

γ -羟丁酸钠抗大鼠实验性胃溃疡的作用

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Anti-ulcer activity of Na- γ -Hydroxybutyrate in rat stomach

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ABSTRACT The antigastric ulcer activity of Na- γ -hydroxybutyrate (GHBA) was studied on various experimental ulcers in rats. The results showed that the number of gastric ulcers induced by sc indomethacin 20 mg/kg, restraint stress or pyloric ligation were markedly diminished by GHBA. Pretreatment with GHBA 10 mg/kg im bid 5 times revealed significant anti-ulcer activity in all of these models. The inhibitory rates of ulcer were 42, 71 and 51% for indomethacin, restraint stress and pyloric ligation, respectively. Chronic gastric ulcer induced by 10% acetic acid was also inhibited by GHBA 10 mg/kg im bid for 8 days. The ulcer inhibitory rate was 73%. The amount of gastric mucus glycoprotein and [³H] TdR incorporation into gastric tissues was increased by GHBA, but the volume of gastric juice, and acid and pepsin output were not influenced. It is suggested that the prevention of GHBA on gastric mucosal damage may be related to its preservation of the gastric mucosal barrier.

KEY WORDS sodium oxybate; stomach ulcer; [³H]thymidine; DNA; pepsin; anti-ulcer agents; glycoproteins; gastric mucosa

摘要 γ -羟丁酸钠(GHBA)能抑制吲哚美辛溃疡、应激溃疡、结扎幽门溃疡等大鼠胃溃疡的形成, 缩小大鼠

乙酸胃溃疡的溃疡面积, 与对照组比差异显著。GHBA能增加胃粘液糖蛋白量、胃组织 DNA 含量、[³H]TdR 参入胃组织 DNA 量, 但不影响胃液、胃酸、胃蛋白酶的分泌, 这表明其增强胃粘膜屏障作用与其防止胃粘膜损伤能力有关。

关键词 羟丁酸钠; 胃溃疡; [³H]胸苷; 脱氧核糖核酸; 胃蛋白酶; 抗溃疡剂; 糖蛋白; 胃粘膜

以氨基酸灌注离体大鼠胃时发现, 如将氨基丁酸的氨基自 α 位移至 γ 位, 则刺激胃蛋白酶分泌的作用减弱⁽¹⁾, 一些碱性氨基酸和其衍生物有保护胃粘膜作用⁽²⁾, γ -氨基丁酸(GABA)有抗大鼠胃溃疡作用⁽³⁻⁶⁾, 其代谢物 γ -羟丁酸钠(Na- γ -hydroxybutyrate, GHBA)亦有抗大鼠乙酸性胃溃疡作用⁽⁴⁾, GABA 有增强胃粘膜屏障功能的作用^(3,6), GHBA 对胃粘膜屏障功能的作用则未见有报道, 本研究的目的是在乙酸性胃溃疡及其它多种实验动物胃溃疡模型上进一步证实 GHBA 的抗实验性胃溃疡的作用, 并着重研究 GHBA 对胃粘膜屏障功能的影响, 进一步探讨其抗胃溃疡作用的机理。

MATERIALS AND METHODS

γ -羟丁酸钠(GHBA)注射液(上海第十三制药厂)2.5 g/10 ml, 吲哚美辛(北京第三制药厂)以 1% 羧甲基纤维素(CMC)液配成 1% 吲哚美辛悬液。10% 乙酸液, 由冰乙酸(杭州叶绿素厂)加蒸馏水制成。

Wistar 大鼠, ♀、♂不拘, 共 144 只, 由本校动物房繁殖。

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GHBA对实验性胃溃疡的作用 将胃溃疡大鼠随机分为治疗组及对照组。治疗组每日8:00及14:00各im GHBA 10 mg/kg, 对照组im生理盐水。对吲哚美辛胃溃疡im GHBA bid, 共5次, 于第3次给GHBA后sc吲哚美辛1次, 末次im GHBA后1h再sc吲哚美辛1次, 5h后处死大鼠; 应激胃溃疡im GHBA bid, 共5次, 于第5次im GHBA后1h给大鼠应激, 应激后16h处死大鼠; 结扎幽门胃溃疡亦im GHBA bid, 共5次, 于第5次im GHBA后5-6h结扎幽门, 术后19h处死大鼠; 对乙酸胃溃疡im GHBA bid, 共8d, 并于d9处死大鼠。

