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Apelin-12 stimulates acid secretion through an increase of histamine release in rat stomachs

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ABSTRACT

Background: Apelin is a peptide that was originally isolated from bovine stomach extract and has been demonstrated to be an endogenous ligand for orphan receptor APJ. Both apelin and the APJ receptor are widely distributed in the whole body. Apelin is supposed to have important regulatory roles in the function of many organs such as in the cardiovascular system; however, the mechanism of apelin function has not been elucidated. In this study, we studied the action of apelin in acid secretion and demonstrated its mechanism of action.
Methods: Gastric lumen-perfused rats were prepared and their stomachs were perfused with a saline solution using a peristaltic pump. Apelin-12, 36 or Pyr[1]-apelin-13, were intravenously injected to examine their effects on acid secretion in rats. In some experiments, rats were pretreated with famotidine (0.33 mg/kg) or atropine sulfate (0.1 mg/kg) intravenously injected 5 or 15 min before apelin injection. Furthermore, isolated vascularly perfused rat stomachs were prepared to examine the effect of apelin on histamine release, which was assayed in the effluent by radioimmunoassay. Messenger RNA of histidine decarboxylase (HDC) in gastric mucosa of isolated stomach was measured by real-time RT-PCR.
Results: Apelin-12 (20–100 μg/kg) dose-dependently increased gastric acid secretion, with a maximum of 203% at 100 μg/kg (n = 5). Neither Pyr[1]-apelin-13 nor apelin-36 caused a significant increase in acid secretion. Famotidine completely blocked the stimulatory action of apelin on acid secretion. Apelin-12 (100 μg/20 ml/10 min) markedly increased histamine release from isolated vascularly perfused rat stomachs by 278%, and also increased the mRNA of HDC by 480% of the control. Atropine sulfate did not abolish the effect of apelin on the secretion of gastric acid. Apelin-12 amplified an increase of acid secretion stimulated by gastrin injection.
Conclusion: These results indicate that apelin-12 stimulates gastric acid secretion through an increase in histamine release and synthesis from gastric mucosa, suggesting that apelin might play a role in the secretion of gastric acid or serve as a regulating factor of the secretion of gastric acid.

1. Introduction

Apelin is a peptide that was recently isolated from bovine stomach extract as an endogenous ligand of the human orphan receptor APJ [1]. The APJ receptor is a G-protein-coupled receptor first identified in 1993 [2] and is expressed in a wide range of tissues, including the endothelium, myocardium, vascular smooth muscle [3,4], adipose tissue [4], and throughout the brain [5]. On the other hand, apelin is distributed in the stomach and also widely in the organs of the body. Preproapelin messenger RNA (mRNA) is distributed in many organs such as in the central nervous system, adrenal gland, vas deferens, kidney, heart, spleen, and liver [6,7]. In an immunohistochemical study, apelin-like immunoreactivity was found in the endothelia of the small arteries of the mesenteria, omentum, heart, lung, gastrointestinal tract, spleen, pancreas, and kidney [8]. Apelin-like immunoreactivity was also found in the stomach wall [8]. In circulation, an immunoenzyme assay estimated the concentration of apelin in humans to be between 3 and 4 ng/ml [9]. To date, the apelin-APJ system has been thought to have relevant pathophysiological effects in those tissues. Recently, apelin was found to have biological functions such as vasodilation [10]. Intravenous apelin administration in rodents reduces mean arterial pressure [11] and systemic venous tone [12]. It has also been demonstrated that apelin is a coronary vasodilator, and when administered at systemic doses, reduces peripheral vascular resistance [10]. These studies imply that apelin has a physiological role in the regulation of circulation [13]. On the other hand, even though it is known that apelin is distributed in the gastrointestinal tract, the actions of apelin there are not fully

Abbreviations: HDC, Histidine decarboxylase; mRNA, messengerRNA; ECL cell, Enterochromaffin-like cell.

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understood. A study by Wang and coworkers showed that apelin stimulated cholecystokinin secretion from a murine enteroendocrine cell line (STC-1) [14]. However, an immunohistochemical study showed that apelin-like immunoreactivity is found in the stomach wall, especially in the oxyntic mucosa, with positivity found in the surface and neck mucous cells and oxyntic cells [8]. These results suggest that apelin may have a function in the gastric mucosa, especially on the secretion of gastric acid. Therefore, in this study, we examined the effect of apelin on the secretion of gastric acid and histamine from gastric mucosa in rats. We also compared differences in acid secretion between different isoforms of apelin.

2. Materials and methods

The study protocol complied with the guidelines for the use of experimental animals of Saitama Medical University.

2.1. Surgical and experimental procedures for gastric-lumen perfused rats

Male Wistar rats weighing 180–220 g (Sankyo Lab Service Co. Tokyo, Japan) were fasted overnight and anesthetized with an intraperitoneal injection of urethan solution (37% wt/vol) of 0.34 ml/100 g body weight and prepared for the gastric acid output study according to a technique described previously [15]. The trachea was exposed, cannulated, and exteriorized. The abdomen was opened through the linea alba, exposing the stomach, and a polyethylene tube for the perfusate was inserted into the esophagus and the tip positioned in the luminal portion of the stomach. The pyloroduodenal junction was then exposed, and another polyethylene tube was introduced into the stomach, via an incision in the duodenum, and secured firmly by a ligature around the pylorus. The stomach lumen was washed with saline until the effluent was clear and then perfused with a saline solution at 37 °C at a rate of 1 ± 0.1 ml/min using a peristaltic pump (Micro tube pump MP-3A, Tokyo Rikakikai Co. Ltd, Tokyo, Japan). The effluent was collected at 15-min intervals and assayed for titratable acid against 0.01 M NaOH using phenolphthalein as an indicator.

