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Stress-induced changes of central GABAergic function in rats

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ABSTRACT The effects of acute and repeated immobilization stress on central GABAergic function were examined in rats by evaluating the changes of the cardiovascular responses to intracerebroventricular injection of GABA. The depressor and bradycardia responses of GABA were attenuated significantly by immobilization stress for 6 h (from −5.5 ± 0.7 kPa and −75 ± 25 bpm to −2.3 ± 0.9 kPa and −27 ± 16 bpm respectively, for GABA 200 μg). These effects of stress were neither mimicked by administration of hydrocortisone, nor abolished by pretreatment with adrenalectomy or diazepam, but they were attenuated significantly by pretreatment with isoniazid. These results indicated that immobilization stress for long duration causes subsensitivity of central GABA receptors, in which the down-regulation mechanism may be involved.

KEY WORDS physical restraint; GABA; hypotension; bradycardia; hydrocortisone; adrenalectomy; diazepam; isoniazid

The neurochemistry of stress has been studied for over two decades. Recently, a lot of attention was paid to GABA, the prevalent inhibitory neurotransmitter in brain. However, apparently conflicting results of both increase and decrease in brain GABA level[1,2] as well as binding characteristics of GABA / benzodiazepine receptor complex[3-5] have been reported. Since most of these results were derived from *in vitro* assay of synaptoneurosomes of cerebral cortex, hippocampus, and striatum, and methods *in vitro* and *in vivo* can yield substantially different results[9], the functional consequences of GABAergic activity related to *in vivo* experiments seem worthwhile to be investigated. In order to identify stress-induced changes of central GABA receptors, we utilized *in vivo* technique evaluating the cardiovascular responses to intracerebroventricular (ICV) injection of GABA, which might minimize artifacts associated with *in vitro* method. Meanwhile, the mechanism responsible for these changes were also explored.

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MATERIALS AND METHODS

Male Sprague-Dawley rats, weighing 264 ± SD 23 g, were housed under 12 h light-dark cycle with free access to food and water. For immobilization stress, the rat was placed inside a narrow, cylindrical acrylic tube, which was perforated for normal breathing and in which only the movements were restricted. This immobilization stress lasted for 1 h, 6 h or 1 h daily for 6 d. After stress treatment, the rat was immediately anesthetized with urethane (1.1 g · kg^{-1}, ip), and the femoral artery was cannulated with PE 50 tubing. Then, the rat was mounted in a stereotaxic instrument. The coordinates for lateral cerebroventricles were AP −0.8, L 1.6, H −2.8 with respect to Bregma. All surgical procedures were completed within 25 min. After a period of stabilization, icv GABA was given about 50 min after the cessation of immobilization stress. At the end of the experiment, toluidine blue solution was similarly injected for verification of the injection site. The above injection was routinely performed at 6:00–9:00 PM to avoid possible circadian variation of GABA receptors. Some rats were adrenalectomized 3 d before injection of GABA.

Injection apparatus consisted of a guide cannula and an injection cannula. Each injection comprised of 5 μl drug solution containing 100 or 200 μg GABA, or the same

volume of vehicle, which was infused over 2 min. GABA (Shanghai 3rd Pharmaceutical Factory) was dissolved in artificial cerebrospinal fluid[6]. Pretreatment drugs used are as follows: glucocorticoids including two different doses, physiologically relevant dose of hydrocortisone (20 mg · kg^{-1}, ip, three times in 6 h) and pharmacologically relevant dose of dexamethasone (10 mg · kg^{-1}, ip); diazepam (8 mg · kg^{-1}, ip) or isoniazid (100 mg · kg^{-1}, ip, twice in 6 h) which were injected along with stress.

Statistical tests employed were *t* test and *F* test.

RESULTS

Effects of stress on cardiovascular responses to GABA icv Systolic, diastolic and mean arterial pressure and heart rate consistently fell after GABA icv, and the responses were dose-dependent (Tab 1). Similar injection of vehicle was ineffective. The depressor and bradycardia responses of GABA were significantly attenuated by immobilization stress for 6 h, whereas repeated stress of 1 h daily for 6 d was less potent, and acute stress for 1 h had little effects (Fig 1, Tab 1).