1 实验性胃溃疡 按文献(3)法制备吲哚美辛胃溃疡、束缚水浸应激胃溃疡、结扎幽门胃溃疡和乙酸诱发胃溃疡。

2 观察胃溃疡数目及面积大小的方法 实验结束时处死大鼠, 取出胃, 将1%福尔马林液10ml注入腹腔内, 再将胃浸入1%福尔马林液内固定10min后, 取出胃沿胃大弯剖开, 洗去胃内容物后, 将胃粘膜朝上平铺于一张白纸上, 以放大镜检数胃粘膜溃疡数, 比较两组之胃溃疡数, 并计算GHBA对胃溃疡的抑制率(抑制率=对照组胃溃疡数-治疗组胃溃疡数/对照组胃溃疡数×100%)。

对乙酸诱发胃溃疡则测量溃疡的纵横径, 取其相乘积表示溃疡面积, 并计算其溃疡面积抑制率。

胃酸及胃蛋白酶活力测定

1 胃酸测定 以酚酞为指示剂, 以H₂O 0.01 mol/L滴定胃总酸量, 并计算胃酸分泌速率(胃酸分泌速率mmol/h=胃总酸量/观察时间)。

2 测定胃蛋白酶活力⁽⁷⁾ 结果以酪氨酸μg/min表示。

胃壁粘液糖蛋白测量 大鼠处死后取出胃, 沿大弯剖开, 以冰冷之0.25 mol蔗糖液淋洗后测量附着于胃壁的粘液中糖蛋白量^(8,9)。

[³H]TdR 参入大鼠胃组织 DNA 的测定

测定[³H]TdR参入胃组织DNA量⁽¹⁰⁾, 闪烁液组成为0.5%PPO+0.005%POPOP+8%萘溶于二甲苯·二氧六环·乙醇等容积的混合液中。

测定胃组织中DNA含量 取胃于冰袋上剪碎成1mm³碎片, 称取200mg, 处理胃组织⁽¹⁰⁾, 取消化液测DNA含量⁽¹¹⁾。

统计分析 按t'检验比较对照组与给药组结果的差异。

RESULTS

GHBA对大鼠实验性胃溃疡的影响

1 对吲哚美辛胃溃疡、应激胃溃疡、结扎幽门胃溃疡的影响 GHBA im能减少大鼠胃溃疡数, 结果见Tab 1。

Tab 1. Effect of Na-γ-hydroxybutyrate (GHBA) 10 mg/kg im bid for 5 times on gastric ulcers in rats. $\bar{x} \pm SD$. * $P > 0.05$, ** $P < 0.05$, *** $P < 0.01$ vs normal saline (NS). %: Inhibitory rate.

Gastric ulcer induced by	n	Number of ulcers		
		NS	GHBA	%
Indomethacin	10	24±9	14±4**	42
Restraint	7	17±6	5±4**	71
Pyloric ligation	10	47±11	23±9***	51

2 对乙酸胃溃疡的影响 GHBA im可使胃溃疡面积缩小, 使胃溃疡面积由0.3±0.12 mm² (n=10)至0.08±0.06 mm² (n=10), 其抑制率为73%, 与对照组比差异显著($P < 0.01$)。

对胃液量、胃酸及胃蛋白酶分泌的影响 GHBA im对结扎幽门大鼠的胃液量、胃酸及胃蛋白酶分泌与对照组相比无明显差异(Tab 2)。

对大鼠胃壁粘液糖蛋白量的影响 GHBA im使结扎幽门胃溃疡及乙酸胃溃疡胃壁粘液糖蛋白量增加, 在结扎幽门胃溃疡, 胃粘液糖蛋白量由0.13±0.02 μg/g胃组织(n=8)增至0.19±0.05 μg/g胃组织(n=10) ($P < 0.05$),

Tab 2. Effects of GHBA 10 mg/kg im on volume of gastric juice, secretion of gastric acid and activity of pepsin, $\bar{x} \pm SD$, * $P > 0.05$ vs NS.

