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96位中国汉族志愿者的美托洛尔 α-羟化能力[1]

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目的：体内及试管内研究汉族人美托洛尔(Met) α-羟化能力. **方法**：反相离子对高效液相色谱法测定 Met 和 α-羟基美托洛尔(HM). **结果**：96名汉族人尿 Met 和 HM 比值(MR)的对数示单基因遗传特征性双态频率分布，仅发现1例弱代谢者(MR＝199.3)，95例强代谢者 MR 为 3.1 ± 2.5. 性别、吸烟、饮茶对尿 Met，HM 及 MR 均无影响. 试管内研究发现 NADH 对人肝微粒体 Met α-羟化酶活性无影响，该酶 K_m 为89.6 μmol L^{-1}，V_{max}为39.5 ng mg^{-1} min^{-1}. 在8例汉族人肝微粒体中未发现该酶缺陷者，测得其活性为31.4 ± 22.1 ng mg^{-1} min^{-1}. **结论**：中国汉族人 Met α-羟化弱代谢者发生率低，NADH 未参与人肝微粒体 Met α-羟化反应.

关键词 美托洛尔；α-羟基美托洛尔；肝微粒体；遗传药理学；多态性

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Effect of intracerebroventricular injection of somatostatin or GABA on pain threshold and contents of GABA or somatostatin in rat brain

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AIM: To study the interactive influence of somatostatin (Som) and GABA in the brain and its relation to the pain modulation. **METHOD**: Using radioimmunoassay, amino acid analyzer and measurement of pain threshold. **RESULTS**: Som 10 μg icv increased the pain threshold (from 4.2±0.2 to 7.0±1.1 s) of the rat, but reduced the content of GABA from 2.3±0.3 to 1.6±0.4 μmol g^{-1} in hippocampus and from 2.4±0.4 to 1.5±0.2 mmol kg^{-1} in brain stem. After depletion of the Som in brain by icv cysteamine (Cys, 600 μg), the content of GABA in hippocampus and brain stem was also reduced without modification of the pain threshold. GABA 1500 μg icv had no effect on the pain threshold, however, caused a decrease of Som content from 55±4 to 37±5 ng g^{-1} in hippocampus and from 84±4 to 55±6 ng g^{-1} in brain stem, which was blocked by bicuculline (10 μg).

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After reduced of the GABA content in brain by subcutaneous injection of isoniazid (300 mg kg^{-1}), Som content of the hippocampus and brain stem was markedly elevated. **CONCLUSION**: Som and GABA inhibited each other, unrelated to their pain modulation.

KEY WORDS somatostatin; GABA; pain threshold; hippocampus; brain stem

After icv[1,2] or intrathecal[3] injection of somatostatin (Som), the rat's pain threshold was increased. Analgesia was produced by microinjection of baclofen (a GABA receptor agonist) into the caudal part of the cerebral aqueduct, as well as into discrate brain stem sites[4]. Both Som and GABA are important inhibitory transmitters in the central nervous system. But there were few reports to describe their interaction in pain modulation. The aim of this experiment is to study the Som and GABA in the brain interactive influence and relation to their pain modulation.

MATERIALS AND METHODS

Drugs Som, GABA, bicuculline and cyproheptadine were purchased from Sigma Co, Cysteamine (Cys) was a product of the Fluka Chemie AG.

The icv The icv microinjection was described in our previous report [2].

Measurement of pain threshold Wistar rats of either sex ($n = 68$, 200 ± s 42 g) were used. Pain threshold was measured by tail-flick method[2].

Measurement of GABA content The hippocampus and brain stem homogenates (10 % sulfosalicylate sodium 30 μL/mg brain tissue) were centrifuged (28 850 × g, 4 C, 40 min). The supernatant was analyzed for GABA by an amino acid analyzer (121 MB type, Beckman Co): column 200 mm × Φ 2.8 mm, flow rate 5 mL h^{-1}, 67 ℃, λ 570 nm[5].

