Title

Cholecystokinin secretion induced by β-conglycinin peptone depends on Gαq- mediated pathways in enteroendocrine cells

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Column title

Peptone sensing mechanism in enteroendocrine cells

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Abstract *Background* Intraduodenal administration of peptone prepared from soybean β-conglycinin (BconP) stimulates cholecystokinin (CCK) secretion from enteroendocrine cells, and suppresses food intake in rats. However, the sensing mechanism of BconP by CCK-producing cells is unknown. *Aim of the study* We investigated signal transduction pathways mediating CCK secretion in response to BconP in the murine CCK-producing cell line, STC-1. *Methods* STC-1 cells were seeded in 48-well culture plates until sub-confluent and CCK secretion was examined under various conditions. CCK concentration was determined by the enzyme immunoassay. *Results* BconP dose-dependently induced CCK secretion in STC-1 cells. Treatment with BAPTA-AM, an intracellular Ca^{2+} chelator, reduced BconP-induced CCK secretion, however, removal of extracellular Ca^{2+} did not affect the secretory response. Treatment with 2-amino borate (2-APB) reduced CCK releasing responses, suggesting

the involvement of IP_3. In addition, BconP failed to induce CCK secretion after treatment with the Gαq protein inhibitor (YM-254890). *Conclusion* These results indicate that Gαq pathway is responsible for BconP-induced CCK secretion in STC-1 cells, and suggest the involvement of a Gαq-coupled GPCR (s) in dietary peptide sensing in enteroendocrine cells.

Key words

Enteroendocrine cells; Cholecystokinin; Dietary peptide; GPCR

Introduction

Enteroendocrine cells secrete gastrointestinal hormones by sensing a variety of luminal information including nutrients [4]. The sensing mechanisms for fatty acids [6,7], glucose [11] and amino acids [12] have been identified in recent studies. However, dietary protein- or peptide-sensing mechanisms in enteroendocrine cells remain unclear. We previously demonstrated that dietary peptides could directly interact with the intestinal epithelium to activate cholecystokinin (CCK) secretion and subsequent physiological responses including the stimulation of pancreatic enzyme secretion and induction of satiety in rats [5,10]. Other reports also suggested that dietary peptides could directly stimulate CCK secretion from CCK-producing cells [9] and the CCK-producing enteroendocrine cell line, STC-1 [8].

We previously reported that peptone prepared from soybean β-conglycinin (BconP) suppressed food intake in rats through CCK secretion from the intestine [10]. However, it was not determined whether BconP directly stimulates CCK-producing cells or not.

The aims of the current study were to examine the direct effect of BconP on CCK secretion and to determine sensing mechanism in CCK-producing enteroendocrine cells.

Materials and Methods

Materials

β-Conglycinin peptone (BconP) was prepared from β-Conglycinin (a gift from Fuji Oil Co., Osaka, Japan) through treatment with pepsin [7]. Bombesin (BBS) and 2-aminoethyldiphenyl borate (2-APB) were purchased from Sigma (St. Louis, MO). YM-254890 was a gift from Astellas Pharma Inc. (Tokyo, Japan). 1,2-*bis*-(*o*-Aminophenoxy)ethane-N,N,N',N'-tetraacetic acid Tetra(acetoxymethyl) Ester (BAPTA-AM) was purchased from Dojindo laboratories (Kumamoto, Japan).

Cell culture

STC-1 cells (a gift from Dr. D. Hanahan, University of California, San Francisco, CA) were grown in Dulbecco's modified Eagle's medium (Invitrogen, Cat. No. 12100-038) supplemented with 10% fetal bovine serum, 50 IU/ml penicillin, and 500 μg/ml streptomycin in a humidified 5% CO_2 atmosphere at 37°C.

CCK- secretion study

STC-1 cells were grown in 48-well culture plates at a density of 1.25 x 10^5 cells/well for 2-3 days until they reached 80-90% confluency. Cells were washed with Hepes buffer, and exposed to test agents dissolved in the same buffer for 60 min at 37°C. The Hepes buffer had the following composition: 140 mM NaCl, 4.5 mM KCl, 20 mM Hepes, 1.2 mM $CaCl_2$, 1.2 mM $MgCl_2$, and 10 mM D-glucose, pH 7.4. In Ca^{2+}-free Hepes buffer, $CaCl_2$ was omitted and 0.2 mM EGTA was included. Supernatants were collected and centrifuged at 800 x g for 5 min at 4°C to remove remained cells, and then stored at -50°C until CCK concentration measurement with a commercial enzyme immuno assay kit (Phoenix Pharmaceuticals Inc., Belmont CA). BconP at 5 mg/ml did not

interfere the assay.

