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1 **Title**

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

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16

17 **Running title**

18 Fatty acid esters release PYY and inhibit pancreatic secretion

19

1 **Abstract**

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

22 **KEY WORDS:**

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

3

4 **Introduction**

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

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

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

7

8 **Methods**

9 **Materials**

10 Guanidinated casein was prepared by a previously described method (Hara *et al.*,
11 1995). The conversion rate of lysyl residues to homoarginine was 96%. Briefly,
12 guanidinated casein (55 g/l) was hydrolyzed with pepsin (0.55 g/l; Sigma Chemical Co.,
13 St. Louis, MO) at pH 1.8 for 10 min at 37°C, and the hydrolysate was then neutralized
14 and desalted (guanidinated casein hydrolysate: HGC). Sucrose fatty acid esters were
15 provided by Dai-Ichi Kogyo Seiyaku Co. (Kyoto, Japan). The water-insoluble sucrose
16 fatty acid ester, F-10, is a mixture of di-, tri- and polyesters without monoesters, and the
17 water-soluble, F-160, contains 70% monoesters and 30% polyesters. These sucrose fatty
18 acid esters contain stearic acid (70%) and palmitic acid (30%) as esterified fatty acids.
19 The lipase inhibitor (Schwizer *et al.*, 1997), orlistat (tetrahydrolipstatin), was a gift from
20 F. Hoffmann-La Roche Ltd. (Basel, Switzerland). This drug inhibits pancreatic lipase,
21 gastric lipase, carboxyl ester lipase (cholesterol esterase) of pancreatic origin and the
22 bile-salt-stimulated lipase of human milk (Borgstrom, 1988)

1 **Animals and Diets in experiments 1 and 2**

2 Male Sprague-Dawley rats (8 weeks old, Japan SLC Inc., Hamamatsu, Japan) were
3 fed a semipurified, sucrose-based diet containing 25% casein for 5 days (American
4 Institute of Nutrition, 1977; Reeves, 1989). After a 24- hr fast, cannulae were implanted
5 into the common bile-pancreatic duct, duodenum, and upper ileum under pentobarbital
6 anesthesia (sodium pentobarbital, 40 mg/kg body weight; Abbott Co., North Chicago,
7 IL), as previously described (Hara *et al.*, 2000; Hira *et al.*, 1997). Briefly, the small tip
8 (7-8 mm) of a polyethylene catheter (SP 28; I.D. 0.4 mm, O.D. 0.8 mm; Natsume
9 Seisakusyo, Tokyo, Japan) was inserted into the common bile-pancreatic duct. The other
10 end of the catheter was connected to silicone tubing (Silascon No.00, I.D. 0.5 mm, O.D.
11 1.0 mm; Dow Corning Co., Kanagawa, Japan). A silicone catheter (Silascon No.00) for
12 returning BPJ to the ileal lumen was placed through a fistula 45 cm distal (middle of the
13 small intestine) from the ligament of Treitz. These catheters were tunneled
14 subcutaneously and connected to each other at the back of the neck to maintain the BPJ
15 flow. Another silicone catheter (Silascon No.00) for administration of the test solution
16 was inserted into the duodenal lumen through a gastric fistula. In the PBD rats, BPJ
17 flow bypasses the proximal small intestine through the catheters, and flows into the ileal
18 lumen. The rats were allowed to recover for 6 days with free access to the semipurified
19 diet described above. The common bile-pancreatic duct was examined after experiments,
20 and rats with a swollen duct due to occlusion of the catheters were excluded from the
21 analysis. The test solution or emulsion was administered into the duodenum through the
22 catheter by a bolus injection (1 ml for 1 min) after twice sampling the BPJ in a fasting

1 state. For BPJ sampling, the bile-pancreatic duct catheter was extended with a
2 polyethylene tube (SP 28; Natsume Seisakusyo), and BPJ was collected from the
3 polyethylene tube via an outlet placed 5 cm from the bottom of the cage. BPJ was
4 collected for 3 min at each time point shown in Figures 1 and 2, and was recirculated
5 continuously into the ileum through the ileal catheter except during the 3-min sampling
6 periods. Rats were allowed to move freely in the cages throughout the experimental
7 period. The experiments were performed in a room controlled at $23 \pm 2^{\circ}\text{C}$, with a 12-hr
8 light-dark cycle (0800-2000, light period). The study was approved by the Hokkaido
9 University Animal Committee, and the animals were maintained in accordance with the
10 guidelines for the care and use of laboratory animals of Hokkaido University.

