Title

Sucrose fatty acid esters suppress pancreatic secretion accompanied by peptide YY release in pancreatico-biliary diverted rats

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Running title

Fatty acid esters release PYY and inhibit pancreatic secretion

Abstract

Our previous study demonstrated that intestinal administration of triglycerides suppressed protein-induced increases in pancreatic exocrine secretion in pancreatico-biliary diverted (PBD) rats, though the mechanism has not been clarified. The present study was conducted to determine whether esterified fatty acids or released fatty acids are responsible for this suppression, and whether an esterified fatty acid stimulates secretion of a pancreatic inhibitory hormone, peptide YY (PYY). We examined the effects of cocoa butter or nondigestible sucrose fatty acid esters on protein-induced pancreatic secretion in conscious PBD rats whose bile-pancreatic juice (BPJ) was diverted from the proximal small intestine through a catheter. Intraduodenal administration of the protein, guanidinated casein hydrolysate (HGC, 150 mg in 1 ml), enhanced pancreatic protein and trypsin secretion. However, administration of HGC with cocoa butter (100 mg/ml) partly, and HGC with a highly esterified sucrose fatty acid ester F-10 (100 mg/ml) completely suppressed the increases in pancreatic secretion. The low-esterified, water-soluble sucrose fatty acid ester, F-160, also completely inhibited protein-induced pancreatic secretion in the presence or absence of the lipase inhibitor, orlistat. Intraduodenal administration of HGC with sucrose ester F-160 induced PYY secretion more strongly than did HGC with sucrose, which was accompanied by the suppression of HGC-induced pancreatic secretion in anesthetized PBD rats. These results suggest that the esterified fatty acid itself stimulates PYY release in the distal intestine, thereby inhibiting protein-induced pancreatic secretion.

KEY WORDS:

Dietary fat, Sucrose fatty acid ester, Peptide YY, Pancreatic secretion, Pancreatico-biliary diversion

Introduction

Dietary fats regulate several gastrointestinal functions including the enhancement of pancreatic exocrine secretion (Green *et al.*, 1989; Olsen *et al.*, 1989). Fatty acids released from triacylglycerol in the upper small intestine are responsible for the promotive effect of fat, and the action of the fatty acids mainly depends on cholecystokinin (CCK) secretion from the upper small intestine (Guimbaud *et al.*, 1997; McLaughlin *et al.*, 1999). In contrast, we previously showed that duodenal instillation of fat suppressed protein-induced increases in exocrine pancreatic secretion in pancreatico-biliary diverted (PBD) rats, but not in normal rats (Hara *et al.*, 1994; 2000). In PBD rats, dietary fats are not digested in the proximal intestine because bile-pancreatic juice (BPJ) is diverted to the distal intestine where the fats are rapidly digested due to the presence of pancreatic enzymes. These results suggest that intact triacylglycerol in the small intestine or digestive products of triacylglycerol in the distal small intestine activate an inhibitory mechanism.

Using non-digestible sucrose fatty acid esters (Noker *et al.*, 1997) in PBD rats, the present study sought to determine whether the inhibitory effects of fat on the pancreatic secretion depend on free fatty acids or esterified fatty acids. We used a stearate-rich digestible triacylglycerol, cocoa butter, as a control fat because the fatty acid moiety of the sugar esters tested is also rich in stearic acid. In order to confirm that there was no

degradation of the sucrose esters used, a lipase inhibitor was applied with the sucrose fatty acid ester. Guanidinated casein, whose lysine residues were converted to homoarginine, was used as a potent stimulant of pancreatic exocrine secretion (Hara *et al.*, 1995). We also examined effects of the sucrose ester on secretion of the gastrointestinal hormone, peptide YY (PYY), which is known to inhibit pancreatic secretion (Jin *et al.*, 1993; Naruse *et al.*, 2002) in anesthetized PBD rats.

Methods

Materials

Guanidinated casein was prepared by a previously described method (Hara *et al.*, 1995). The conversion rate of lysyl residues to homoarginine was 96%. Briefly, guanidinated casein (55 g/l) was hydrolyzed with pepsin (0.55 g/l; Sigma Chemical Co., St. Louis, MO) at pH 1.8 for 10 min at 37°C, and the hydrolysate was then neutralized and desalted (guanidinated casein hydrolysate: HGC). Sucrose fatty acid esters were provided by Dai-Ichi Kogyo Seiyaku Co. (Kyoto, Japan). The water-insoluble sucrose fatty acid ester, F-10, is a mixture of di-, tri- and polyesters without monoesters, and the water-soluble, F-160, contains 70% monoesters and 30% polyesters. These sucrose fatty acid esters contain stearic acid (70%) and palmitic acid (30%) as esterified fatty acids. The lipase inhibitor (Schwizer *et al.*, 1997), orlistat (tetrahydrolipstatin), was a gift from F. Hoffmann-La Roche Ltd. (Basel, Switzerland). This drug inhibits pancreatic lipase, gastric lipase, carboxyl ester lipase (cholesterol esterase) of pancreatic origin and the bile-salt-stimulated lipase of human milk (Borgstrom, 1988)

