JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

Potato Extract (Potein) Suppresses Food Intake in Rats through Inhibition of Luminal Trypsin Activity and Direct Stimulation of Cholecystokinin Secretion from Enteroendocrine Cells

Shingo Nakajima,[†] Tohru Hira,[*,‡] Masahito Tsubata,[§] Kinya Takagaki,[§] and Hiroshi Hara[‡]

[†]Division of Applied Biosciences, Graduate School of Agriculture, and [‡]Division of Applied Biosciences, Research Faculty of Agriculture, Hokkaido University, Kita-9, Nishi-9, Kita-ku, Sapporo 060-8589, Japan

[§]Department of Research and Development, Toyo Shinyaku Company, Ltd., 2-19-27 Hakataekimae, Hakata-ku 812-0011, Fukuoka, Japan

ABSTRACT: Dietary proteins and trypsin inhibitors are known to stimulate the secretion of the satiety hormone cholecystokinin (CCK). A potato extract (Potein) contains 60% carbohydrate and 20% protein including trypsin inhibitory proteins. In this study, we examined whether Potein suppresses food intake in rats and whether it directly stimulates CCK secretion in enteroendocrine cells. In fasted rats, food consumption was measured up to 6 h after the oral administration of Potein or soybean trypsin inhibitor (SBTI). CCK-releasing activities of Potein and SBTI were examined in the murine CCK-producing cell line STC-1. Potein inhibited the trypsin activity in vitro with a potency 20-fold lower than that of SBTI. Oral administration of Potein dose-dependently suppressed food intake for 1−6 h. Potein, but not the SBTI, dose-dependently induced CCK secretion in STC-1 cells. These results suggest that Potein suppresses food intake through the CCK secretion induced by direct stimulation on enteroendocrine cells and through inhibition of luminal trypsin.

KEYWORDS: trypsin inhibitor, cholecystokinin, enteroendocrine cell line STC-1

INTRODUCTION

Overeating is a prominent contributor to several metabolic diseases, including obesity and diabetes. Sensations of satiety are triggered by postprandial gut−brain signals including the secretion of gut hormones and the activation of the vagus nerve.[1] Understanding the primary response of "nutrient sensing" in the gut could aid in the development of orally ingested functional foods or medicines to modulate appetite.

Cholecystokinin (CCK) is a gut−brain satiety hormone secreted from the enteroendocrine cells (I cells). It also regulates gallbladder contraction, pancreatic enzyme secretion, and gastric emptying.[2,3] CCK-A receptor-deficient rats (Otsuka Long-Evans Tokushima Fatty rats) are a typical model of obesity induced by overeating.[4] The role peripheral CCK plays in satiety is evident from studies using CCK receptor antagonists and vagotomized models.[5,6]

Trypsin inhibitors (TIs) derived from plants as well as synthetic TIs have been shown to stimulate CCK secretion.[7,8] These compounds protect endogenous trypsin-sensitive CCK-releasing peptides from proteolytic inactivation in the lumen, allowing the CCK-releasing peptides to stimulate the release of CCK from the enteroendocrine I cells. Luminal dietary proteins also protect the CCK-releasing peptides by competitive inhibition of trypsin.[9] However, several studies have demonstrated that luminal dietary components (proteins, peptides, and fatty acids) directly stimulate CCK-producing cells to release CCK in pancreaticobiliary-diverted rats,[10] isolated mucosal cells,[11] and the murine CCK-producing enteroendocrine cell line STC-1.[12−15]

A previous study in humans demonstrated that boiled potatoes have the highest satiety index among several common foods.[16] Potatoes contain several protease inhibitors, including a trypsin inhibitory protein.[17−19] In humans, oral administration of the potato protease inhibitor reduced energy intake.[20] This result may be explained at least partially by the increase in CCK secretion that is induced by luminal trypsin inhibition. In addition, it has been shown that hydrolysates of potato proteins induced CCK secretion from STC-1 cells.[21]

A potato extract (Potein) that includes the trypsin inhibitor protein is coproduced during starch production and is further used for potato protein production. Because dietary peptides/proteins including trypsin inhibitor proteins, as well as fatty acids, are a potent stimulator of CCK secretion,[8,10−15] it was expected that Potein has the potential of stimulating CCK secretion in the intestine and consequently suppresses food intake. In the present study, we examined the effect of oral administration of Potein on food consumption in rats. In addition, we have examined whether Potein directly induces CCK secretion in the CCK-producing cell line STC-1.

