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Exacerbation of cold restraint-induced gastric ulcer by GABA in mice

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KEY WORDS GABA; cold; physical restraint; stomach ulcer; bicuculline; baclofen; atropine; phentolamine; gastric mucin

AIM: To study the effect of GABA on cold restraint-induced gastric ulcer (CRGU) and its mechanism. **METHODS**: CRGU was produced in adult ♂ mice at 1 ℃ in cold room under restraint for 1 h. The ulcer index indicated the severity of gastric mucosa lesion. Gastric mucin was determined with alcian blue dye. **RESULTS**: GABA 2 μmol (icv) accelerated CRGU, Bicuculline (Bic) 0.2 μmol (icv) did not affect CRGU, whereas Baclofen (Bac) 4 μmol (icv) exacerbated CRGU. Bic did not modify the exacerbation of CRGU by GABA and Bac. Atropine (Atr) 0.2 mg·kg^{-1} (sc) and Phentolamine (Phen) 2.5 mg·kg^{-1} inhibited CRGU, and abolished the exacerbation of CRGU by GABA. Cold restraint (CR) decreased the amount of the gastric mucin, but GABA 2 μmol had no effect on CR gastric mucin. **CONCLUSION**: While GABA-B receptor in brain was activated, exogenous GABA exacerbated CRGU via vagal and sympathetic nerves, bearing no relation to decrease of the gastric mucin or weakening of the gastric mucosal barrier.

Some neurotransmitters and neuromodulaters participate in stress-induced gastric ulcer[1-3]. GABA is a main inhibitory neurotransmitter in mammal CNS. GABA (im) inhibited the water immersion-restraint gastric ulcer[4]. GABA (icv) inhibited the cold restraint-gastric ulcer (CRGU). Bicuculline (Bic) and picrotoxin (Pic), the blockers of GABA-A receptor in brain, reversed the inhibitory effect of GABA[5]. Baclofen (Bac), an agonist of GABA-B receptor, exacerbated the stress gastric ulcer, and aminooxyacetic acid (AOAA), an inhibitor of GABA-T, also exacerbated RGU by enhancing GABA level in brain[6]. We showed that GABA 2 μmol (icv) exacerbated CRGU in mice, but had no effect on RGU. To explore the mechanism of exacerbation of CRGU by GABA in mice, we used several drugs to study whether GABA-A and GABA-B receptors or the vagal nerve and the sympathetic nerve would participate in the effect of GABA.

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MATERIALS AND METHODS

CRGU Adult ♂ Kunming mice weighing 24 - 25 g from the Laboratory Animal Center of Suzhou Medical College were fasted except water for 15 h, then the fore and hind limbs of mice were fixed on 12 cm × 5 cm plastic plates (with abdomen up and trunk not movable) and mice were stressed at 1 ℃ in cold room for 1 h. Stomach was examined under an XTL-3 type microscope with a microruler of 0.01 mm precision for bleeding spot masses.

Ulcer index To measure the bleeding spot mass, when the length ≤ 1 mm, the ulcer index = 1; when the length was 1 - 2 mm, the index = 2; when the length was 2 - 3 mm, the index = 3, and so on. When the width > 1 mm, the index was doubled.

Mucin determination The stomachs were dissected, opened along the greater curvature, rinsed by ice-cold sucrose 0.25 mol·L^{-1}, and rumen was discarded. Having been weighed, the stomachs were incubated in alcian blue 1 mL (1 g·L^{-1}) in a sucrose solution (0.15 mol·L^{-1}), pH 5.8, at 20 ℃ for 1.5 h. The dye solution was freshly made up and filtered before use. The stomachs were transferred into sucrose 5 mL, 0.25 mol·L^{-1} for 15 min. Finally, the stomachs were incubated in magnesium chloride solution 5 mL, 0.5 mol·L^{-1} for 2 h, and removed and the magnesium chloride solution was shaken briefly with diethyl ether 5 mL. Absorbance of the aqueous layer was read at 605 nm. Results are expressed as A/mg tissue[7].

Chemicals GABA was from Shanghai Third Reagent Factory. Bic and Bac were from Sigma. Atropine (Atr) was from Suzhou Chang-Zheng Medicine Factory. Phentolamine (Phen) was the product of Ciba-Geigy Limited, Basle, Switzerland. Alcian blue dye, NaAc and $MgCl_2$ were from Shanghai Chemical Reagent Factory.

Statistical analysis The *t* test was used for data of icv and ip GABA effects. The Newman-Keul's test was used for the other data.

RESULTS

GABA The ulcer index obtained from icv GABA 2 μmol group was larger than that of control group of icv artificial cerebrospinal fluid (CSF), $P<0.01$. If the dose of GABA (100 μmol, ip) was 50-fold higher than that of icv GABA, the ulcer index had no significant change, as compared with that of the saline (ip) group, $P>0.05$ (Tab 1).

Tab 1. Effects of GABA, Bic, Bac, Atr, Phen on cold restraint-induced gastric ulcer in mice. $\bar{x} \pm s$.
[a] $P>0.05$, [b] $P<0.05$, [c] $P<0.01$ *vs* control.
[e] $P<0.05$, [f] $P<0.01$ *vs* GABA.