Measurement of Som content The radioimmunoassay was used[6].

All data were analyzed with ANOVA and t-test.

RESULTS

Effect of Som on pain threshold and brain GABA The pain threshold began to elevate 10 min after icv Som 10 μL (1 g L^{-1}) and continued to increase ($P<0.01$) (Tab 1) for 20 min, the GABA contents in hippocampus and brain stem were decreased (Tab 2), as compared with icv ACSF (artificial cerebrospinal fluid).

Tab 1. Pain threshold in radiant heat test after icv Som (1 g L^{-1}), cysteamine (Cys, 60 g L^{-1}) or GABA (100 g L^{-1}) in rats. $n = 6-8$, $\bar{x} \pm s$. $^{a}P>0.05$, $^{c}P<0.01$ *vs* **artificial cerebrospinal fluid (ACSF).**

	Tail-flick latency/s			
	ACSF	Som	Cys	GABA
Basal	4.2±0.4	4.2±0.2	4.2±0.4	4.3±0.4
10 min	4.3±0.4[a]	5.8±0.7[c]	4.4±0.3[a]	5.3±1.3[a]
20 min	4.2±0.4[a]	6.3±1.1[c]	4.3±0.3[a]	5.2±1.2[a]
30 min	4.2±0.5[a]	7.0±1.1[c]	4.3±0.4[a]	5.1±1.0[a]

Tab 2. Effect of icv injection of Som (1 g L^{-1}), cysteamine (Cys, 40 g L^{-1}), GABA (100 g L^{-1}), and isoniazid (300 mg kg^{-1}) on contents of GABA and Som in hippocampus and brain stem of rat. $n = 5-10$, $\bar{x} \pm s$. $^{c}P<0.01$ *vs* **ACSF.**

	GABA/μmol g^{-1}		Somatostatin/ng g^{-1}	
	Hippocampus	Brain stem	Hippocampus	Brain stem
ACSF	2.3±0.3	2.2±0.4	55±4	84±4
Som	1.6±0.4[c]	1.5±0.2[c]		
Cys	1.6±0.2[c]	1.5±0.1[c]	34±3[c]	43±4[c]
GABA			37±5[c]	55±6[c]
Isoniazid	1.6±0.4[c]	1.1±0.2[c]	74±6[c]	112±9[c]

After icv Cys 15 μL (a Som depletor, 40 g L^{-1}) at 4 h in rats, the Som and GABA contents in hippocampus and brain stem were decreased ($P<0.01$) (Tab 2), without any alteration of the pain threshold, as compared with icv ACSF ($P>0.05$) (Tab 1).

Effect of GABA on pain threshold and

brain Som After icv GABA 15 μL (100 g L^{-1}), the pain threshold did not show remarkable change ($P>0.05$) (Tab 1), as compared with ACSF group. Thirty min after icv GABA, the contents of Som in hippocampus and brain stem were less than that of ACSF group ($P<0.01$) (Tab 2). The level of Som in brain decreased by icv GABA was elevated by pretreatment (5 min before icv GABA) of bicuculline 10 μL (1 g L^{-1}, $GABA_A$ receptor antagonist), but not of cyproheptadine 10 μL (5 g L^{-1}, 5-HT receptor antagonist), naloxone 10 μL (1 g L^{-1}, opiate receptor blocker), or atropine 10 μL (0.5 g L^{-1}, muscarinic receptor antagonist). Comparing with ACSF group, no obvious difference was present between them ($P>0.05$) (Tab 3).

Tab 3. Effect of icv various receptor antagonists (bicuculline, 1 g L^{-1}. cyproheptadine, 5 g L^{-1}. naloxone, 1 g L^{-1}. atropine, 0.5 g L^{-1}) on icv GABA (100 g L^{-1}) in decreasing Som content in hippocampus and brain stem of rat. $n=6-10$, $\bar{x}\pm s$. [c] $P>0.05$, [d] $P<0.01$ *vs* ACSF; [e] $P>0.05$, [f] $P<0.01$ *vs* GABA.