MATERIALS AND METHODS

Materials. Potein was produced by Toyo Shinyaku Co., Ltd. (Fukuoka, Japan). Potein consisted of 4.1% water, 19.9% protein, 59.8% carbohydrate, 4.2% fiber, 0.2% fat, and 11.8% ash (analyzed by Japan Food Research Laboratories, Tokyo, Japan). Soybean trypsin inhibitor (SBTI, type 2) was purchased from Sigma (St. Louis, MO). Other reagents were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), unless specified.

Received: March 11, 2011
Revised: August 2, 2011
Accepted: August 3, 2011
Published: August 03, 2011

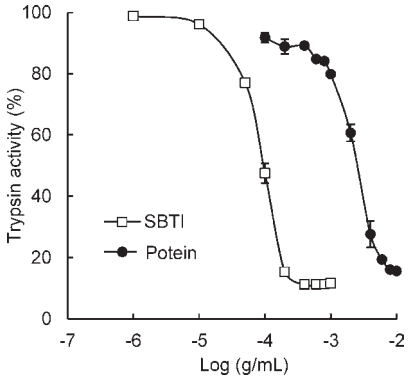

Figure 1. Trypsin activities in the presence of various concentrations of Potein or SBTI. Trypsin activity was expressed as 100% when BAPA hydrolysis was measured without test compounds. Values are the mean of two repetitions.

Pepsin Treatment.[11] Potein was suspended (50 g/L) in 0.02 N H_3PO_4, and the pH was adjusted to 1.85 with 20 N H_3PO_4. Pepsin (from porcine gastric mucosa, Sigma) was added at 0.5% w/w against substrate, and the suspension was shaken for 10 or 60 min at 37 °C followed by boiling for 20 min to terminate the reaction.

Pepsin and Pancreatin Treatment. Potein was treated with pepsin for 10 or 60 min as described above but without the heat-inactivation step. The pH of the suspension was adjusted to 8.2 with $Ca(OH)_2$. Pancreatin (from porcine pancreas, Sigma) was added at 4% w/w against substrate, and the suspension was incubated for 2 h at 37 °C followed by boiling for 20 min to terminate the reaction.

After boiling, the suspensions were neutralized and desalted by centrifugation and filtration (0.2 μm pore size). The filtrates were lyophilized and labeled as Potein digestions.

Measurement of Trypsin Inhibitory Activity. This assay was conducted using purified trypsin (from bovine pancreas type 1, Sigma) and benzoyl-L-arginine-*p*-nitroanilide (BAPA; Peptide Institute, Osaka, Japan) as the substrate.[22] Twenty microliters of the solution of Potein (0.1−10 mg/mL) and SBTI (0.001−1 mg/mL) was added to 500 μL of a solution of BAPA solution (400 μg/mL in 5 mM Tris-HCl at pH 8.1). The mixture was warmed to 37 °C. After warming, 200 μL of a trypsin solution (20 μg/mL) was added, and the mixture was incubated for 10 min at 37 °C. The reaction was terminated by adding 300 μL of 30% acetic acid, and the absorbance was measured at 410 nm. Trypsin activity in the absence of inhibitors was expressed as 100% (Figure 1).

Animal Experiments. Male Sprague−Dawley rats (8 weeks old) were purchased from Japan SLC (Hamamatsu, Japan) and were fed a semipurified diet containing 25% casein based on the AIN-93G formulation.[23] The diet consisted of 250 g/kg casein, 602.5 g/kg sucrose, 50 g/kg soybean oil, 50 g/kg cellulose, 35 g/kg mineral mixture (AIN-93G), 10 g/kg vitamin mixture (AIN-93G), and 2.5 g/kg choline bitartrate. The experiments were performed in a temperature-controlled room maintained at 23 ± 2 °C with a reversed 12 h light−dark cycle (10:00 p.m.−10:00 a.m., light period). Rats had free access to the diet during a 3-day acclimation period and to water throughout the experiment. The study was approved by the Hokkaido University Animal Committee, and the animals were maintained in accordance with the guidelines for the care and use of laboratory animals of Hokkaido University.

Experimental Protocol for Food Intake Studies. Rats were fed the diet between 9:20 a.m. and 9:20 p.m. and were trained daily by an oral administration of distilled water through a feeding tube (Safeed Feeding tube Fr.5, 40 cm; Terumo, Tokyo, Japan) immediately before the diet was provided. Experiments with test samples were performed after a stable daily food intake had been obtained.