Statistical analysis

Results are expressed as means ± SEM. Statistical significance was assessed using one-way ANOVA and significant differences among mean values were determined by the Student-Newman-Keuls post hoc test ($P < 0.05$).

Results and Discussion

The cellular mechanisms by which dietary peptides induce CCK secretion in enteroendocrine cells are still uncharacterized. The present study has demonstrated that dietary peptides derived from soybean β-conglycinin, directly stimulate CCK secretion via the activation of α subunit of heterotrimeric G protein, Gαq, dependent on intracellular Ca^{2+} in the murine CCK-producing enteroendocrine cell line STC-1. These results suggest the existence of putative G protein-coupled receptor(s) (GPCR) which sense dietary peptides in enteroendocrine cells. GPCRs are one of the largest receptor family that has seven transmembrane domains, and have function in broad tissues to sense specific molecules (ligands) including neurotransmitters, hormones, cytokines, taste, smells and also nutrients.

Firstly, we demonstrated that STC-1 cells secrete CCK on exposure to BconP (Fig. 1A). Under our experimental system, 2 mg/ml is the minimum concentration to induce a statistically significant level of CCK secretion. Therefore, BconP at 2 or 5 mg/ml was used for following studies. Previously, we reported that BconP suppresses food intake in rats through CCK secretion [10]. The present study confirms that CCK-producing cells are able to sense some active peptides in dietary peptides.

The experiment using intracellular Ca^{2+} chelator BAPTA-AM (Fig.

1B) suggests that intracellular Ca^{2+} mobilization is responsible for BconP-induced CCK secretion. We measured intracellular Ca^{2+} concentrations in STC-1 cells loaded with fura-2 by using a spectrafluorophotometer, and could detect small increase in intracellular Ca^{2+} concentration (~60 nM) at lower doses of BconP (~ 500 μg/ml) (data not shown). However, experiments at higher doses of BconP (> 2 mg/ml) required for significant CCK secretion were not possible due to an interference of fluorescent signal by BconP itself.

To clarify the source of Ca^{2+} responsible for the BconP-induced CCK secretion, Ca^{2+} in the extracellular milieu was omitted. Removal of extracellular Ca^{2+} did not attenuate BconP-induced CCK secretion (Fig. 1C). This suggests that BconP-induced CCK secretion depends on intracellular Ca^{2+}, but does not depend on Ca^{2+} entry via Ca^{2+} channels.

The treatment with an inositol triphosphaet (IP_3) receptor blocker inhibited BconP-induced CCK secretion (Fig. 2A). The drug, 2-APB is reported to inhibit store-operated Ca^{2+} channels (SOCs) and transient receptor potential channels (TRPCs) [1]. However, the data in Fig. 1C suggests that influx of extracellular Ca^{2+} does not involved. Therefore inhibition of CCK secretion by 2-APB might be due to blockage of IP_3 pathways.

IP_3 is produced by phospholipase C from phosphatidylinositol 4,5-biphosphate, and this is mainly regulated by the αq subunit of heterotrimeric G protein. To examine the involvement of Gαq proteins, we used the specific inhibitor, YM-254890 [14]. As expected [13], YM-254890 treatment abolished bombesin-induced CCK secretion (Fig. 2B) and $[Ca^{2+}]_i$ (data not shown), confirming that the inhibitor blocks Gαq pathways in our experimental system. BconP-induced CCK secretion was significantly reduced by YM-254890 (Fig. 2B), indicating the involvement of the Gαq protein and, in turn, suggesting the

involvement of a Gαq-coupled receptor(s). Previous report [8] demonstrated pertussis toxin-sensitive G protein (s) (Gαi,o) mediates CCK secretion by peptone (egg albumin hydrolysate) in STC-1 cells. Involvement of different G-proteins between our and previous data may come from the source of protein (β-conglycinin and egg albumin). Recent paper suggested that a GPCR, GPR93 mediates peptone-induced CCK secretion in STC-1 [3] cells, and the receptor couples Gαq protein in other cell systems [2]. These reports raise the possibility that BconP also activates GPR93 to induce CCK secretion in our study. The involvement of GPR93 in peptone-sensing in STC-1 cells or *in vivo* should be confirmed by knock-down or knock-out of the receptor.