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

13 The PBD rats were divided into three groups on the basis of body weight after a
14 6-day recovery period and a 24-hr fast. One group received 1 ml of HGC (150 mg/ml)
15 solution containing sodium caseinate (10 mg/ml) as an emulsifying agent. The second
16 group received this solution supplemented with cocoa butter (100 mg/ml), and the third
17 group received this solution supplemented with fatty acid ester F-10 (100 mg/ml). The
18 test solution was administered into the duodenum of PBD rats through the duodenal
19 catheter. BPJ was collected before and after the administration as described above.

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

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

1 solutions contained HGC (150 mg/ml) with sucrose (100 mg/ml), HGC (150 mg/ml)
2 with the sucrose fatty acid ester, F-160 (100 mg/ml), or HGC (150 mg/ml) with F-160
3 (100 mg/ml) and the lipase inhibitor, orlistat (0.4 mg/ml), respectively. One ml of the
4 test solution was administered into the duodenum and BPJ was collected before and
5 after administration as described above.

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

8 Two groups of PBD rats were prepared as in experiment 1, 2 with some
9 modifications. The catheter redirecting the BPJ from the bile-pancreatic duct to the
10 distal intestine was left in the abdominal cavity for a 6 day-recovery period to prevent
11 physical damage by rats. On the day of the experiment, another catheter was implanted
12 into the jugular vein to collect blood samples under anesthesia (sodium pentobarbital,
13 40 mg/kg body weight). The catheter circulating the BPJ in the abdominal cavity was
14 cut around the middle after a small midline incision, and an extension catheter (20 cm)
15 was connected to the end of the bile-pancreatic catheter to collect BPJ at the basal state
16 for 3 min. At the same time, blood samples (100 μ L) for PYY measurement were drawn
17 into a syringe containing aprotinin (final concentration at 200 kIU/ml) and heparin
18 (final concentration at 50 IU/ml) through the jugular catheter. After BPJ collection for 3
19 min, the extension catheter was replaced with a short (1 cm) polyethylene tube to
20 reconnect of catheters between the bile-pancreatic duct and the ileum. Then, two ml of
21 HGC (150 mg/ml) solution containing either sucrose (100 mg/ml) or F-160 (100 mg/ml)
22 was directly injected into the duodenum for 2 min. We injected 2 ml of the test solutions

1 in experiment 3 because intestinal motility is known to be suppressed under anesthesia.
2 BPJ (for 3 min) and blood samples (100 μ l) were collected at 30, 60 and 120 min after
3 the duodenal injection, and were kept on ice until the end of the experiment. During the
4 experiment, additional pentobarbital (20 mg/kg body weight) was injected to keep rats
5 under anesthesia, and body temperature was maintained using a heating pad. Plasma
6 was separated from blood samples by centrifugation at 2,500 x g for 15 min at 4°C, and
7 then frozen at -80°C until PYY measurement. Plasma PYY concentrations were
8 measured using a commercial enzyme immuno assay (EIA) kit (Yanaihara Institute Inc.,
9 Shizuoka, Japan). The antiserum cross-reacts 100% with intact PYY (1-36, rat), 0%
10 with PYY (19-36, human), 0.7% with neuro peptide Y (NPY, human), 0% with NPY
11 (1-19, human), and 0% with pancreatic polypeptide (human).