In the first experiment, 40 min before the beginning of the dark period (9:20 a.m.), water (2 mL of deionized water), SBTI (0.1 g in 2 mL of deionized water), or Potein (0.1 g in 2 mL of deionized water) was administered orally through the feeding tube. After test liquids were administered, the diet was made available to the animals. Food consumption was measured at 1, 2, 3, and 6 h after the oral administration. The experiment was conducted until each rat received all three treatments. In the second experiment, different doses of Potein (0.2 or 0.4 g in 2 mL of deionized water) were administered, and food consumption was measured with the same protocol. In the third experiment, a mixture of 0.04 g of casein sodium and 0.12 g of potato starch in 2 mL of deionized water was used as control treatment, which mimicked the protein and carbohydrate composition of Potein. This control solution or 0.2 g of Potein was orally administered, and food consumption was measured as described above. In the fourth experiment, sample (water, 0.1 g of SBTI, or 0.1 g of Potein) was orally administered 2 h before feeding the diet (8:00 a.m.), and rats were allowed access to the diet between 10:00 a.m. and 10:00 p.m. Food consumption was measured at 1, 2, 3, and 6 h after the start of feeding.

Cell Experiments. STC-1 cells (a gift from Dr. D. Hanahan, University of California at San Francisco) were grown in Dulbecco's modified Eagle's medium (Invitrogen, Carlsbad, CA) 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. Cells were routinely subcultured by trypsinization upon reaching 80−90% confluency.

STC-1 cells were grown in 48-well culture plates at a density of 1.25×10^5 cells/well for 2−3 days until they reached 80−90% confluency. Cells were washed twice with Hepes buffer to remove the culture media, followed by exposure to test agents (dissolved in Hepes buffer) for 60 min at 37 °C. The Hepes buffer (pH 7.4) had the following composition: 140 mM NaCl, 4.5 mM KCl, 20 mM Hepes, 1.2 mM $CaCl_2$, 1.2 mM $MgCl_2$, 10 mM D-glucose, and 0.1% BSA. Following the incubation, supernatants were collected and centrifuged at 800*g* for 5 min at 4 °C to remove the remaining cells. Supernatants were then stored at −50 °C until the measurement of CCK concentration using a commercial enzyme immunoassay (EIA) kit (Phoenix Pharmaceuticals Inc., Belmont, CA). The primary antiserum provided in this kit cross-reacts 100% with sulfated and nonsulfated CCK (26−33), CCK-33 (porcine), caerulein, gastrin-1 (human), and big gastrin-1 (human); 12.6% with CCK (30−33); and 0% with pancreatic polypeptide (human) and Vasoactive intestinal peptide (human, porcine, rat). Because STC-1 cells do not express detectable levels of gastrin,[13] we selected an EIA kit in which the antibody cross-reacts with gastrin. The coefficients of intra-assay and interassay variation were <5 and <14%, respectively.

Cytotoxic effects on STC-1 cells were determined by the release of lactate dehydrogenase (LDH) in the supernatant of STC-1 cells exposed to Potein as described above (by using a cytotoxicity detection kit, Roche, Basel, Switzerland).

Statistical Analysis. All results are expressed as the mean ± SEM. Statistical significance was assessed using one-way ANOVA, and significant differences among mean values were determined by Duncan's multiple-range test ($P < 0.05$) and paired *t* test ($P < 0.05$).

RESULTS

Trypsin Inhibition by Potein and SBTI. Tryptic activity (BAPA hydrolysis) was reduced in the presence of Potein or SBTI in a dose-dependent manner (Figure 1). The IC_{50} values of SBTI and Potein were calculated as 0.14 and 3.02 mg/mL, respectively. Thus, the inhibitory potency of SBTI was 20-fold higher than that of Potein.

Food Intake in Rats after Oral Administration of Potein or SBTI. At 1 and 3 h after oral administration of Potein (0.1 g), food intake was significantly decreased compared with water