Effects of glucocorticoids level on cardiovascular responses to GABA icv Hydrocortisone (20 mg · kg^{-1}, ip, three times in 6 h) failed to attenuate the responses of GABA. On the contrary, adrenalectomy or administration of dexamethasone (10 mg · kg^{-1}, ip) attenuated them significantly (Tab 2).

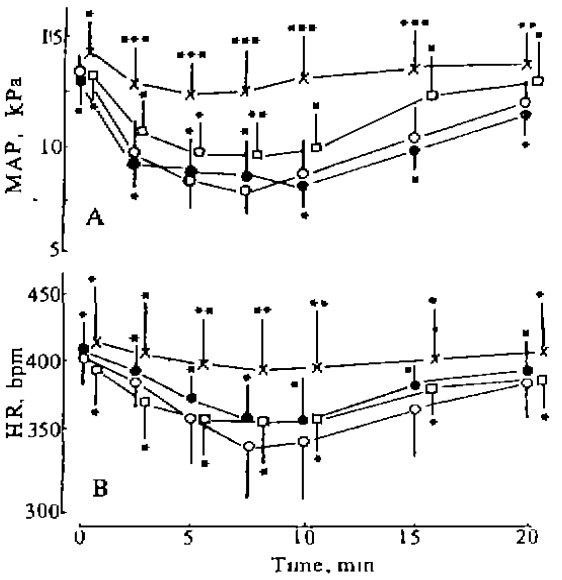

Fig 1. Influences of immobilization stress on the depressor response (A) and bradycardia (B) to GABA icv (200 μg) in rats. (○) **unrestrained;** (●) **1 h stress;** (×) **6 h stress;** (□) **1 h stress daily for 6 d.** $n=6-8$, $\bar{x} \pm SD$, $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* **unrestrained rats. The values at 0 min are baselines before icv GABA.**

Influences of pretreatment with diazepam, isoniazid, and adrenalectomy on stress-induced attenuation of cardiovascular responses to GABA icv Effects of stress were not abolished by pretreatment with diazepam (8 mg · kg^{-1}, ip) or adrenalectomy, they were attenuated significantly by pretreatment with isoniazid (100 mg · kg^{-1}, ip, twice in 6 h) (Tab 3).

Tab 1. Stress-induced maximal changes of cardiovascular responses to GABA icv in rats. $n=6-8$, $\bar{x} \pm SD$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* **unrestrained rats.**

	GABA μg	Unrestrained rats	Stressed rats 1 h	6 h	1 h × 6 d
Depressor	100	−3.1 ± 0.8	−3.5 ± 1.1*	−1.2 ± 0.5**	−2.2 ± 0.5**
kPa	200	−5.5 ± 0.7	−5.5 ± 0.9*	−2.3 ± 0.9***	−4.2 ± 0.8***
Bradycardia	100	−29 ± 19	−25 ± 16*	−9 ± 4**	−17 ± 9*
bpm	200	−71 ± 25	−58 ± 26*	−27 ± 16***	−44 ± 18**

Tab 2. Effects of glucocorticoids and adrenalectomy on cardiovascular responses to GABA icv (200 μg) in rats. $n=6-8$, $\bar{x}\pm SD$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* unrestrained rats. $^{+}P>0.05$, $^{\#}P<0.05$, $^{\#\#}P<0.01$ *vs* 6 h stress rats.