In conclusion, the present study demonstrated that CCK-producing enteroendocrine cells directly sense a dietary peptide β-conglycinin peptone, resulting in CCK secretion. The intracellular signal transduction pathways involve the Gαq-mediated pathways. Our data provides evidence of the involvement of a Gαq-coupled GPCR in dietary peptide sensing mechanism in enteroendocrine cells, though further studies are required to identify the GPCR.

Acknowledgements

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Figure legends

Fig. 1. BconP stimulates CCK secretion in STC-1 cells intracellular Ca^{2+}-dependnetly

A: STC-1 cells cultured for 2-3 days were exposed to BconP (0-10 mg/ml). CCK concentration in the supernatant was measured after incubation for 60 min. Values are means of 3-4 wells. +: $P < 0.05$, ++: $P < 0.01$ vs. control (0 mg/ml). (B) Cells were treated with BAPTA-AM (25 μM, closed bar) or vehicle (0.1 % DMSO, open bar) for 15 min before BconP exposure. The cells were then exposed to BconP (5 mg/ml) for a further 60 min in the presence of BAPTA or vehicle. Values are means ± SEM of 8-12 wells. (C) STC-1 cells were exposed to BconP for CCK secretion study in the presence or absence of extracellular Ca^{2+}. Values

are means ± SEM of 4 wells. Values not sharing a common letter differ significantly ($P < 0.05$).

Fig. 2. Effects of signal transduction inhibitors on BconP-induced CCK secretion

A: STC-1 cells were pretreated with 100-150 μM 2-APB or vehicle (0.1% DMSO) for 5 min, and exposed to BconP (5 mg/ml) in the presence of 2-APB or vehicle. B: STC-1 cells were pretreated with a Gαq inhibitor YM-254890 (10 μM) or vehicle (0.1% DMSO) for 30 min, then challenged with 2 mg/ml BconP or 10 nM (BBS) for the CCK secretion study. Values are means ± SEM of 4 wells. Values not sharing a common letter differ significantly ($P < 0.05$).