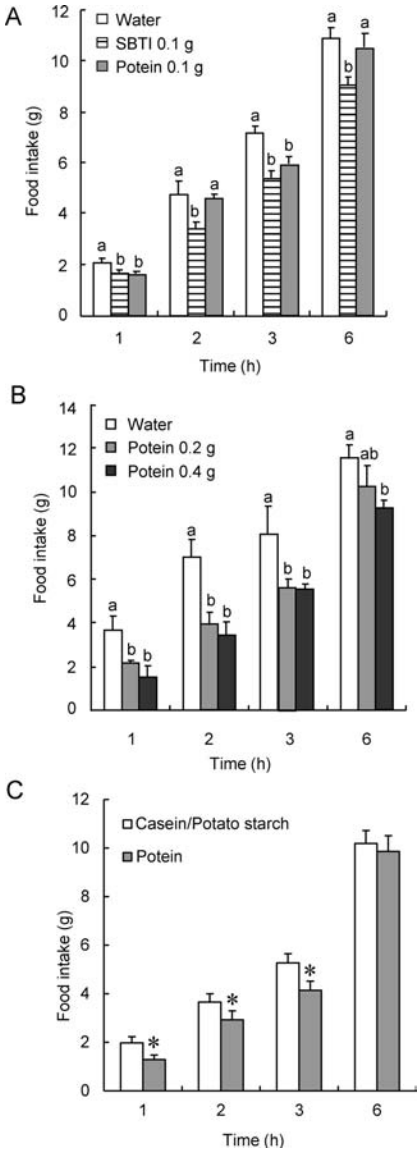

Figure 2. Effects of oral administration of Potein or SBTI on food intake in rats. (A) Food intake at 1, 2, 3, and 6 h after oral administration of Potein (0.1 g) or SBTI (0.1 g). Values are the mean ± SEM (n = 11). Bars not sharing the same letters differ significantly (P < 0.05 by Duncan's multiple-range test) at the same time points. (B) Food intake after oral administration of 0.2−0.4 g of Potein. Values are the mean ± SEM (n = 3). Bars not sharing the same letters differ significantly (P < 0.05 by Duncan's multiple-range test). (C) Food intake after oral administration of 0.2 g of Potein or 0.04 g of casein/0.12 g of potato starch as Potein mimic. Values are the mean ± SEM (n = 16). Asterisks (∗) indicate significant difference between Potein and casein/potato starch treatment (P < 0.05 by paired t test).

administration (control) (Figure 2A). Oral administration of SBTI significantly suppressed food intake until 6 h as compared with control.

Increased amounts of Potein (0.2 and 0.4 g/rat) suppressed food intake in a dose-dependent fashion. A significant reduction was observed until 3 and 6 h after oral administration of 0.2 and 0.4 g of Potein, respectively (Figure 2B). When compared to oral administration of the solution containing casein (0.04 g) and potato starch (0.12 g), food intake was lower at 1, 2, and 3 h after oral administration of Potein (Figure 2C).

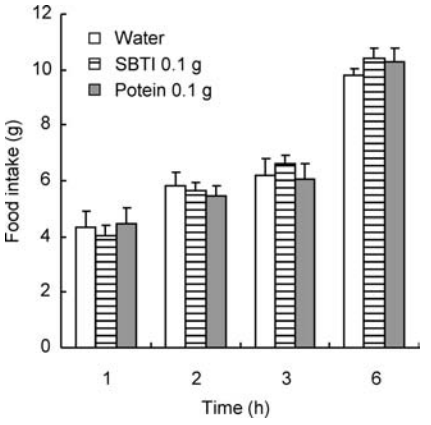

Figure 3. Effect of oral administration of Potein and SBTI 2 h prior to feeding the diet on food intake in rats. Diet was given 2 h after the oral administration of Potein (0.1 g) or SBTI (0.1 g). Food intake was measured 1, 2, 3, and 6 h after feeding the diet. Values are the mean ± SEM (n = 11).

In every experiment, rats immediately started to eat the diet after oral administration of test samples (water, Potein, SBTI, casein/potato starch). No aversive behaviors[24] were observed during eating of the diet after preload of Potein, and no abnormal behaviors such as diarrhea were seen during or after the experiment.

Food Intake in Rats When Potein or SBTI Was Administered 2 h before Feeding the Diet. Potein (0.1 g) or SBTI (0.1 g) was administered 2 h before feeding the diet to examine whether the effects of Potein or SBTI lasted for a long period. However, this treatment did not reduce food intake for 6 h (Figure 3).

CCK Secretion in Response to Potein or SBTI in the Enteroendocrine Cell Line STC-1. Although Potein had apparently lower potency ($^1/_{20}$) of tyrpsin inhibition compared to SBTI (Figure 1), Potein at 0.2 g reduced food intake to the same degree as SBTI at 0.1 g dose (Figure 2). This might be due to the high potency of Potein to directly stimulate CCK secretion from CCK-producing cells. As shown in Figure 4A, exposure to Potein (1−20 mg/mL) induced a dose-dependent increase in CCK concentration in supernatants from STC-1 cells. Treatment with SBTI (1−20 mg/mL) did not induce the secretion of CCK (Figure 4A). The CCK-releasing activity of Potein was almost equivalent to that of the positive control (soybean β-conglycinin peptone[25]). No cytotoxic effect was observed by treatment with Potein (1−20 mg/mL) because no increase in LDH release was detected (data not shown).