12 **Analysis**

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

21 **Calculations and statistical analysis**

22 One unit of trypsin was defined as the activity necessary to hydrolyze 1 μ mole of

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

6

7 **Results**

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

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

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

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

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

1

2 **Discussion**

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

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

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

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

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

1 is associated with CCK release independent of proximal luminal proteases as described
2 in our previous studies (Hara *et al.*, 1994; Hira *et al.*, 1997). PYY is released by fatty
3 acids in the distal intestine (Fu-Cheng *et al.*, 1995; Dumoulin *et al.*, 1998; Onaga *et al.*,
4 2002), and inhibits pancreatic secretion (Jin *et al.*, 1993; Naruse *et al.*, 2002). To
5 investigate the involvement of PYY in the suppression mechanism, plasma PYY levels
6 were measured in PBD rats after the intraduodenal injection of HGC with sucrose or
7 F-160. We used anesthetized rats after chronic BPJ diversion in order to reduce physical
8 stress on rats during the collection of BPJ and blood. In the case of conscious rats, four
9 catheters for BPJ collection and return, duodenal infusion and blood collection are
10 needed to be implanted. These are difficult to maintain during the recovery period, and
11 are also difficult to handle during experiment. In this experiment, the F-160 sucrose
12 ester suppressed pancreatic secretion induced by HGC (Fig. 3) under anesthesia to the
13 same degree as experiment 2. A duodenal administration of F-160 with HGC induced
14 higher PYY release than did HGC with sucrose. This is the first finding that a sucrose
15 ester stimulates PYY secretion *in vivo*. Fatty acids induce PYY release as demonstrated
16 in previous papers (Anini *et al.*, 1999; Aponte *et al.*, 1989). However, sucrose esters are
17 indigestible and we confirmed that the suppression of pancreatic secretion is triggered
18 by sucrose ester itself in experiment 2. These results suggest that sucrose esters itself,
19 possibly the esterified fatty acid moieties, stimulate PYY release in the mid and distal
20 intestine. A recent study has demonstrated that PYY-producing L-cells locate not only in
21 the ileum but also in the jejunum in rats (Mortensen *et al.*, 2003).

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

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

9 Interestingly, HGC + sucrose also induced transient PYY secretion, which peaked at
10 60 min after injection. This result agrees with those from previous reports showing
11 peptone-induced PYY release in the rat ileum (Dumoulin *et al.*, 1998) and in humans
12 (Calbet & Holst, 2004). Our findings show that HGC also stimulates PYY release in the
13 mid and distal intestine, and also show that the effects of esterified fatty acids are more
14 potent than and additive to the protein's effects.

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

1 reduction of the risk of the adverse effects of fat.

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

5

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12

1 **Figure legends**

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

4 BPJ volume (A), protein (B), and trypsin secretion (C) were monitored at the indicated
5 times after the intraduodenal instillation of 1 ml of test solution {150 mg guanidinated
6 casein hydrolysate (HGC) with 10 mg sodium caseinate (open circles, n = 5), or HGC
7 containing either 100 mg cocoa butter (closed squares, n = 6) or 100 mg sucrose fatty
8 acid ester F-10 (closed circles, n = 9)}. The value at each time represents volume,
9 protein concentration, or trypsin activity, respectively, in BPJ secreted for 3 min.
10 Two-way ANOVA *P*-values of BPJ volume, protein, and trypsin secretion were 0.014,
11 < 0.001, and < 0.001 for administration (A), 0.054, < 0.001, and < 0.001 for time (T),
12 and 0.934, 0.024, and 0.033 for A x T, respectively. +: Significantly different from the
13 value at 0 min in each group (LSD, *P* < 0.05). Values at the same time point not
14 sharing the same letter differ significantly (LSD, *P* < 0.05).

15

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

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

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

5

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

8 BPJ volume (A) and protein secretion (B) after the intraduodenal instillation of 2 ml of
9 test solution {300 mg guanidinated casein hydrolysate (HGC)} containing either 200
10 mg sucrose (open circles, $n = 7$), or 200 mg sucrose fatty acid ester F-160 (closed
11 circles, $n = 8$). Two-way ANOVA P -values of BPJ volume and protein secretion were
12 0.4874 and 0.0087 for administration (A), 0.119 and 0.0469 for time (T), and 0.4513
13 and 0.7099 for A x T, respectively. +: Significantly different from the value at 0 min in
14 each group (LSD, $P < 0.05$).

15

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

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

1 *P*-values were 0.0113 for administration (A), 0.0005 for time (T), and 0.1316 for A x T,
2 respectively. +: Significantly different from the value at 0 min in each group (LSD, *P* <
3 0.05). *: Significantly different at the same time point between the two groups (LSD, *P*
4 < 0.05).