Animals and Diets in experiments 1 and 2

Male Sprague-Dawley rats (8 weeks old, Japan SLC Inc., Hamamatsu, Japan) were fed a semipurified, sucrose-based diet containing 25% casein for 5 days (American Institute of Nutrition, 1977; Reeves, 1989). After a 24- hr fast, cannulae were implanted into the common bile-pancreatic duct, duodenum, and upper ileum under pentobarbital anesthesia (sodium pentobarbital, 40 mg/kg body weight; Abbott Co., North Chicago, IL), as previously described (Hara *et al.*, 2000; Hira *et al.*, 1997). Briefly, the small tip (7-8 mm) of a polyethylene catheter (SP 28; I.D. 0.4 mm, O.D. 0.8 mm; Natsume Seisakusyo, Tokyo, Japan) was inserted into the common bile-pancreatic duct. The other end of the catheter was connected to silicone tubing (Silascon No.00, I.D. 0.5 mm, O.D. 1.0 mm; Dow Corning Co., Kanagawa, Japan). A silicone catheter (Silascon No.00) for returning BPJ to the ileal lumen was placed through a fistula 45 cm distal (middle of the small intestine) from the ligament of Treitz. These catheters were tunneled subcutaneously and connected to each other at the back of the neck to maintain the BPJ flow. Another silicone catheter (Silascon No.00) for administration of the test solution was inserted into the duodenal lumen through a gastric fistula. In the PBD rats, BPJ flow bypasses the proximal small intestine through the catheters, and flows into the ileal lumen. The rats were allowed to recover for 6 days with free access to the semipurified diet described above. The common bile-pancreatic duct was examined after experiments, and rats with a swollen duct due to occlusion of the catheters were excluded from the analysis. The test solution or emulsion was administered into the duodenum through the catheter by a bolus injection (1 ml for 1 min) after twice sampling the BPJ in a fasting

state. For BPJ sampling, the bile-pancreatic duct catheter was extended with a polyethylene tube (SP 28; Natsume Seisakusyo), and BPJ was collected from the polyethylene tube via an outlet placed 5 cm from the bottom of the cage. BPJ was collected for 3 min at each time point shown in Figures 1 and 2, and was recirculated continuously into the ileum through the ileal catheter except during the 3-min sampling periods. Rats were allowed to move freely in the cages throughout the experimental period. The experiments were performed in a room controlled at 23 ± 2°C, with a 12-hr light-dark cycle (0800-2000, light period). 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.

Experiment 1: Effect of the sucrose fatty acid ester, F-10, on protein–induced pancreatic secretion in PBD rats compared to that of cocoa butter

The PBD rats were divided into three groups on the basis of body weight after a 6-day recovery period and a 24-hr fast. One group received 1 ml of HGC (150 mg/ml) solution containing sodium caseinate (10 mg/ml) as an emulsifying agent. The second group received this solution supplemented with cocoa butter (100 mg/ml), and the third group received this solution supplemented with fatty acid ester F-10 (100 mg/ml). The test solution was administered into the duodenum of PBD rats through the duodenal catheter. BPJ was collected before and after the administration as described above.

Experiment 2: Effect of the water-soluble sucrose fatty acid ester, F-160, on protein-induced pancreatic secretion in the presence of a lipase inhibitor

Three groups of PBD rats were prepared as in experiment 1. The three test

solutions contained HGC (150 mg/ml) with sucrose (100 mg/ml), HGC (150 mg/ml) with the sucrose fatty acid ester, F-160 (100 mg/ml), or HGC (150 mg/ml) with F-160 (100 mg/ml) and the lipase inhibitor, orlistat (0.4 mg/ml), respectively. One ml of the test solution was administered into the duodenum and BPJ was collected before and after administration as described above.

Experiment 3: Effect of the sucrose fatty acid ester, F-160, on protein-induced pancreatic secretion and plasma PYY in anesthetized PBD rats