Osmotic stimulus such as glycine (2.5−20 mg/mL) and NaCl (5−20 mg/mL) did not cause significant increase in CCK secretion (Figure 4B), indicating the specific effect of Potein on CCK secretion.

Potein was treated with pepsin and pancreatin in vitro to mimic luminal protein digestion. At 5 mg/mL, Potein treated with pepsin (10 min) and pancreatin (2 h) induced higher secretion of CCK than did the untreated Potein. Other treatments induced similar increases in CCK secretion compared to the untreated Potein (Figure 5). At 10 mg/mL, the 10 min pepsin-treated Potein stimulated the secretion of higher CCK than did the untreated Potein. The other treatments induced CCK secretion to levels similar to those associated with the untreated Potein.

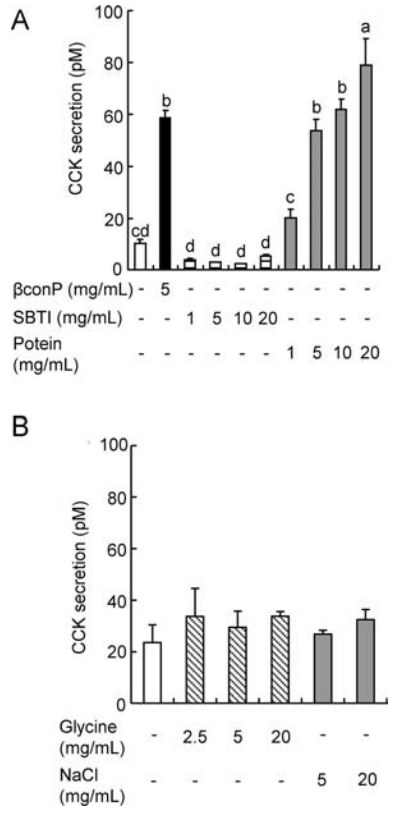

Figure 4. CCK secretion in response to Potein and to SBTI in STC-1 cells. (A) STC-1 cells cultured in 48-well plates were exposed to β-conglycinin peptone (βconP; 5 mg/mL), Potein (1−20 mg/mL), or SBTI (1−20 mg/mL) for 60 min. (B) STC-1 cells cultured in 48-well plates exposed to glycine (2.5−20 mg/mL) and NaCl (5−20 mg/mL) for 60 min. CCK concentrations in the supernatant were measured by EIA. Values are the mean ± SEM of three to four wells. Bars not sharing the same letters differ significantly ($P < 0.05$ by Duncan's multiple-range test).

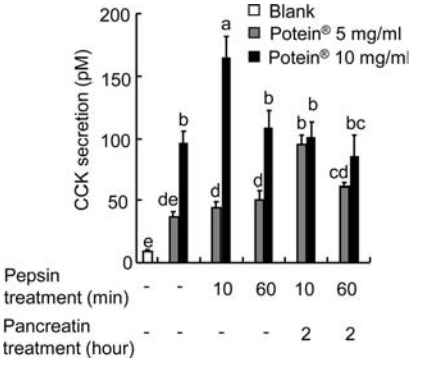

Figure 5. Effects of an in vitro digestion of Potein on CCK-releasing activity in STC-1 cells. Potein was treated with pepsin and pancreatin for various lengths of time. STC-1 cells were exposed to digested Poteins at 5 and 10 mg/mL for 60 min. Values are the mean ± SEM of three to four wells. Bars not sharing the same letters differ significantly ($P < 0.05$ by Duncan's multiple-range test).

DISCUSSION

Dietary proteins and peptides are potent satiety-inducing nutrient as well as fats.[26−28] Potato proteins containing TIs may be responsible for the high satiety index of potato compared with various other common foods.[15,19] Because the secretion of the satiety hormone CCK is increased when the luminal CCK-releasing factor is protected from tryptic degradation,[29,30] we examined the effects of Potein, which possesses trypsin inhibitory protein, on the food intake of rats. We also tested whether Potein directly induces CCK secretion from enteroendocrine cells.

Food intake in rats was significantly decreased at 1 and 3 h after oral administration of 0.1 g of Potein (Figure 2A). A dose-dependent decrease in food intake was observed; the administration of 0.2 and 0.4 g of Potein was associated with larger and longer reductions in food intake compared with the 0.1 g treatment (Figure 2B). The degree of decreased food intake (1−2 g/rat) was much greater than the doses of Potein (0.1−0.4 g/rat), and food intake was significantly lower after preload of Potein compared to preload of a mixture of casein and potato starch equivalent to the protein and carbohydrate composition of Potein (Figure 2C). Therefore, the reduction in food intake is not due to the additional energy (macronutrients) fed to the rats in the form of Potein.