	Baselines		Maximal changes	
	MAP, kPa	HR, bpm	Depressor, kPa	Bradycardia, bpm
Unrestrained	$13.1\pm0.8^{+}$	$403\pm23^{+}$	$-5.5\pm0.7^{***}$	$-71\pm25^{***}$
6 h stress	$14.4\pm1.7^{*}$	$413\pm39^{*}$	$-2.3\pm0.9^{*\#\#}$	$-27\pm16^{*\#\#}$
Hydrocortisone	$13.2\pm0.8^{*+}$	$394\pm18^{*+}$	$-5.1\pm0.8^{*+\#\#}$	$-54\pm24^{*+\#\#}$
Dexamethasone	$13.7\pm1.0^{*+}$	$404\pm17^{*+}$	$-2.5\pm0.6^{*\#\#+}$	$-44\pm15^{*\#\#+}$
Adrenalectomy	$12.8\pm1.6^{*+}$	$385\pm37^{*+}$	$-2.2\pm1.0^{*\#\#+}$	$-30\pm10^{*\#\#+}$

Tab 3. Influences of pretreatment with diazepam, isoniazid, and adrenalectomy on stress-induced decreases of cardiovascular responses to GABA icv (200 μg) in rats. After pretreatment, the rats were immobilized for 6 h. $n=6-8$, $\bar{x}\pm SD$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* saline group.

	Baselines		Maximal changes	
Pretreatment	MAP, kPa	HR, bpm	Depressor, kPa	Bradycardia, bpm
Saline	13.9 ± 1.5	408 ± 35	-2.2 ± 0.9	-29 ± 17
Diazepam	$13.3\pm0.4^{*}$	$379\pm22^{*}$	$-1.8\pm0.8^{*}$	$-26\pm15^{*}$
Isoniazid	$13.5\pm1.2^{*}$	$383\pm34^{*}$	$-3.8\pm0.9^{*\#\#}$	$-45\pm12^{*\#\#}$
Adrenalectomy	$11.4\pm1.2^{*}$	$362\pm31^{*}$	$-1.7\pm0.5^{*}$	$-9\pm6^{*}$

DISCUSSION

Our data suggested that the rats continuously exposed to immobilization stress develop a subsensitivity of central GABA receptors related to cardiovascular regulation, whereas acute and repeated stress of short duration were less potent. In fact, although not statistically different, a slight enhancement of the responses of GABA by 1 h stress was observed in a dose of 100 μg.

In previous studies, both hypersensitivity[7,8] and subsensitivity[4,10] of GABA receptor complex following various stresses were reported. Failure to find consistent effects of stress on GABAergic function may be due to the variability of the type and the duration of stress, the time delay between the cessation of stress and measuring, and the evaluating methods. *In vivo* method may be more suitable since animals are under a more "physiological" condition. Our results derived from *in vivo* technique are consistent with the viewpoint of subsensitivity of GABA receptor after stress.

The role of glucocorticoid in stress-induced changes of GABAergic function has been assessed by increasing and decreasing its level. The results indicated that glucocorticoid did not play a key role. In fact, glucocorticoids and their metabolites potentiate rather than inhibit GABA binding and GABA receptor-mediated Cl^{-} flux[11,12]. Pretreatment with diazepam failed to abolish the effects of immobilization stress as observed in ours, which was inconsistent with the results derived from footshock stress[4,13].

Immobilization stress could cause an increase of GABA concentration in brain[1], hence, modulating GABA receptor by down-regulation mechanism. The result of

pretreatment with isoniazid for inhibiting the synthesis of GABA supported this possibility. Other mechanism responsible for our results remained to be explored.

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大鼠应激产生的中枢γ-氨基丁酸能功能变化

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提要 大鼠连续制动应激 6 h 可显著减弱 icv GABA 的降压和心动过缓反应，200 μg GABA 的反应由未应激组的−5.5±0.7 kPa 和−71±25 bpm 分别降至−2.3±0.9 kPa 和−27±16 bpm. 该应激效应不被 ip 氢化可的松模拟，亦不被预先肾上腺切除或 ip 安定取消，但可被 ip 异烟肼显著减弱. 提示持续制动应激可通过受体向下调节机制使脑内 GABA 受体脱敏.

关键词 身体的约束；γ-氨基丁酸；低血压；心动过缓；氢化可的松；肾上腺切除术；安定；异烟肼

Instructions to authors

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