Two groups of PBD rats were prepared as in experiment 1, 2 with some modifications. The catheter redirecting the BPJ from the bile-pancreatic duct to the distal intestine was left in the abdominal cavity for a 6 day-recovery period to prevent physical damage by rats. On the day of the experiment, another catheter was implanted into the jugular vein to collect blood samples under anesthesia (sodium pentobarbital, 40 mg/kg body weight). The catheter circulating the BPJ in the abdominal cavity was cut around the middle after a small midline incision, and an extension catheter (20 cm) was connected to the end of the bile-pancreatic catheter to collect BPJ at the basal state for 3 min. At the same time, blood samples (100 μL) for PYY measurement were drawn into a syringe containing aprotinine (final concentration at 200 kIU/ml) and heparin (final concentration at 50 IU/ml) through the jugular catheter. After BPJ collection for 3 min, the extension catheter was replaced with a short (1 cm) polyethylene tube to reconnect of catheters between the bile-pancreatic duct and the ileum. Then, two ml of HGC (150 mg/ml) solution containing either sucrose (100 mg/ml) or F-160 (100 mg/ml) was directly injected into the duodenum for 2 min. We injected 2 ml of the test solutions

in experiment 3 because intestinal motility is known to be suppressed under anesthesia. BPJ (for 3 min) and blood samples (100 µl) were collected at 30, 60 and 120 min after the duodenal injection, and were kept on ice until the end of the experiment. During the experiment, additional pentobarbital (20 mg/kg body weight) was injected to keep rats under anesthesia, and body temperature was maintained using a heating pad. Plasma was separated from blood samples by centrifugation at 2,500 x g for 15 min at 4°C, and then frozen at -80°C until PYY measurement. Plasma PYY concentrations were measured using a commercial enzyme immuno assay (EIA) kit (Yanaihara Institute Inc., Shizuoka, Japan). The antiserum cross-reacts 100% with intact PYY (1-36, rat), 0% with PYY (19-36, human), 0.7% with neuro peptide Y (NPY, human), 0% with NPY (1-19, human), and 0% with pancreatic polypeptide (human).

Analysis

The volume of BPJ was measured gravimetrically, with 1 µl of BPJ taken as 1 mg as the basis for the measurement of pooled BPJ (100 µl = 100 mg). Trypsinogen in BPJ diluted with 0.9% NaCl containing 0.1 % Triton X-100 was activated by purified enterokinase (Sigma Chemical Co., St. Louis, MO) at 30°C for 20 min in a 15 mM Tris buffer (pH 8.1). Trypsin activities were estimated photometrically (Rick, 1976) using the synthetic substrate, *N* α-*p*-toluene-sulfonyl-L-arginine methyl ester (TAME). The protein concentration in BPJ was quantified with a modified version of Lowry's method (Lowry *et al.*, 1951; Sugawara, 1975).

Calculations and statistical analysis

One unit of trypsin was defined as the activity necessary to hydrolyze 1 µmole of

substrate for 1 min at 30°C. Values for the basal state (0 min) were calculated as the average of two samplings before the administration of the test solution. The influence of administration and time on the secretion profiles was determined by two-way analysis of variance (ANOVA). The significance of differences among means was determined by least significant difference (LSD) test ($P < 0.05$).

Results

Figure 1 shows pancreatic secretory profiles after the duodenal administration of HGC solution in the presence or absence of cocoa butter or F-10 in conscious rats (experiment 1). Only the HGC solution induced a significant increase in the volume of BPJ secretion at 90 min after the duodenal administration (Fig. 1A). HGC induced 2-4 fold increases in protein and trypsin secretion at 30, 60 and 90 min after administration (Fig. 1B, C). Values at 60 and 90 min after administration of HGC with cocoa butter or F-10 were much lower than those for HGC alone at the same time points. HGC containing cocoa butter induced a small, delayed increase in trypsin secretion at 120 min after administration. HGC containing F-10 did not induce any increases in either pancreatic protein or trypsin secretion.

In experiment 2, only HGC containing sucrose induced significant increases in the volume of BPJ secretion at each time point after the duodenal administration (Fig. 2A). The administration of HGC with sucrose induced increases in protein and trypsin secretion 2-3 fold higher than basal values (Fig. 2B, C), but neither protein nor trypsin secretion were changed after the duodenal administration of HGC containing F-160. In

the presence of the lipase inhibitor, orlistat, HGC containing F-160 did not induce any increases in either pancreatic protein or trypsin secretion. Values for protein and trypsin secretion in both the HGC + F-160 and HGC + F-160 + orlistat groups were much lower than values in the HGC group at almost all time points.

In experiment 3 (Fig. 3A and B), basal BPJ volume and protein secretion were lower than those in experiment 1 and 2, possibly due to the anesthesia. Trypsin secretion was not measured in experiment 3 as the secretory pattern was found to always be similar to that of protein secretion in experiment 1, 2 as well as in the results of previous studies (Hira *et al.*, 1997; Hira *et al.*, 2003). Intraduodenal injection of HGC + sucrose tended to increase BPJ volume, though not significantly. Protein secretion was increased in the HGC + sucrose treated group at 60 and 90 min compared to basal value (0 min). However, HGC + F-160 did not cause any significant increase in protein secretion throughout the experimental period. This result is consistent with that of experiment 2 in which pancreatic protein secretion was lower in the HGC + F-160 group than that in the HGC + sucrose group.