Drug	n	Ulcer index
CSF (icv)	8	24.0±2.1
GABA 2 μmol (icv)	8	37.5±2.1[c]
Saline (ip)	8	20.0±1.6
GABA 100 μmol (ip)	8	21.2±1.4[a]
CSF (icv)	8	33.6±1.6
GABA 2 μmol (icv)	8	44.4±2.5[b]
Bic 0.2 μmol (icv)	8	37.2±3.1[a]
Bac 4 μmol (icv)	8	47.7±6.1[b]
Bic 0.2 μmol (icv) + GABA 2 μmol (icv)	8	44.9±4.1[b]
Bic 0.2 μmol (icv) + Bac 4 μmol (icv)	8	46.4±2.5[b]
CSF (icv)	7	30.8±2.2
GABA 2 μmol (icv)	7	43.2±2.3[c]
Atr 0.2 mg·kg−1 (sc)	7	10.6±2.8[c]
Atr 0.2 mg·kg−1 (sc) + GABA 2 μmol (icv)	7	22.4±2.2[ae]
CSF (icv)	7	31.0±2.2
GABA 2 μmol (icv)	7	43.0±2.1[c]
Phen 2.5 mg·kg^{-1}(im)	7	25.0±1.5[b]
Phen 2.5 mg·kg^{-1}(im) + GABA 2 μmol (icv)	7	25.0±1.9[bf]

Bic and Bac Bic (icv), an antagonist of GABA-A receptor, did not affect the development of CRGU, as compared with the control, $P>0.05$. In contrast, Bac (icv), an agonist of GABA-B receptor, increased the ulcer index ($P<0.05$). In the group of icv Bic + GABA or Bic + Bac, the indexes were larger than that of the control ($P<0.05$). Biological statistical analysis indicated that there were not abvious differences among the groups of Bac, GABA, Bic + GABA and Bic + Bac (Tab 1).

Atr Atr (sc), a blocker of cholinergic M receptor, markedly decreased the ulcer index, $P<0.01$. Saline (sc) + GABA (icv) increased the index, $P<0.01$. The index of Art (sc) + GABA (icv) group was markedly less than that of the GABA (icv) group, suggesting that the blockade of M receptor by Atr may inhibit CRGU, and abolish the exacerbating effect of GABA (icv) on CRGU (Tab 1).

Phen Phen (im), a blocker of adrenergic α receptor, markedly decreased the index, $P<0.05$, whereas saline (im) + GABA (icv) increased the index, $P<0.01$. But Phen (im) + GABA (icv) decreased the index, which was less than that of the control ($P<0.05$) and GABA group ($P<0.01$). Results show that the blockade of α receptor by Phen may inhibit CRGU and block the exacerbating effects of GABA on CRGU (Tab 1).

CR and GABA on gastric mucin The amount of gastric mucin in the control group (icv CSF) was 0.4 ± 0.3 *A*/g, but that in the animals suffered from CR + icv CSF was 0.25 ± 0.07 *A*/g, which was lower than that of the control, $P<0.05$, and the amount of gastric mucin after CR + icv GABA 2 μmol was 0.29 ± 0.04 *A*/g, which was still lower than that of the control, $P<0.05$. These findings indicate that CR may decrease the amount of gastric mucin, and GABA (icv) does not modify the effect of CR on the gastric mucin amount.

DISCUSSION

Our experiments demonstrated that CR for 1 h induced gastric ulceration in mice, and the pretreatment of icv GABA 2 μmol exacerbated the effect of CR on ulceration, but the pretreatment of ip GABA 100 μmol did not modify CRGU. Because GABA is difficult to be transported through the blood-brain barrier, the effect of exogenous GABA on CRGU is not the direct action on peripheral tissues via the blood circulation, but rather through a special GABAergic mechanism in CNS. Our results showed that the blockade of

GABA-A receptor by Bic did not affect the development of CRGU, but the activation of GABA-B receptor by Bac exacerbated CRGU. The effect of Bac was identical with that of icv GABA. The activation of GABA-B receptor might alter the release of other central neurotransmitters[8,9], which were also related to the stress-response and the gastric ulceration[2,7,10,11]. We conjecture that the exacerbation of CRGU by exogenous GABA is based on GABA-B receptor-transmited physiological and pharmacological effects.

Atr and Phen inhibited CRGU. The mechanism of their action might abolish the exacerbation of CRGU by GABA. this suggested that the activity of CNS during CR induced the gastric ulceration through both the vagal nerve and the sympathetic nerve to stomach. After the activation of central GABA-B receptor by exogenous GABA, the exacerbation of CRGU was dependent on the both pathways simultaneously.

Our results showed that CR decreased the amount of gastric mucin, which was in agreement with Green's results. As a result of weakening the gastric mucus-HCO_3^- barrier, the gastric mucosa was damaged by gastric acid and pepsin and the ulcer was induced. Because icv GABA did not decrease the effect of CR on the mucin amount, the exacerbation of GABA on CRGU is out of all relation to the weakening of gastric mucus-HCO_3^- barrier.

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γ-氨基丁酸对小鼠冷束缚致胃溃疡的加剧效应

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关键词 γ-氨基丁酸；冷；身体的约束；胃溃疡；比枯枯灵碱；巴氯芬；阿托品；酚妥拉明；胃粘蛋白

目的：研究 γ-氨基丁酸(GABA)对冷束缚致胃溃疡(CRGU)的影响及其机制. **方法**：成年♀小鼠在 1 ℃冷室内束缚 1 h 诱发 CRGU. 用阿尔新兰染料测定胃粘蛋白. **结果**：GABA 2 μmol (icv)加剧 CRGU，GABA 100 μmol (ip)和比枯枯灵碱 0.2 μmol (icv)不影响 CRGU，而巴氯芬 4 μmol (icv)加剧 CRGU，比枯枯灵碱不改变 GABA 和巴氯芬对 CRGU 的加剧效应. 阿托品 0.2 mg·kg^{-1}和酚妥拉明 2.5 mg·kg^{-1}均能抑制此溃疡，并阻断 GABA 的加剧效应. GABA 对 CR 减少胃粘蛋白量的效应无影响. **结论**：外源性 GABA 在脑中激活 GABA-B 受体后，通过迷走和交感神经来加剧 CRGU，此效应与削弱胃粘液屏障无关

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