Although SBTI has 20 times higher potency to inhibit trypsin compared to Potein (Figure 1), oral SBTI (0.1 g) suppressed food intake to the same degree as 0.2 g of Potein (Figure 2A,B). In the CCK-producing enteroendocrine cell line STC-1, Potein induced CCK secretion in a dose-dependent manner (Figure 4), with potency similar to that of β-conglycinin peptone. This peptone has been reported to stimulate CCK secretion in STC-1 cells[25] and to suppress food intake after duodenal administration in rats.[31] In contrast, purified SBTI did not increase the secretion of CCK. Because our experiments in STC-1 cells did not include luminal trypsin or trypsin-sensitive CCK-releasing peptides,[32] the results shown in Figure 3 indicate that Potein, but not SBTI, directly triggers CCK secretion from enteroendocrine cells. These results suggest that the suppressive effect of Potein on food intake depends not only on trypsin inhibition but also on the CCK secretion induced by Potein's direct stimulation on CCK-producing cells. Komarnytsky et al. recently showed that potato protease inhibitor concentrate did not stimulate CCK secretion in STC-1 cells.[33] Because Potein contains various proteins, carbohydrates, and unknown components, it is likely that some of these components are responsible for direct stimulation of CCK secretion. Luminal CCK-releasing factors have not been found in humans, and the purified potato protease inhibitor failed to suppress human food intake.[34] However, such dual actions of Potein to stimulate CCK secretion suggest the possibility of its applicability in appetite control in humans.

In vitro digestion by pepsin and pancreatin did not attenuate the CCK-releasing activity of Potein (Figure 5), suggesting that the active components in Potein are resistant to luminal protein digestion. Interestingly, the potency of Potein to induce CCK secretion was enhanced by some pepsin and pancreatin treatments (Figure 5). It is possible that the active components or structures were liberated by partial and adequate digestion of potato proteins. Carbohydrates do not stimulate CCK secretion,[35] and potato peptides are reported to stimulate CCK secretion.[20] Therefore, protein fractions might be responsible for the CCK releasing potency of Potein. However, we cannot exclude the involvement of carbohydrates in this study. Further studies are needed to identify the CCK-releasing factors in Potein. Although active components responsible for direct stimulation of CCK secretion are still unclear, these results

suggest that luminal digestion enhances the ability of Potein to induce CCK secretion.

Although the suppressive effect was observed for >3 h, oral administration of Potein or SBTI (0.1 g) 2 h prior to providing the test diet did not reduce subsequent food consumption (Figure 3). This result suggests that the ingested diet is also responsible for the suppression of food intake. The secretion of CCK might be additionally or synergistically enhanced by the presence of the administered samples (Potein or SBTI) and the ingested diet in the intestinal lumen. Other factors such as glucagon-like peptide-1 (GLP-1) and peptide-YY (PYY) may also explain the reduction in food intake that we observed. GLP-1 and PYY are anorexic gut hormones produced by enteroendocrine L cells located mostly in the distal small intestine and the large intestine. The secretion of these hormones is stimulated by ileal nutrients including proteins/peptides.[36,37] Inhibiting luminal trypsin activity might prevent the digestion of ingested proteins in the proximal small intestine, thus allowing dietary proteins/peptides to reach the distal small intestine. As a result, the secretion of GLP-1 or PYY could be enhanced. Further in vivo studies are required to investigate the involvement of these hormones in the anorexic effect associated with Potein.

In summary, the present study demonstrates that oral administration of Potein reduces food intake for 1−6 h in rats in a dose-dependent manner. Potein inhibited trypsin activity and induced CCK secretion in vitro. Digestion of Potein with protease did not attenuate its ability to induce CCK secretion in STC-1 cells. These results suggest that oral administration of Potein in rats suppresses food intake via CCK secretion induced both by direct stimulation on CCK-producing cells and by luminal trypsin inhibition.

AUTHOR INFORMATION

Corresponding Author

*Phone: +81-11-706-2811. Fax: +81-11-706-2504. E-mail: hira@chem.agr.hokudai.ac.jp.

REFERENCES

(1) Cummings, D. E.; Overduin, J. Gastrointestinal regulation of food intake. *J. Clin. Invest.* **2007**, *117*, 13–23.

(2) Grider, J. R. Role of cholecystokinin in the regulation of gastrointestinal motility. *J. Nutr.* **1994**, *124* (8 Suppl.), 1334S–1339S.

(3) Owyang, C. Negative feedback control of exocrine pancreatic secretion: role of cholecystokinin and cholinergic pathway. *J. Nutr.* **1994**, *124* (8 Suppl.), 1321S–1326S.