Plasma PYY levels at 0 min were 1.16 ± 0.06 and 1.10 ± 0.16 ng/ml in the HGC + sucrose and HGC + F-160 group, respectively. Intraduodenal injection of the HGC + F-160 solution induced a rapid, continuous increase in PYY secretion (Fig. 4). However, HGC + sucrose only increased PYY secretion at 60 min. PYY levels in HGC + F-160 treated rats were significantly higher at 30, 60 and 90 min than the basal values, and values at 30 and 90 min in the HGC + F-160 group were higher than those in the HGC + sucrose group.

Discussion

The present study demonstrated that sucrose fatty acid esters inhibit protein induced-pancreatic secretion in PBD rats, and that the inhibition was greater than that with cocoa butter. We also found that the sucrose esters stimulate release of PYY, which is known to inhibit pancreatic exocrine secretion. Although we previously reported that dietary fats (corn oil and soybean oil) suppress protein-induced pancreatic secretion in PBD rats (Hara *et al.*, 1994; 2000), it had been not clarified whether the fat (triglyceride) itself or fatty acids and monoacylglycerol released by fat digestion in the ileal lumen triggered the inhibitory mechanism. To address this issue, we used sucrose fatty acid esters from which free fatty acids are not liberated in the intestinal lumen because these compounds are resistant to digestive enzymes. The highly esterified sucrose fatty acid ester, F-10, completely suppressed the stimulatory effect of HGC on pancreatic secretion (Fig. 1). The effect is thought to have been induced by the esterified fatty acids as the sucrose had no effect and no free fatty acids were liberated from the sucrose ester.

In experiment 1, we emulsified test liquids as the sucrose ester and cocoa butter were not water-soluble. It is possible that physicochemical masking of HGC by its dispersion into the particles is responsible for the suppression. To clarify this issue, we used another sucrose ester, F-160, which is soluble in water and does not require emulsification in experiment 2. Non-emulsified, water-soluble sucrose ester F-160 completely suppressed the enhancement of the pancreatic secretion induced by HGC

(Fig. 2) as well as by the emulsified fatty acid ester. This finding reveals that the sucrose fatty acid ester itself, not the masking of HGC, is responsible for the suppression. In addition, we assessed the effect of sucrose itself on protein-induced pancreatic secretion as sucrose is another fundamental component of sucrose fatty acid esters. We observed that HGC with sucrose still strongly stimulated pancreatic secretion, showing that sucrose is not involved in the suppression.

Sucrose esters are resistant to lipase; however, previous reports showed that some sucrose esters can be digested in the intestine (Noker *et al.*, 1997; Shigeoka *et al.*, 1984). If the suppression depends on fatty acids released from sucrose esters, inhibition of lipase activity should reverse the sucrose ester-induced inhibition of pancreatic secretion. However, the sucrose ester continued to suppress the protein-induced pancreatic secretion in the presence of the lipase inhibitor orlistat. These results indicate that protein-induced pancreatic secretion is inhibited by sucrose esters, not by free fatty acids. The F-10 and F-160 sucrose esters contained 2.2% (wt/wt) and 1.7% (wt/wt) free fatty acids, respectively. These sucrose esters liberated only 1.9% (F-10) and 0.2% (F-160) of the free fatty acids after *in vitro* digestion in Tris buffer (pH 8.2) containing fresh BPJ for 60 min at 37°C, whereas triolein released 20% (wt/wt) fatty acids. These amounts of free fatty acids released from the sucrose esters thought to be too small to induce significant PYY release as previous reports have shown that large amounts of fatty acids (> 100 mg/rat) are required to induce significant PYY release in rats (Anini *et al.*, 1999).

Administration of HGC strongly stimulated pancreatic secretion in PBD rats, which

is associated with CCK release independent of proximal luminal proteases as described in our previous studies (Hara *et al.*, 1994; Hira *et al.*, 1997). PYY is released by fatty acids in the distal intestine (Fu-Cheng *et al.*, 1995; Dumoulin *et al.*, 1998; Onaga *et al.*, 2002), and inhibits pancreatic secretion (Jin *et al.*, 1993; Naruse *et al.*, 2002). To investigate the involvement of PYY in the suppression mechanism, plasma PYY levels were measured in PBD rats after the intraduodenal injection of HGC with sucrose or F-160. We used anesthetized rats after chronic BPJ diversion in order to reduce physical stress on rats during the collection of BPJ and blood. In the case of conscious rats, four catheters for BPJ collection and return, duodenal infusion and blood collection are needed to be implanted. These are difficult to maintain during the recovery period, and are also difficult to handle during experiment. In this experiment, the F-160 sucrose ester suppressed pancreatic secretion induced by HGC (Fig. 3) under anesthesia to the same degree as experiment 2. A duodenal administration of F-160 with HGC induced higher PYY release than did HGC with sucrose. This is the first finding that a sucrose ester stimulates PYY secretion *in vivo*. Fatty acids induce PYY release as demonstrated in previous papers (Anini *et al.*, 1999; Aponte *et al.*, 1989). However, sucrose esters are indigestible and we confirmed that the suppression of pancreatic secretion is triggered by sucrose ester itself in experiment 2. These results suggest that sucrose esters itself, possibly the esterified fatty acid moieties, stimulate PYY release in the mid and distal intestine. A recent study has demonstrated that PYY-producing L-cells locate not only in the ileum but also in the jejunum in rats (Mortensen *et al.*, 2003).