	NS	GHBA
Rats	8	10
Gastric juice (ml)	13 ± 5	13 ± 6*
Acid output (mmol/L)	4 ± 2	3 ± 2*
Gastric acid (mmol/h)	0.22 ± 0.1	0.17 ± 0.09*
Pepsin (tyrosine $\mu\text{g}/\text{min}$)	26 ± 20	29 ± 32*

而在乙酸胃溃疡则由 $0.05 \pm 0.01 \mu\text{g}/\text{g}$ 胃组织 ($n = 10$) 增至 $0.12 \pm 0.02 \mu\text{g}/\text{g}$ 胃组织 ($n = 10$), 与对照组比有显著差异 ($P < 0.01$).

对 [^3H]TdR 参入大鼠胃组织 DNA 及胃组织 DNA 含量的影响 GHBA im 使 [^3H]TdR 参入乙酸性胃溃疡的胃组织 DNA 的量增加, 由 $330 \pm 19 \text{ cpm}/100 \text{ mg}$ 胃组织增至 $401 \pm 59 \text{ cpm}/100 \text{ mg}$ 胃组织 ($P < 0.05$), 并使胃组织 DNA 含量增高, 由 $191 \pm 59 \mu\text{g}/100 \text{ mg}$ 胃组织增至 $271 \pm 25 \mu\text{g}/100 \text{ mg}$ 胃组织 ($P < 0.01$), 与对照组比差异显著 ($n = 7$).

DISCUSSION

本文结果表明 GHBA im 对多种实验性大鼠胃溃疡有效, 对大鼠乙酸胃溃疡的疗效与文献⁽¹⁾报道一致, GHBA im 能明显增加胃壁粘液糖蛋白量, 而附于胃壁的为 HCO_3^- 所饱和的粘液不动层是胃粘膜屏障的重要成份⁽¹²⁾, 说明 GHBA 可通过增强胃粘膜屏障机能而发挥其抗实验胃溃疡作用. GHBA im 有增加胃组织内 ATP 含量及促进胃组织蛋白质合成的作用⁽⁴⁾, GHBA im 又能使 [^3H]TdR 参入胃组织 DNA 量增加和提高胃组织 DNA 含量, 这些结果说明 GHBA 有促进胃组织能量代谢及合成代谢过程, 因而有增强胃粘膜抵抗致溃疡因素的作用, 但 im GHBA 10 mg/kg 对胃酸及胃蛋白酶分泌既无促进亦无抑制作用, 即使 iv GHBA 40 mg/kg 亦不减少基础胃酸分泌⁽¹³⁾, 所以 GHBA 抗实验性胃溃疡作用与其对胃酸及胃蛋白酶的分泌作用无关.

由上可知 GHBA 确有抗实验性胃溃疡作用, 其作用在增加胃壁粘液糖蛋白量, 提高胃组织内 ATP、DNA 含量和促进胃组织蛋白质合成, 故其作用与增强胃粘膜屏障机能有关.

GABA 在细胞内可转化为 GHBA⁽¹⁴⁾, GHBA 和 GABA^(3,4) 均有抗实验性胃溃疡作用, GABA 所用剂量为 100 mg/kg, 而 GHBA 剂量为 10 mg/kg, 剂量小于 GABA 10 倍, 这是否表明 GABA 在体内转化为 GHBA 起作用, 有待研究.