Fig. 1

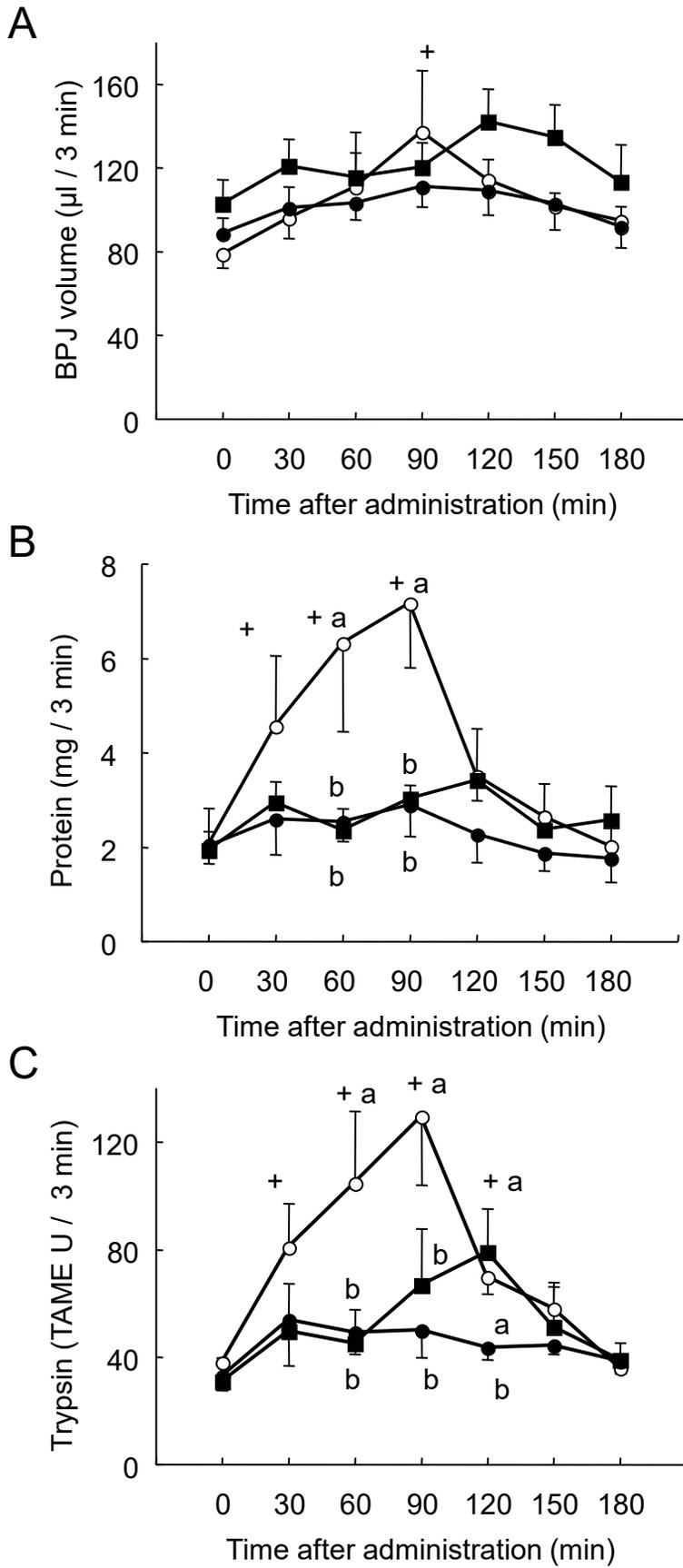


Fig. 2

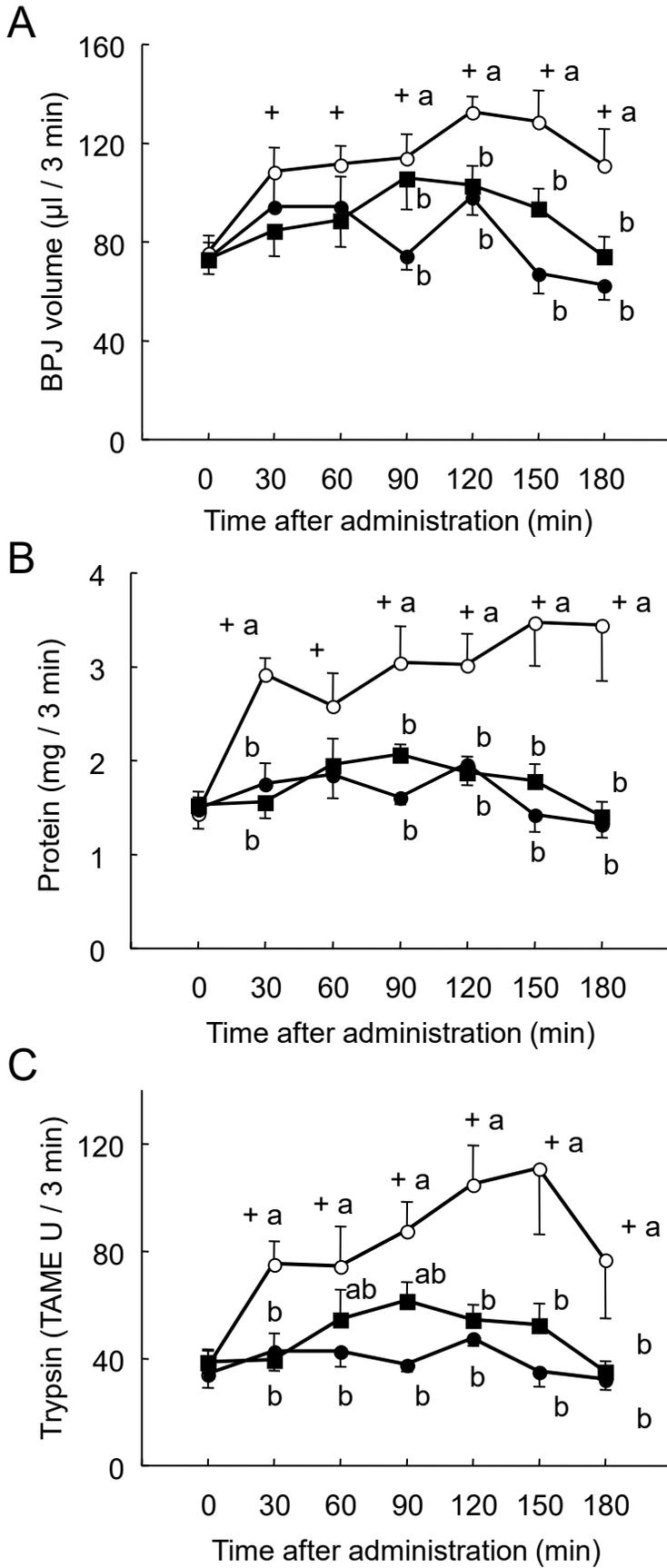