It is unclear how sucrose fatty acid esters stimulate PYY release from

enteroeondocrine L cells. The mechanism for free fatty acid-induced PYY release from L cells is also poorly understood. It has been shown that carboxyl group and carbon chain length are responsible for fatty acid sensing in CCK releasing-enteroendocrine I cells (McLaughlin *et al.*, 1998; Hira *et al.*, 2004). However, free fatty acids were found not to be responsible for PYY release in the present study as sucrose esters contain only small amounts of free fatty acids and do not liberate free fatty acids by luminal digestion as described above. Further investigations on the mechanism involved in sucrose ester-induced PYY release are necessary.

Interestingly, HGC + sucrose also induced transient PYY secretion, which peaked at 60 min after injection. This result agrees with those from previous reports showing peptone-induced PYY release in the rat ileum (Dumoulin *et al.*, 1998) and in humans (Calbet & Holst, 2004). Our findings show that HGC also stimulates PYY release in the mid and distal intestine, and also show that the effects of esterified fatty acids are more potent than and additive to the protein's effects.

The present results suggest that undigested lipids activate PYY release to inhibit pancreatic secretion. PBD eliminates pancreatic enzymes from the proximal small intestine, so that could be a model of digestive dysfunction, such as dyspepsia. Excessive amounts of medium- and long-chain fatty acids in the distal intestine could increase epithelial permeability, permitting drug, allergen, toxin, and virus passage through the intestinal barriers (Mine & Zhang, 2003; Soderholm *et al.*, 2002; Usami *et al.*, 2001; Yata *et al.*, 2001). Therefore, the physiological relevance of the suppression of the pancreatic secretion triggered by fat in the distal small intestine may be the

reduction of the risk of the adverse effects of fat.

In conclusion, the duodenal administration of sucrose fatty acid esters stimulates PYY release and suppresses pancreatic secretion without hydrolysis by lipase. The inhibitory mechanism may be triggered by the esterified fatty acids themselves.

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Figure legends

Figure 1. Sucrose fatty acid ester F-10 and cocoa butter inhibit pancreatic secretion induced by guanidinated casein hydrolysate

BPJ volume (A), protein (B), and trypsin secretion (C) were monitored at the indicated times after the intraduodenal instillation of 1 ml of test solution {150 mg guanidinated casein hydrolysate (HGC) with 10 mg sodium caseinate (open circles, n = 5), or HGC containing either 100 mg cocoa butter (closed squares, n = 6) or 100 mg sucrose fatty acid ester F-10 (closed circles, n = 9)}. The value at each time represents volume, protein concentration, or trypsin activity, respectively, in BPJ secreted for 3 min. Two-way ANOVA *P*-values of BPJ volume, protein, and trypsin secretion were 0.014, < 0.001, and < 0.001 for administration (A), 0.054, < 0.001, and < 0.001 for time (T), and 0.934, 0.024, and 0.033 for A x T, respectively. +: Significantly different from the value at 0 min in each group (LSD, $P < 0.05$). Values at the same time point not sharing the same letter differ significantly (LSD, $P < 0.05$).

Figure 2. Sucrose fatty acid ester F-160 inhibits protein-induced pancreatic secretion independent of luminal lipase activity

BPJ volume (A), protein (B), and trypsin secretion (C) after the intraduodenal instillation of 1 ml of test solution {150 mg guanidinated casein hydrolysate (HGC)} containing either 100 mg sucrose (open circles, n = 6), 100 mg sucrose fatty acid ester F-160 (closed squares, n = 7), or 100 mg F-160 with 0.4 mg lipase inhibitor orlistat (closed circles, n = 9)}. Two-way ANOVA *P*-values of BPJ volume, protein, and

trypsin secretion were all < 0.001 for administration (A), < 0.001, 0.043, and 0.001 for time (T), and 0.167, 0.095, and 0.066 for A x T, respectively. +: Significantly different from the value at 0 min in each group (LSD, $P < 0.05$). Values at the same time point not sharing the same letter differ significantly (LSD, $P < 0.05$).