2.2. Determination of acid output

Acid output was expressed in terms of H^+ μEq/15 min or 60 min. Acid output was measured after administering apelin-12, (apelin-12 [human, rat] was a gift from Professor Kazuhiko Tatemoto that was synthesized by a solid-phase synthetic technique using an automatic peptide synthesizer [Model 431, Applied Biosystems] and purified by HPLC after deprotection [8], and pyroglutamated form Pyr^1-apelin-13 [human, rat] (Pyr^1-apelin-13) and apelin-36 [human] were purchased from the Peptide Institute, Inc., Osaka, Japan, each of these peptides has the purity of more than 99%) intravenously as a bolus at 1 h after the beginning of gastric lumen perfusion. To examine the dose-dependent effects of apelin-12, apelin-12 at 4, 20, or 100 μg/kg was administered intravenously as a bolus at 1 h after the beginning of gastric lumen perfusion. Pyr^1-apelin-13 or −36 at 100 μg/kg was also administered intravenously as a bolus, and their effects on acid secretion were examined.

To investigate the role of histamine in apelin-stimulated acid secretion, famotidine (0.33 mg/kg, Astellas Pharm. Inc. Co., Ltd, Tokyo, Japan) was intravenously administered to gastric lumen-perfused rats 15 min before apelin injection. The effluents of the gastric lumen were collected at 15-min intervals and assayed for titratable acid against 0.01 M NaOH.

To evaluate the involvement of the vagal cholinergic nerve in apelin-stimulated acid secretion, atropine sulfate (Sigma Chemical Co., St Louis, MO, USA) was intravenously administered to gastric lumen-perfused rats 5 min before apelin injection. The effluents of the gastric lumen were collected at 15-min intervals and assayed for titratable acid against 0.01 M NaOH.

2.3. Surgical and experimental procedures for isolated vascularly perfused rat stomachs to study the effect of apelin-12 on histamine release

Isolated, vascularly perfused rat stomachs were prepared as previously described [16]. Rats were anesthetized with pentobarbital sodium (40 mg/kg, Abbott Laboratories, North Chicago, IL, USA) administered intraperitoneally. The abdominal aorta was carefully dissected free, and all branches except the celiac trunk were ligated and the spleen was removed. The aorta was ligated proximal to the superior mesenteric artery and proximal to the celiac axis immediately before a polyethylene catheter with a diameter of 1 mm was inserted and secured at the level of the celiac axis. No more than 30 s was permitted to elapse between ligation of the aorta and initiation of the gastric vascular perfusion. Thereafter, the portal vein was isolated and cannulated with a polyethylene catheter with a diameter of 1.8 mm, and all branches except those to the stomach were ligated. The completely isolated, perfused stomach was transferred to an organ bath filled with Krebs-Ringer buffer (KRB, pH 7.4). The vascular bed was perfused through the arterial catheter with KRB (pH 7.4), which contained 80 mg/dl glucose (Waco Pure Chemical Co., Osaka, Japan), 4% dextran (Amersham Pharmacia Biosciences AB, Uppsala, Sweden) and 0.2% bovine serum albumin (Sigma Chemical Co.). The perfusate was gassed with 95% O_2/5% CO_2 and the stomachs were perfused at a rate of 2 ml/min. The vascular effluent was not recirculated. The gastric lumen was perfused with 1% N-2-hydroxyethylpiperazine-N′-ethanesulfonic acid (HEPES, Wako Pure Chemical Co.) solution (pH 7.4) gassed with 100% O_2 at 2 ml/min. The perfusate and the organ bath were kept at 37 °C. The vascular perfusion was maintained at 2 ml/min. After a 20-min equilibration period, the venous effluent was collected at 2-min intervals into tubes on ice, and an aliquot of 1 ml was pipetted into tubes. Samples were stored at −20 °C until assayed. Histamine in the effluent was assayed by radioimmunoassay after acetylation using a commercial specific kit (Immunotech. S.A., Marseille Cedex, France).

2.4. Experimental design to study the effect of apelin on histamine release

To measure basal release of histamine, the isolated rat stomachs were vascularly infused with KRB for 30 min. To measure apelin-12-stimulated histamine release, apelin-12 at a concentration of 10 μg/2 ml/min was continuously infused into the isolated vascularly perfused rat stomachs for 10 min beginning 10 min after the initiation of continuous injection of KRB. The venous effluent was collected at 2-min intervals into tubes on ice. Histamine in the effluent was assayed by radioimmunoassay.

2.5. Quantitative detection of rat HDC mRNA in rat stomachs by real-time reverse transcription polymerase chain reaction (RT-PCR)

To investigate the effect of apelin-12 on histamine synthesis in the gastric mucosa, HDC mRNA was assayed by real time RT-PCR using a LightCycler system. Apelin-12 at a concentration of 10 μg/2 ml/min was continuously infused into isolated vascularly perfused rat stomachs for 10 min, and 2 h after the injection, the mucosa of the gastric corpus was scraped off. As a control, saline was injected instead of apelin-12 solution. Total RNA was isolated from the mucosa using the FastPrep system, which included the BIO 101 FP120 FastPrep instrument and the FastRNA Pro Green kit (BIO 101 Inc., Carlsbad, CA, USA). The concentration of HDC RNA was determined by measuring the absorbance at 260 and 280 nm. The RNA was reverse transcribed into complementary DNA (cDNA) with a 1st Strand cDNA Synthesis kit for RT-PCR (Roche Diagnostics Corp., Indianapolis, IN, USA).