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学位論文題目	Mechanisms in regulating the release of serotonin from the perfused rat stomach （ex vivo　ラット胃灌流実験によるセロトニン分泌の制御機構の研究）
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論文内容の要旨

【ABSTRACT】
Immunohistochemical study of the rat stomach showed that serotonin-containing enterochromaffin (EC) cells were densely packed in the antral mucosa, sparsely scattered in the corpus, and not found in the fundus. Such morphological findings suggest that serotonin detected in this study may have originated from antral EC cells. Luminal acidification stimulated the vascular release of serotonin but did not affect the luminal release of serotonin. The basal release of serotonin into the vasculature was 10 times higher than that into the gastric lumen at intragastric pH 2. The vascular release of serotonin is regulated by stimulation from cholinergic nicotinic mechanisms, whereas inhibitory neurotransmitters such as vasoactive intestinal peptide and NO are probably not involved. Somatostatin and peptide YY originating from endocrine cells may exert direct inhibitory effects, possibly via somatostatin and peptide YY receptors on the EC cells, and a cholinergic muscarinic mechanism may exert indirect effects on the vascular release of serotonin via the muscarinic receptor on the endocrine cells.

【BACKGROUND】
Evidence has shown that serotonin regulates gastric motility as well as gastric acid secretion in both in vitro and in vivo models of various animals. Despite a number of studies that investigated the functional role of serotonin in the stomach, few previous studies have examined the neuronal or hormonal mechanism to regulate the release of serotonin from the stomach.

【PURPOSE】
In the present study, we investigated the effects of intragastric pH, neuronal and hormonal mechanisms on the release of serotonin from the isolated vascularly and luminally perfused rat stomach.

【MATERIALS AND METHODS】
Male Wistar rats weighing 200-300g were used. The immunohistochemistry for serotonin in the corpus and antrum of the rat stomach was compared. The rats were fasted and anesthetized. Arterial perfusion was achieved through aortic cannula with the tip lying adjacent to the celiac artery. The vascular perfusate consisted of Krebs solution containing 3% dextran, 0.2% bovine serum albumin, etc. Vascular effluents were collected through portal vein. Citrate-phosphate buffer at pH 2 was used as a luminal perfusate. Each of following chemicals was introduced into the vasculature for a 12- to 21-min period of perfusion; 1 μM of tetrodotoxin (TTX), 100 μM of hexamethonium bromide, 1 μM of atropine sulfate, 1 μM of acetylcholine, 0.1 μM of vasoactive intestinal peptide (VIP), 1 μM of the VIP receptor antagonist VIP(10-28), 1 μM sodium nitroprusside (NaNP), 100 μM of N^G-nitro-l-arginine(l-NNA), 0.1 μM of gastrin, 0.1 μM of somatostatin or 0.1 μM of peptide YY (PYY). In some experiments, 1 μM acetylcholine was infused combined with 1 μM atropine; atropine was infused for 9- to 21-min period, and acetylcholine was infused for 12- to 21-min period. In some experiments, 0.1 μM of somatostatin or 0.1 μM of PYY was infused combined with 1 μM TTX; TTX was infused for 9- to 21-min period, and somatostatin or PYY was infused for a 12- to 21-min

period. The determination of serotonin was performed by HPLC. Vascular effluents were filtrated with Ultrafree-MC by centrifuging for 30 min at 10,000 rpm at 4oC. Luminal effluents were filtrated manually with a 0.22-(m-pore disk filter, 100-(l aliquots of filtrates were injected into HPLC, and serotonin content was measured. Statistical analysis of the data was performed by use of single-factor ANOVA for repeated measures followed by the Scheffe's F test. A paired t-test (two-tail) was used to compare the values of mean basal release and mean serotonin release during drug infusion in the experiments and a value of $p < 0.05$ was considered statistical significant.

【RESULTS】

Luminal acidification stimulated the vascular release of serotonin but did not affect the luminal release of serotonin. The basal release of serotonin into the vasculature was 10 times higher than that into the gastric lumen at intragastric pH 2. The vascular release of serotonin is regulated by stimulation from cholinergic nicotinic mechanisms, whereas inhibitory neurotransmitters such as vasoactive intestinal peptide and NO are probably not involved. Somatostatin and peptide YY originating from endocrine cells may exert direct inhibitory effects, possibly via somatostatin and peptide YY receptors on the EC cells, and a cholinergic muscarinic mechanism may exert indirect effects on the vascular release of serotonin via the muscarinic receptor on the endocrine cells.

【DISCUSSION】

The serotonin-contaning EC cells were located in the antrum than corpus, and released more serotonin into vasculature than lumen. The released amount of serotonin is highest at pH 2 intraluminally. The TTX and hexamethonium reduced the vascular release of serotonin, although atropine had no effect. These results suggest that the cholinergic nicotinic pathway seems to be involved. Neither VIP, VIP(10-28), NaNP, nor l-NNA affected serotonin release.These findings are quite different from those observed in the rat duodenum. The inhibitory effects of somatostatin and PYY were not antagonized by TTX, suggesting both of them may exert direct inhibitory effects on serotonin release without the mediation of neuronal pathway. Because it has been shown that cholinergic muscarinic receptors are located on antral D cells, the cholinergic muscarinic pathways may act indirectly on EC cells through somatostatin-containing D cells in the stomach. This might be a partial explanation for the inhibitory effect of acetylcholine on the release of serotonin.

【CONCLUSION】

The serotonin-containing EC cells were densely distributed in the antrum, and these cells release more serotonin into the vasculature than into the gastric lumen. Vascular release of serotonin was increased at low pH levels of gastric lumen. The vascular release of serotonin was regulated by cholinergic nicotinic mechanisms but not regulted by VIP and NO pathways. Somatostatin and PYY originating from endocrine cells appeared to exert direct inhibitory effects, possibly via somatostatin and PYY receptors on EC cells, and the cholinergic muscarinic mechanism might exert indirect effects on serotonin release, possibly via muscarinic receptors on somatostatin-containing D cells.

論文審査の結果の要旨

本研究は免疫組織化学法によりラット胃壁内のセロトニンを含むenterochromaffin cell（EC細胞）の分布を調べ、またex vivoラット胃灌流モデルを用いて胃管腔内酸性刺激時のセロトニン分泌量を高速液体クロマトグラフィ法で計測して以下の点を明らかにした。

1．EC細胞は胃前庭部粘膜に密に、胃体部にはまばらに分布し、胃底部にはほとんど分布していなかった。

2．胃管腔内に酸性刺激を加えると酸性度に応じて門脈中へのセロトニン分泌量は増加したが、胃管腔内への分泌量は変化しなかった。また、門脈血中へのセロトニン分泌はテトロドトキシン（TTX）およびヘキサメトニウム（hexamethonium）により減少したため、コリン作動性神経の関与が示唆された。

3．ソマトスタチンならびにペプチドYY（peptide YY）はセロトニン分泌量を減少させ、またこれらの抑制性作用はTTXにより影響を受けなかったためEC細胞への直接作用によるものと考えられた。

このように、本論文は胃管腔内酸性刺激によるセロトニンの門脈内への分泌亢進ならびにそのメカニズムについて重要な知見を与えたものであり、博士（医学）の学位論文に値するものである。

なお、本学位授与申請者は平成13年９月４日実施の論文内容とそれに関連した試問を受け、合格と認められたものである。