Figure 3. Sucrose fatty acid ester F-160 inhibits protein-induced pancreatic secretion in anesthetized PBD rats

BPJ volume (A) and protein secretion (B) after the intraduodenal instillation of 2 ml of test solution {300 mg guanidinated casein hydrolysate (HGC)} containing either 200 mg sucrose (open circles, n = 7), or 200 mg sucrose fatty acid ester F-160 (closed circles, n = 8)}. Two-way ANOVA *P*-values of BPJ volume and protein secretion were 0.4874 and 0.0087 for administration (A), 0.119 and 0.0469 for time (T), and 0.4513 and 0.7099 for A x T, respectively. +: Significantly different from the value at 0 min in each group (LSD, $P < 0.05$).

Figure 4. Sucrose fatty acid ester F-160 induces PYY secretion in anesthetized PBD rats

Blood samples were collected from the jugular catheter at the indicated times after the intraduodenal instillation of 2 ml of test solution {300 mg guanidinated casein hydrolysate (HGC)} containing either 200 mg sucrose (open circles, n = 7), or 200 mg sucrose fatty acid ester F-160 (closed circles, n = 8)}. The values at each time were expressed as relative values of basal PYY levels in individual rats. Two-way ANOVA

P-values were 0.0113 for administration (A), 0.0005 for time (T), and 0.1316 for A x T, respectively. +: Significantly different from the value at 0 min in each group (LSD, $P < 0.05$). *; Significantly different at the same time point between the two groups (LSD, $P < 0.05$).

Fig. 1

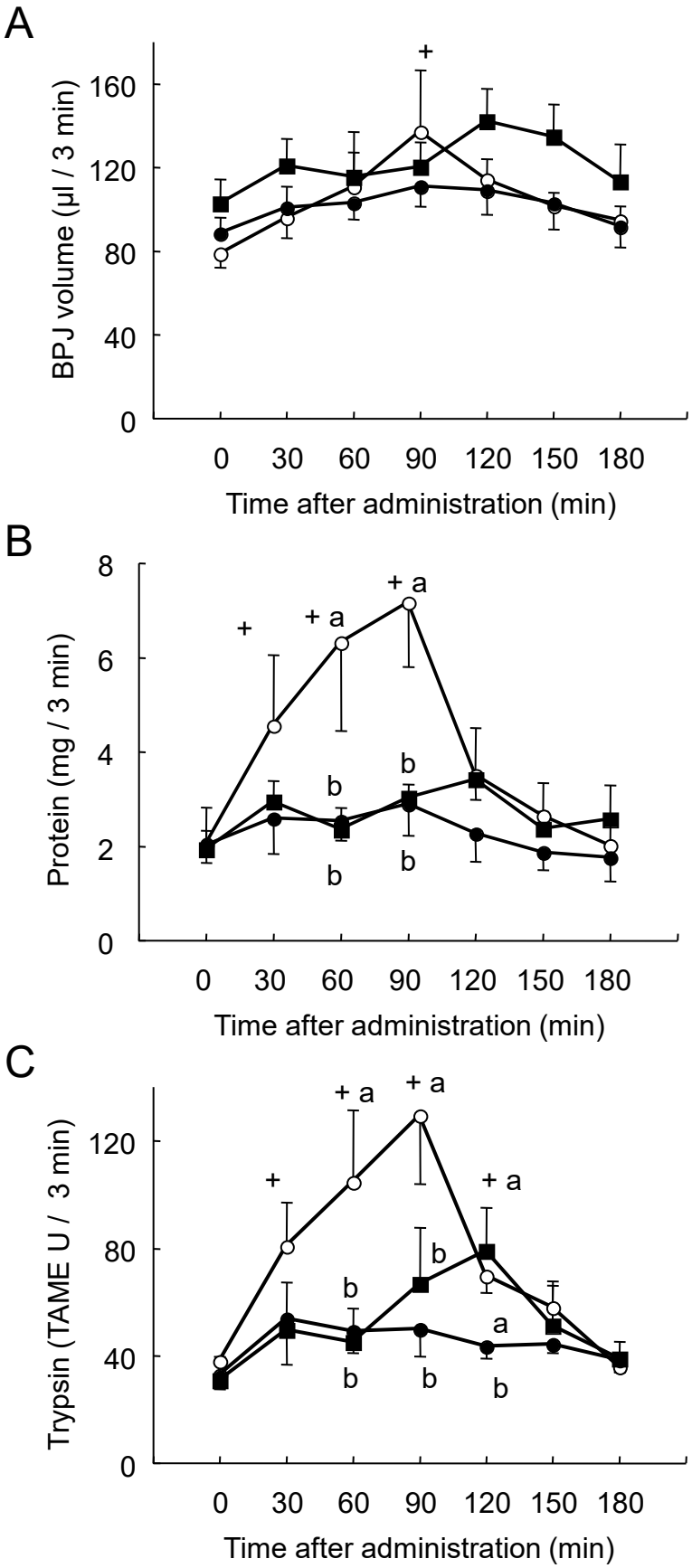

A
BPJ volume (µl / 3 min)
160
120
80
40
0
+
0 30 60 90 120 150 180
Time after administration (min)
B
Protein (mg / 3 min)
8
6
4
2
0
+
+ a
+ a
b
b
b
b
0 30 60 90 120 150 180
Time after administration (min)
C
Trypsin (TAME U / 3 min)
120
80
40
0
+
+ a
+ a
+ a
b
b
b
b
a
b
0 30 60 90 120 150 180
Time after administration (min)

