牛磺酸在鼠胃腸生理角色之研究

Physiological Roles of Taurine in Rat Gastrointestinal Tract

中文摘要

牛磺酸(Taurine)是哺乳動物體內抑制性胺基酸(inhibitory amino acids)之一種。本研究之第一部分爲使用免疫組織化學方法(Immunohistochemistry)，以牛磺酸合成酵素(CSAD)之抗體，探討在胃之分佈情況及其分泌細胞存在的位置，發現在鼠胃之腸經叢(Myenteric plexus)及黏膜下神經叢(Submucosal plexus)含CSAD 抗體免疫反應之神經纖維存在。

另一方面，探討牛磺酸對胃酸分泌及其信號傳訊系統方面的影響。結果顯示在離體鼠胃，牛磺酸於 10^{-9}M-10^{-4}M 時產生劑量依存性(dose-dependence)誘導胃酸分泌，且在 10^{-5}M 可以引起最大誘發分係作用，約爲自發性胃酸份密的 1.6 倍。而牛磺酸所誘發胃酸作用，可被 GABA 受體 A 型的拮抗劑 antagonist)bicuculline，抗膽素藥 atropine，和抑制鈉離子通道作用劑 tetrodotoxin 完全抑制，而組織胺 H_2 受體拮 cimetidine 及胃泌素 gastrin 的拮抗劑 proglumide 則只呈現部份抑制現象。而對於甘胺酸 glycine 受體拮抗劑 strychnine 則完全沒有影響。在牛磺酸 10^{-6}M 以下所誘發胃酸分泌可完全被 atropine 及 tetrodotoxin 所抑制，而在牛磺酸 10^{-5}M 以上所誘發胃酸分泌對 atropine 及 tetrodotoxin 則呈現部份抑制的現象。至於其信號傳訊方面，我們將鼠胃分成肌肉層及黏膜層(包括 submucosal connective tissues) 以牛磺酸處理 2 分鐘，發現牛磺酸可以使胃黏膜層細胞內 cAMP 含量隨劑量依存性增加且與牛磺酸濃度呈現高相關性($r=0.859, P < 0.001$)。

本研究之第二部份在探討牛磺酸對胃及長側間隙通透影響，過去有研究報告顯示胃黏膜層在致酸環境下會誘發側間隙傷害。然而，牛磺酸於離體鼠胃黏膜側間隙通透的機制尚未被研究。因此本篇探討牛磺酸對胃及十二指腸側間隙通透影響，以【^{14}C】mannitol 分子做指標決定側間隙通透。結果顯示，在離體鼠胃部份，牛磺酸 10^{-9}M-10^{-4}M 產生劑量依存性降低穿透率，且在 10^{-5}M 最大抑制作用；而再十二指腸側間隙通透方面，牛磺酸 10^{-9}M-10^{-4}M 則產生劑量依存性降低穿透率；以 10^{-6}M 爲最大促進作用。另一方面，牛磺酸影響側間隙路徑和細胞外鈣離子、及細胞內 cAMP 關係在此也被討論。結果顯示，牛磺酸影響胃側間隙通透與細胞外鈣離子較無關而與細胞內 cAMP 含量有關。而在十二指腸側間隙通透與細胞外鈣離子較有關而與細胞內 cAMP 含量變化無關。

綜合以上結果證實 1〉胃內含有牛磺酸基導性神經存在並參與調節酸分泌。2〉牛磺酸誘發酸分泌乃經過 GABA 受體 A 型，主要可能由胃中膽素激導性神經(cholinergic neuron)。3〉牛磺酸呈現劑量依存性抑制胃側間隙通透，此所以發之細胞內 cAMP 上升，正如同與牛磺酸誘導酸分泌上生細胞內 cAMP 機制相似。4〉而牛磺酸對於十二指腸側間通透性呈現劑量依存性升高，可能與細胞外鈣較有關，而與細胞內 cAMP 較無關。

英文摘要
The effect of Taurine(Tau) on acid secretion, mucosal permeability, signal transduction, and immunohistochemical localization of taurinergic neurons in rat stomach were localized with immunohistochemical methods using antibody against Tau synthesizing enzyme,.cysteine sulfinic acid decarboxylase (CSAD). Myenteric plexus and submucosal plexus were found to contain CSAD-positive nerve fibers.

The effect of Tau on gastric acid secretion was investigated in an everted preparation of isolated rat stomach. Tau at 10^{-9}M to 10^{-4} M induced acid secretion, and the maximum secretion was obtained at 10^{-5} M, which was approximately 1.6 fold of the spontaneous secretion . The Tau-induced acid secretion was inhibited by bicuculline, atropine but not bycimetidine, proglumide and strychnine. Atropine and tetrodotoxin completely inhibited the acid secretion induced by low concentrations of Tau and partially induced by high concentrations of Tau. Intracellular cAMP contents in the stomach was significantly increased by Tau treatment in a dose- dependent manner. A high correlation($r=0.859$, $p<0.001$)between concentration of Tau and cAMP was observed. Therefore, it is suggested that Tau-induced acid secretion may act through A type of GABA receptor, which located mainly on the cholinergic neurons and partially on the noneuronal cells in the rat stomach.

On the other hand, the effect of Tau on the paracellular permeability of the mucosal layer was investigated in the isolated rat stomach and the duodenal segment. Several researchers have reported that acidification can induce paracellular mucosal layers were studied using transepithelial flux of 【^{14}C】mannitol. Mannitol is a useful size probe to determine mucosal permeability of tight junction and its movement across intact epithelium was limited to the paracellular pathway. The concentration of Tau from 10^{-9}M to 10^{-4}M produced a dose-dependent decrease of permeability coefficient of mannitol in the stomach, and the maximal inhibition of permeability of mannitol was obtained at 10^{-5}M. In the absence of extracellular Ca^{2+}, the paracellular permeability of stomach is lower than that in the physiological condition, and it cannot be further decreased by Tau. In the duodenal segment, Tau ranging form 10^{-9}M to10^{-4} M produced a dose-dependent increase of permeability coefficient of mannitol, and the maximal stimulation was obtained at 10^{-6}M. The absence of extracellular Ca^{2+} also decrease the basal level of duodenal permeability, and it can also decrease the basal level of duodenal permeability, and it can also be stimulated by Tau in a minor degree. In addition, intracellular cAMP contents was significantly increased by Tau treatment in the stomach but not in duodenal segment. As for the effect on Ca^{2+} free

solution, there were no apparent difference in the presence of the Tau in the stomach. In the duodenal segment there were apparently different in the presence of the Tau. These results indicate that Tau-mediated apical acidification decrease the paracellular permeability in the stomach. The mechanisms of Tau on the permeability were involved with increase of intracellular cAMP contents as well as acid secretion. Extracellular Ca^{2+} may not involved in the action of Tau on the paracellular permeability in the isolated stomach. In the duodenal segment, Tau increased paracellular permeability of mucosal layer in a Ca^{2+} - dependent manner, no correlation with cellular cAMP was observed.

All these results indicate that 1〉 taurinergic neurons are involved in the modulation of gastric acid secretion. 2〉 Tau induced acid secretion via GABA receptor probably located mainly in the cholinergic neurons. 3〉Tau produced a dose dependent decrease of permeability coefficient in the rat stomach.The mechanisms of Tau on the permeability included the increase of intracellular cAMP as well as acid secretion. 4〉 Tau produced a dose dependent increase of permeability coefficient in the rat duodenal segment. The action of Tau on the paracellular permeability in the duodenal segment required extracellular Ca^{2+} but had no participation of intracellular cAMP.