Figures

FIGURE 1

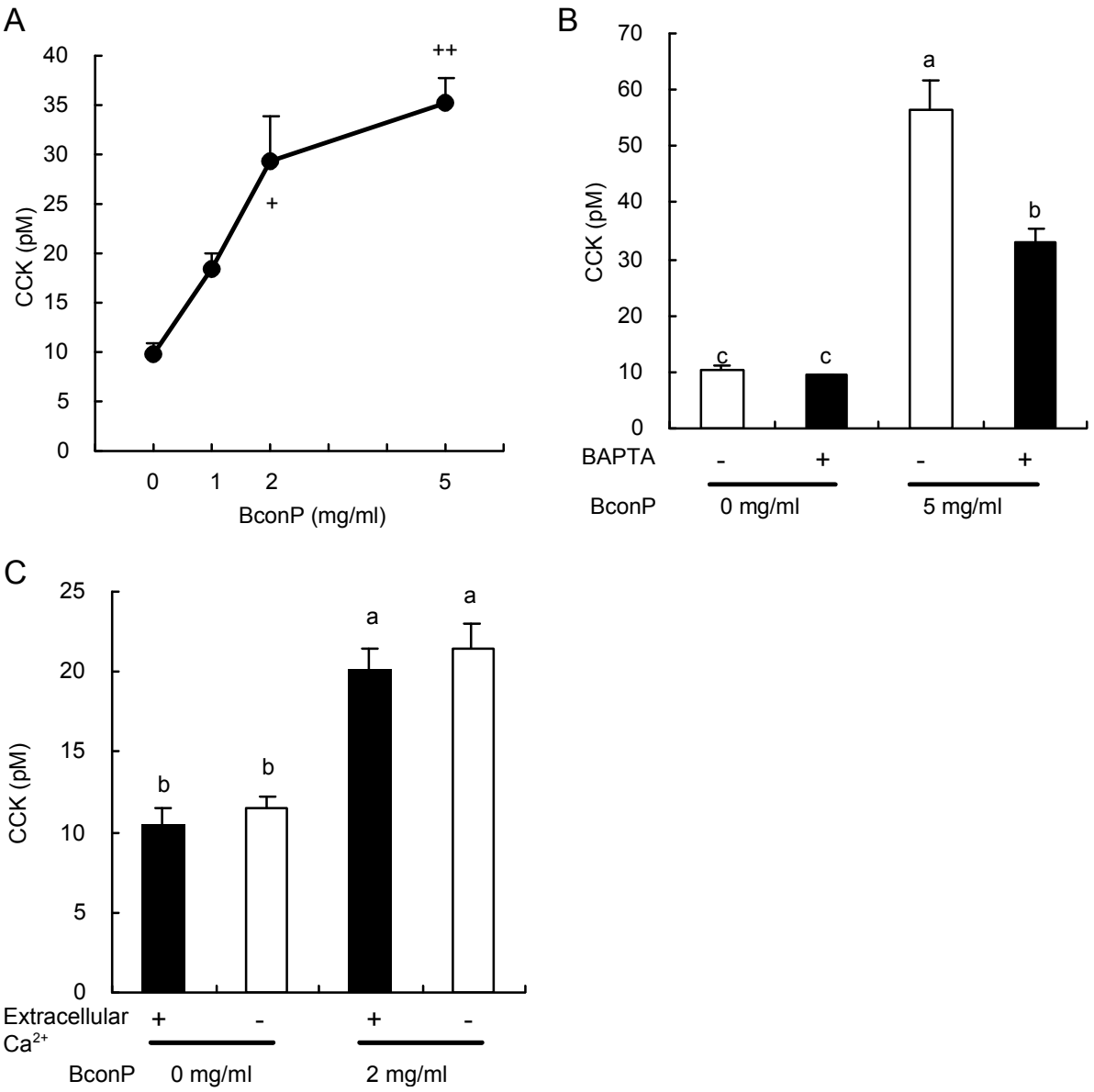
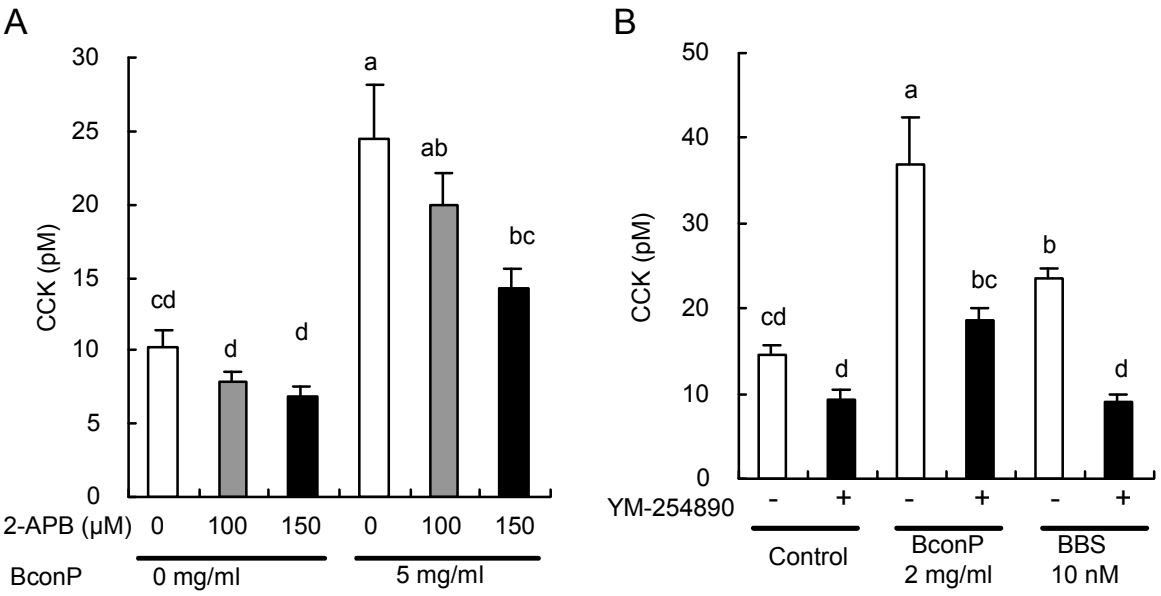


FIGURE 2


A
CCK (pM)
30
25
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15
10
5
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cd
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bc
2-APB (μM) 0 100 150 0 100 150
BconP 0 mg/ml 5 mg/ml
B
CCK (pM)
50
40
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0
cd
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a
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d
YM-254890 - + - + - +
Control
BconP 2 mg/ml
BBS 10 nM