Fig. 2

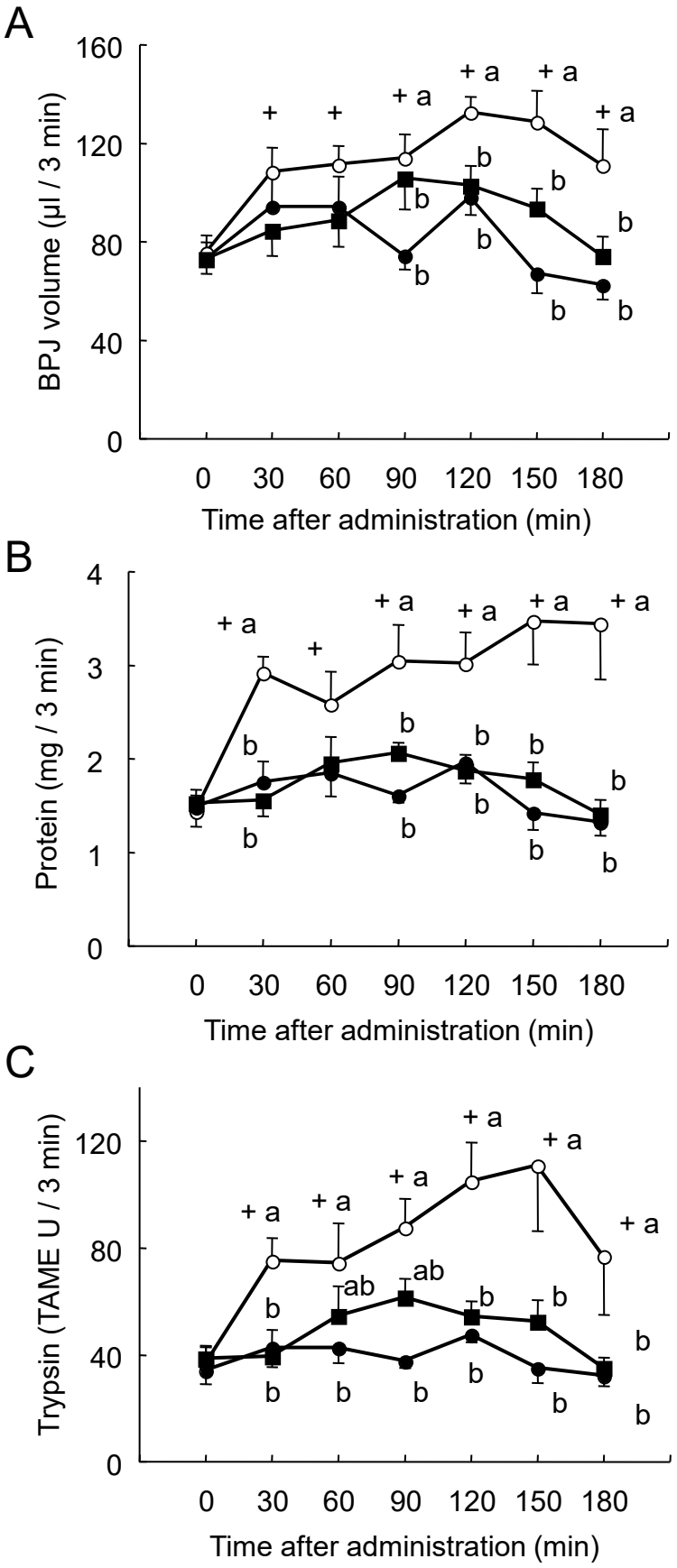

A
BPJ volume (μl / 3 min)
160
120
80
40
0
0 30 60 90 120 150 180
Time after administration (min)
B
Protein (mg / 3 min)
4
3
2
1
0
0 30 60 90 120 150 180
Time after administration (min)
C
Trypsin (TAME U / 3 min)
120
80
40
0
0 30 60 90 120 150 180
Time after administration (min)