REFERENCES

- 1 Aono M, Moriga M, Uchino H. Effect of topical application of amino acids on gastric pepsin secretion in the rat. *Gastroenterol Jpn* 1981; 16 : 100
- 2 Fromm D. Gastric mucosal barrier. In: Johnson LR, ed. *Physiology of the gastrointestinal tract*; vol 1, 1st ed. NY: Raven Press, 1981: 733-48
- 3 Yong DG, Geng BQ, Gu GG. Effects of GABA on gastric ulcers and gastric mucosal barrier in rats. *Acta Pharmacol Sin* 1985; 4 : 273
- 4 Татевосян АТ, Геворкян ЖС, Оганесян АС, Мирзоян СА. Биохимические основы противоязвенного действия ГАМК и натрия оксibuтирата. *Фармакол Токсикол* 1982; 45 (2) : 48
- 5 Bhargava KP, Gupta GP, Gupta MB. Central GABA-ergic mechanism in stress-induced gastric ulceration. *Br J Pharmacol* 1985; 84 : 619
- 6 Minano FJ, Serrano JS, Durán JA, Sancibriañ M. Protective effect of GABA and sodium valproate on stress-induced gastric lesions in guinea-pigs. *J Pharm Pharmacol* 1985; 37 : 675
- 7 Anson ML. The estimation of pepsin, trypsin, papain, and cathepsin with hemoglobin. *J Gen Physiol* 1938; 22 : 79
- 8 Green AP, Lander JE, Turner DH. The effect of stress and indomethacin, carbenoxolone, salbutamol, metiamide, zolimidine and CF 19415 on rat gastric mucus. *J Pharm Pharmacol* 1981; 33 : 348
- 9 Taché Y. Bombesin : Central nervous system action to increase gastric mucus in rats.

- Gastroenterology* 1982; 83 : 75
- 10 Johnson LR, Guthrie P. Effect of cholecystokinin and 16, 16-dimethyl prostaglandin E₂ on RNA and DNA of gastric and duodenal mucosa. *Ibid* 1976; 70 : 59
- 11 Giles Kw, Myers A. An improved diphenylamine method for the estimation of deoxyribonucleic acid. *Nature* 1965; 206 : 93
- 12 Allen A, Garner A. Mucus and bicarbonate secretion in the stomach and their possible role in mucosal protection. *Gut* 1980; 21 : 249
- 13 Watanabe K, Watanabe H, Goto Y, Shimizu M, Maeda-Hagiwara M. γ -Butyrolactone enhances the activity of GABA in the gastric acid secretion of anesthetized rats. *Jpn J Pharmacol* 1983; 33 : 1163
- 14 Vayer P, Mandel P, Maitre M. Conversion of γ -hydroxybutyrate to γ -aminobutyrate in vitro. *J Neurochem* 1985; 45 : 810

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五味子乙素对原代培养大鼠肝细胞脂质过氧化的抗氧化作用

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Action of schizandrin B, an antioxidant, on lipid peroxidation in primary cultured hepatocytes

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ABSTRACT The action of schizandrin B (Sin B) was observed in freshly isolated hepatocytes damaged by FeSO₄/cysteine and CCl₄. Two types of free radicals, ·OH and ·CCl₃, generated from FeSO₄/cysteine and CCl₄, respectively, induced lipid peroxidation in hepatocytes. It was found that the speed of lipid peroxidation (MDA production) and the degree of alteration in hepatocyte morphology were closely related to the type of free radicals. MDA production and membrane protrusion of hepatocytes injured by FeSO₄/cysteine were faster and more severe than those observed with CCl₄. Sin B was shown to decrease the production of MDA and the release of GPT and LDH, and to increase hepatocyte viability as well as maintaining the integ-

rity of the hepatocyte membrane surface. These actions of Sin B were stronger than vitamin E at the same concentration. It was observed that no inhibitory effect of phenobarbital, a typical inducer of cytochrome P-450, as Sin B induced liver cytochrome P-450, on MDA production in hepatocytes damaged by FeSO₄/cysteine. The results suggest that Sin B possesses antioxidant activity.

KEY WORDS free radicals; lipid peroxides; malondialdehyde; antioxidants; schizandrin B

摘要 将新鲜分离的大鼠肝细胞体外培养, 分别用可产生自由基的 FeSO₄/半胱氨酸系统及 CCl₄ 引起肝细胞膜的脂质过氧化。五味子乙素对这两种不同自由基产生系统所引起的肝细胞膜脂质过氧化损伤均有保护作用, 使肝细胞丙二醛的生成及 LDH 和 GPT 酶的释放均减少, 肝细胞存活率提高, 细胞膜形态保持完整。表明五味子乙素有抗氧化作用。

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