	Somatostatin content/ng g^{-1}	
	Hippocampus	Brain stem
Control	53±4	84±5
ACSF	55±4	58±4
GABA	37±5[d]	55±6[d]
GABA + bicuculline	53±3[e,f]	83±6[e,f]
GABA + cyproheptadine	37±3[d]	55±4[d]
GABA + naloxone	37±3[d]	56±5[d]
GABA + atropine	37±5[d]	52±5[d]

Besides, 40 min after sc isoniazid (a GABA synthetase inhibitor, 300 mg kg^{-1}), the GABA contents in hippocampus and brain stem were decreased ($P<0.01$), but the Som contents were elevated ($P<0.01$) (Tab 2), as compared with the ACSF group.

DISCUSSION

The results indicated that the GABA contents of hippocampus and brian stem were reduced but the pain threshold was increased following icv Som. The effect of electroacupuncture analgesia would be elevated when the function of GABAergic neurons was attenuated, on the contrary, the effect of electroacupuncture analgesia could be decreased when the function of GABAergic neurons was enhanced[7]. It seems that analgesia of icv Som in this study might be related to the decrease of function of GABAergic neurons.

In this study after decrease of Som content in brain by Cys, the pain threshold did not change significantly, but the GABA contents in hippocampus and brain stem were markedly decreased. This finding was similar to the phenomena that the reduced release of GABA due to decrease of endogenous Som by Cys or Som anti-serum *in vitro* from slices of rat caudatoputamen[8]. As to its mechanism, the report[9] indicated that the Cys could decrease the Som content in tissue but enhance the Som-like immunoreactivity, and it was considered that it might be a reason to decresase GABA content in brain by Cys as Som did.

GABA inhibited the release of Som from hypothalamic neurons cultured fetal rat[10], and GABA could inhibit the potassium-stimulated release of Som from rat spinal cord slices, the inhibitory effect could be attenuated by bicuculline. This results were consistent with ours. After icv GABA, the Som contents of hippocampus and brain stem were reduced, and the effect was also induced via $GABA_A$ receptor. It showed no relationship with the pain modulation. There have been controversial reports about GABA-induced analgesia, although GABA had analgesic effect, the doses used here were relatively large[4,12].

Som and GABA are *coexistant in* the

brain[13], the relationship between them was either inhibiting the release or inhibiting the effective of each other[11,14]. In the present investigation, we also found that the inhibitory effect was present between Som and GABA, however, the inhibitory effect was not related to their pain modulation.

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脑室注射生长抑素或 GABA 对大鼠痛阈和脑内 GABA 或生长抑素含量的影响

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A **目的**：研究脑内 Som 和 GABA 的相互影响及其与痛觉调制的关系. **方法**：应用放射免疫，氨基酸分析仪和痛阈测定法. **结果**：发现 Som 10 μg icv 可使大鼠海马，脑干内 GABA 含量显著减少，分别由对照组的2.3±0.3和2.2±0.4 μmol g^{-1}降至1.6±0.4和1.5±0.2 μmol g^{-1}，痛阈由对照组的4.2±0.2 s 升高到7.0±1.1 s；半胱胺 600 μg icv 降低脑内 Som 后，海马和脑干内 GABA 含量也明显减少，但痛阈不变. GABA 1500 μg icv 后，痛阈变化不明显，而海马、脑干内 Som 含量均显著减少，分别由对照组的55±4和84±4 ng g^{-1}降至37±5和55±6 ng g^{-1}这一效应可被荷包牡丹碱10 μg 阻断；以异烟肼300 mg kg^{-1}降低脑内 GABA 后，海马和脑干内 Som 含量即明显增多. **结论**：脑内 Som 和 GABA 之间存在着相互抑制作用，但与它们在痛觉调制中的作用无关.

关键词 生长抑素；γ-氨基丁酸；痛阈；海马；脑干