(4) Moran, T. H.; Katz, L. F.; Plata-Salaman, C. R.; Schwartz, G. J. Disordered food intake and obesity in rats lacking cholecystokinin A receptors. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **1998**, *274*, R618–R625.

(5) Reidelberger, R. D.; Castellanos, D. A.; Hulce, M. Effects of peripheral CCK receptor blockade on food intake in rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2003**, *284*, R429–R437.

(6) Reidelberger, R. D.; Hernandez, J.; Fritzsch, B.; Hulce, M. Abdominal vagal mediation of the satiety effects of CCK in rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2004**, *286*, R1005–R1012.

(7) Guan, D.; Rivard, N.; Maouyo, D.; Gettys, T. W.; Morisset, J. Importance of cholecystokinin in peptide-YY release in response to pancreatic juice diversion. *Regul. Pept.* **1993**, *43*, 169–176.

(8) Göke, B.; Printz, H.; Koop, I.; Rausch, U.; Richter, G.; Arnold, R.; Adler, G. Endogenous CCK release and pancreatic growth in rats after feeding a proteinase inhibitor (camostate). *Pancreas* **1986**, *1*, 509–515.

(9) Herzig, K. H. Cholecystokinin- and secretin-releasing peptides in the intestine − a new regulatory interendocrine mechanism in the gastrointestinal tract. *Regul. Pept.* **1998**, *73*, 89–94.

(10) Nishi, T.; Hara, H.; Aoyama, Y. Guanidinated casein hydrolysate stimulation of cholecystokinin release via pancreatic enzyme- and cholinergic-independent mechanisms in rats. *Biosci., Biotechnol., Biochem.* **1999**, *63*, 1070–1074.

(11) Nishi, T.; Hara, H.; Hira, T.; Tomita, F. Dietary protein peptic hydrolysates stimulate cholecystokinin release via direct sensing by rat intestinal mucosal cells. *Exp. Biol. Med. (Maywood)* **2001**, *226*, 1031–1036.

(12) Liddle, R. A. Cholecystokinin cells. *Annu. Rev. Physiol.* **1997**, *59*, 221–242.

(13) McLaughlin, J. T.; Lomax, R. B.; Hall, L.; Dockray, G. J.; Thompson, D. G.; Warhurst, G. Fatty acids stimulate cholecystokinin secretion via an acyl chain length-specific, Ca^{2+}-dependent mechanism in the enteroendocrine cell line STC-1. *J. Physiol.* **1998**, *513*, 11–18.

(14) Nakajima, S.; Hira, T.; Eto, Y.; Asano, K.; Hara, H. Soybean beta 51−63 peptide stimulates cholecystokinin secretion via a calcium-sensing receptor in enteroendocrine STC-1 cells. *Regul. Pept.* **2010**, *159*, 148–155.

(15) Geraedts, M. C.; Troost, F. J.; Fischer, M. A.; Edens, L; Saris, W. H. Direct induction of CCK and GLP-1 release from murine endocrine cells by intact dietary proteins. *Mol. Nutr. Food Res.* **2011**, *55*, 476–484.

(16) Holt, S. H.; Miller, J. C.; Petocz, P.; Farmakalidis, E. A satiety index of common foods. *Eur. J. Clin. Nutr.* **1995**, *49*, 675–690.

(17) Kim, M. H.; Park, S. C.; Kim, J. Y.; Lee, S. Y.; Lim, H. T.; Cheong, H.; Hahm, K. S.; Park, Y. Purification and characterization of a heat-stable serine protease inhibitor from the tubers of new potato variety "Golden Valley". *Biochem. Biophys. Res. Commun.* **2006**, *346*, 681–686.

(18) Park, Y.; Choi, B. H.; Kwak, J. S.; Kang, C. W.; Lim, H. T.; Cheong, H. S.; Hahm, K. S. Kunitz-type serine protease inhibitor from potato (*Solanum tuberosum* L. cv. Jopung). *J. Agric. Food Chem.* **2005**, *53*, 6491–6496.

(19) Pearce, G.; Sy, L.; Russell, C.; Ryan, C. A.; Hass, G. M. Isolation and characterization from potato tubers of two polypeptide inhibitors of serine proteinases. *Arch. Biochem. Biophys.* **1982**, *213*, 456–462.

(20) Hill, A. J.; Peikin, S. R.; Ryan, C. A.; Blundell, J. E. Oral administration of proteinase inhibitor II from potatoes reduces energy intake in man. *Physiol Behav.* **1990**, *48*, 241–246.

