMP contents in the pons-MeOb and cerebellum but also cGMP contents in the pons-MeOb and cerebral cortex, while 6β-AN 12 μg kg^{-1} only lowered cAMP content. The decreases of cGMP and cAMP induced by 6β-AN were antagonized by ip AF-DX 116 or atropine, respectively. **CONCLUSION**: Stimulation of M_1-R causes the increase of cGMP and that of M_2-R induces the decreases of both cGMP and cAMP in the pons-MeOb.

KEY WORDS cyclic GMP; cyclic AMP; muscarinic receptors; pilocarpine; nortropanes; pirenzepine; scopolamine; atropine

Pons and medulla oblongata (MeOb) play an important role in regulation of respiration. We previously found that there were M_1 muscarinic receptor (M_1-R, 30 %— 40 %) and M_2-R (60 %— 70 %) subtypes in pons-MeOb[1], and that the excitation of M_1-R stimulated respiration and excitation of M_2-R inhibited respiration[1,2]. However, it is not known why the effects of M_1 and M_2 receptors on respiration are so distinct. Although M_1 and M_2 receptors were separately coupled to the elevation of cGMP level and the inhibition of cAMP formation *in vitro*[3,4], the relationship between M-R subtypes and the two cyclic nucleotides in pons-MeOb remains to be defined. In the present study, the contents of cGMP and cAMP in rat pons-MeOb, cerebral cortex, and cerebellum were assayed after ip injections of M_1-R agonist pilocarpine[5,6] and

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