Fig. 3

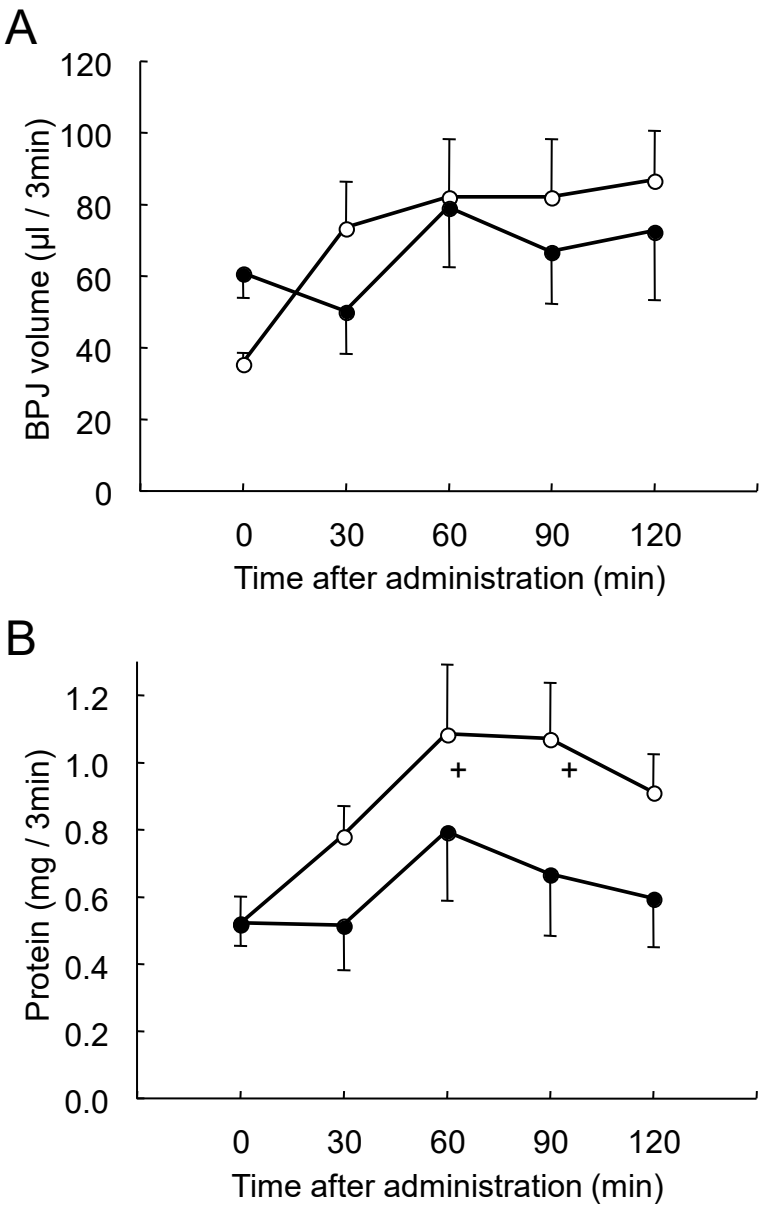
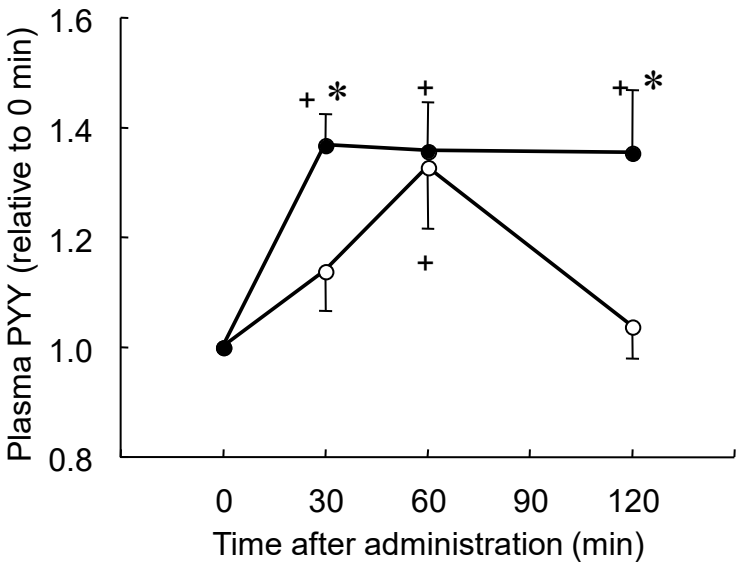

A
120
100
80
60
40
20
0
BPJ volume (µl / 3min)
0
30
60
90
120
Time after administration (min)
B
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Protein (mg / 3min)
+
+
0
30
60
90
120
Time after administration (min)

Fig. 4


Plasma PYY (relative to 0 min)
1.6
1.4
1.2
1.0
0.8
0
30
60
90
120
Time after administration (min)
+
*
+
+
*
+