(21) Foltz, M.; Ansems, P.; Schwarz, J.; Tasker, M. C.; Lourbakos, A.; Gerhardt, C. C. Protein hydrolysates induce CCK release from enteroendocrine cells and act as partial agonists of the CCK1 receptor. *J. Agric. Food Chem.* **2008**, *56*, 837–843.

(22) Kakade, M. L.; Rackis, J. J.; McGhee, J. E.; Puski, G. Determination of trypsin inhibitor activity of soy products: a collaborative analysis of an improved procedure. *Cereal Chem.* **1974**, *51*, 376–382.

(23) Reeves, P. G.; Nielsen, F. H.; Fahey, G. C., Jr. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition Ad Hoc Writing Committee on the Reformulation of the AIN-76A Rodent Diet. *J. Nutr.* **1993**, *123*, 1939–1951.

(24) Bensaïd, A.; Tomé, D.; L'Heureux-Bourdon, D.; Even, P.; Gietzen, D.; Morens, C.; Gaudichon, C.; Larue-Achagiotis, C.; Fromentin, G. A high-protein diet enhances satiety without conditioned taste aversion in the rat. *Physiol. Behav.* **2003**, *78*, 311–320.

(25) Hira, T.; Maekawa, T.; Asano, K.; Hara, H. Cholecystokinin secretion induced by β-conglycinin peptone depends on Gαq-mediated pathways in enteroendocrine cells. *Eur. J. Nutr.* **2009**, *48*, 124–127.

(26) Bensaid, A.; Tome, D.; Gietzen, D.; Even, P.; Morens, C.; Gaussercs, N.; Fromentin, G. Protein is more potent than carbohydrate for reducing appetite in rats. *Physiol. Behav.* **2002**, *75*, 577–582.

(27) Reid, M.; Hetherington, M. Relative effects of carbohydrates and protein on satiety: a review of methodology. *Neurosci. Biobehav. Rev.* **1997**, *21*, 295–308.

(28) Tome, D. Protein, amino acids and the control of food intake. *Br. J. Nutr.* **2004**, *92* (Suppl.), S27–S30.

(29) Niederau, C.; Liddle, R. A.; Williams, J. A.; Grendell, J. H. Pancreatic growth: interaction of exogenous cholecystokinin, a protease inhibitor, and a cholecystokinin receptor antagonist in mice. *Gut* **1987**, *28* (Suppl.), 63–69.

(30) Miyasaka, K.; Nakamura, R.; Kitani, K. Effects of trypsin inhibitor (camostate) on pancreas and CCK release in young and old female rats. *J. Gerontol.* **1989**, *44*, M136–M140.

(31) Nishi, T.; Hara, H.; Tomita, F. Soybean β-conglycinin peptone suppresses food intake and gastric emptying by increasing plasma cholecystokinin levels in rats. *J. Nutr.* **2003**, *133*, 352–357.

(32) Chang, C. H.; Chey, W. Y.; Sun, Q.; Leiter, A.; Chang, T. M. Characterization of the release of cholecystokinin from a murine neuroendocrine tumor cell line, STC-1. *Biochim. Biophys. Acta* **1994**, *1221*, 339–347.

(33) Komarnytsky, S.; Cook, A.; Raskin, I. Potato protease inhibitors inhibit food intake and increase circulating cholecystokinin levels by a trypsin-dependent mechanism. *Int. J. Obes. (London)* **2011**, *35*, 236–243.

(34) Peters, H. P.; Foltz, M.; Kovacs, E. M.; Mela, D. J.; Schuring, E. A.; Wiseman, S. A. The effect of protease inhibitors derived from potato formulated in a minidrink on appetite, food intake and plasma cholecystokinin levels in humans. *Int. J. Obes. (London)* **2011**, *35*, 244–250.

(35) Liddle, R. A. Regulation of cholecystokinin secretion in humans. *J. Gastroenterol.* **2000**, *35*, 181–187 (review).

(36) Batterham, R. L.; Heffron, H.; Kapoor, S.; Chivers, J. E.; Chandarana, K.; Herzog, H.; Le Roux, C. W.; Thomas, E. L.; Bell, J. D.; Withers, D. J. Critical role for peptide YY in protein-mediated satiation and body-weight regulation. *Cell Metab.* **2006**, *4*, 223–233.

(37) Hira, T.; Mochida, T.; Miyashita, K.; Hara, H. GLP-1 secretion is enhanced directly in the ileum, but indirectly in the duodenum by a newly identified potent stimulator, zein hydrolysate in rats. *Am. J. Physiol. Gastrointest. Liver Physiol.* **2009**, *297*, G663–G671.