Fig. 3

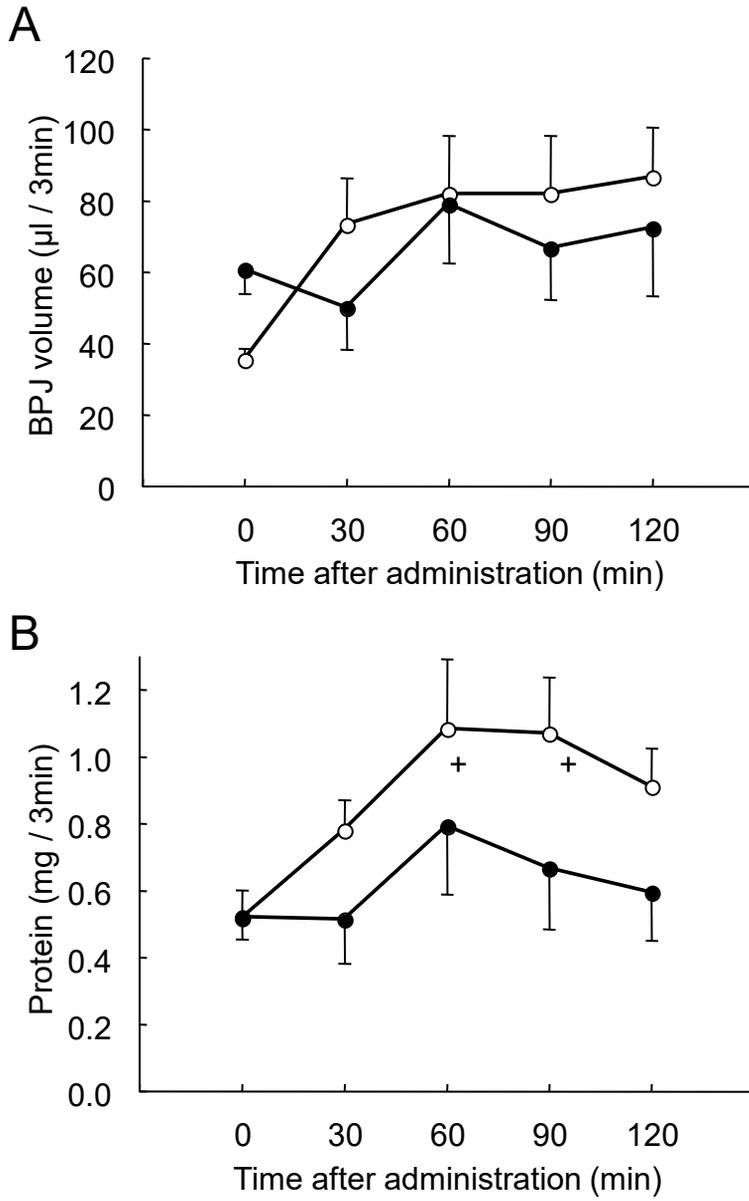


Fig. 4

