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The Effect of Oleate on Pancreatic and Bile Secretion in the Conscious Rat (42785)

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Abstract. The effects of sodium oleate infused into either the duodenum or the terminal ileum on bile and pancreatic secretion were examined in the conscious rat. Rats were prepared with cannulae draining pure bile and pancreatic juice separately, and with an ileal and two duodenal cannulae. A 40 m*M* taurocholate solution containing 7 mg/ml bovine trypsin was infused into the duodenum throughout the experiment to replace diverted bile–pancreatic juice to maintain the normal regulation of pancreatic secretion. The intraduodenal infusion of sodium oleate significantly increased pancreatic juice flow, protein, and bicarbonate outputs, whereas it did not affect bile secretion. Intravenous infusion of proglumide (300 mg/kg/hr) did not inhibit pancreatic secretion stimulated by intraduodenal infusion of sodium oleate. An intravenous infusion of atropine (100 μg/kg/hr) attenuated protein and fluid secretions but not that of bicarbonate in response to intraduodenal oleate. In contrast, the intraileal infusion of oleate had no effect on pancreatic secretion, whereas it decreased bile flow, bicarbonate, and bile salt outputs. In conclusion, sodium oleate introduced in the duodenum stimulates pancreatic secretion but oleate in the terminal ileum inhibits bile secretion.

It has been known that intraduodenal fatty acid is a strong stimulant of pancreatic exocrine secretion, but in the distal intestine or colon, it inhibits pancretic and/or bile secretion (1–10). However, the mechanism of its stimulatory effect was not fully elucidated. Mott *et al.* (1) reported that in man the intraduodenal infusion of oleic acid increased pancreatic juice flow and bicarbonate and protein outputs, effects similar to those of an intravenous injection of cholecystokinin (CCK-PZ). A similar phenomenon was also observed in dogs (2). Because of the marked increase in bicarbonate secretion when oleic acid was infused into the intestine (3), the involvement of secretin or VIP release was suggested. Despite these suggestions, an increase in plasma secretin level has not been proved (4). Recently, the release of secretin by the digestive products of fat was demonstrated in dogs (5). Singer *et al.* (6) reported that oleate and tryptophan in the duodenum stimulate pancreas by a vagovagal cholinergic reflex in the early pancreatic enzyme response in dogs. Cholinergic component was also reported in humans (7).

In contrast to these stimulatory effects of oleic acid on the pancreas, it was reported for dogs (8) that oleic acid infused into the colon decreased pancreatic secretion. Furthermore, in cats, oleic acid infused into the ileum or into the colon decreased pancreatic secretion (9). Recently, Owyang *et al.* found that the intracolonic infusion of oleic acid suppressed pancreatic and bile secretions in man (10).

In rats, we have recently reported that sodium oleate strongly stimulated pancreatic juice flow and protein output, and that its stimulatory effect on fluid secretion was greater than the effect of casein (11). In order to clarify the underlying mechanism for these phenomena, we examined in the present study whether proglumide, a competitive inhibitor for CCK, or atropine could inhibit protein secretion produced by sodium oleate. Furthermore, it was examined whether oleate in the distal ileum inhibits pancreatic secretion in the conscious rat as was observed in other species (8–10). Finally since the bile was essentially involved in fat digestion (12), the effects of oleate in duodenum and distal ileum on bile secretion were also examined.

Methods. Male Wistar strain rats (13–16 week-old) were obtained from Shizuoka Jikken Dobutsu, Shizuoka, Japan. Proglumide was a generous gift from Kaken Pharmaceu-

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tical Co., Tokyo, Japan. Sodium oleate and bovine trypsin were purchased from Sigma Chemical (St. Louis, MO) and taurocholate was purchased from Calbiochem (La Jolla, CA). Rats were fed commercial rat chow (CRF-1; Oriental, Tokyo, Japan) before surgery, during recovery, and between experiments. Rats were prepared with cannulae draining pure bile and pancreatic juice separately, and with two duodenal cannulae. In some animals, an additional cannula was inserted into the ileum (10 cm proximal from the ileal end) or another additional cannula was inserted into the right jugular vein. The operative procedures used have been described in detail in previous publications (13, 14). Briefly, a midline incision was made under Enflurane anesthesia (Abbott, North Chicago, IL). The common bile duct was ligated proximal to the pancreas below the hilum of the liver and a cannula (Silastic Medical Grade Tubing, Dow Corning, Midland, MI; 0.025 inches i.d. × 0.037 inches, o.d.) was inserted above the ligature to collect pure bile and another cannula was inserted into the common bile duct proximal to the ampulla of Vater to collect pure pancreatic juice. Two other cannulae were inserted into the duodenum so that the outlet tip could be located at the site of the ampulla of Vater, and another was inserted into the terminal ileum (10 cm proximal from the cecum). Finally, the last cannula was inserted into the right jugular vein to infuse proglumide or atropine. Cannulae were initially brought into the abdominal cavity through a subcutaneous channel starting at the back near the tail. After the operation, rats were placed in modified Bollman-type restraint cages. Bile and pancreatic juice (BPJ) were continuously returned to the duodenum by a servo-system consisting of a collection tube (Corning, NY) in a liquid-level photodetector (SKAN-A-MATIC Co., Elbridge, NY) coupled to a peristaltic pump (Gilson France S.A., France) (13).

Animals were placed in a room with a 12-hr light–dark cycle at 24°C in filtered air.

Experiments were conducted between the third and seventh postoperative days after a 5-hr fast. Infusion of 40 m*M* taurocholate solution containing bovine trypsin (7 mg/ml), pH 7.8, was started at a rate of 1 ml/hr by a syringe pump (Harvard Apparatus Compact infusion pump, Harvard Apparatus, Millis, MA) in place of BPJ. BPJ was collected separately at 30-min intervals. After a 90-min basal collection, a 2% sodium oleate solution (pH 9.2) was infused through the other duodenal cannula into the duodenum at a rate of 1 ml/hr. In some animals, after a basal collection, sodium oleate was infused into the ileum.

The volume of pancreatic juice was determined with a Hamilton syringe, bile was collected in a Wintrobe's hematocrit tubing and its volume was measured. Protein concentration was estimated by determining the optical density at 280 nm (15) of samples diluted 1:200 in 0.04 Tris buffer, pH 7.8. Purified bovine trypsinogen (Worthington Biochemical, Freehold, NJ) was used as a standard. The concentration of bicarbonate in pancreatic juice and bile was measured by a Natelson microgasometer (16, 17) and the output was calculated. The total bile acid concentration in the bile was measured enzymatically using 3α-hydroxysteroid dehydrogenase (Worthington Biochemical) (18). In some experiments, to examine whether CCK was released by sodium olate in the duodenum and affected pancreatic secretion and to examine the involvement of cholinergic mechanism, a competitive inhibitor for CCK, proglumide, or atropine was simultaneously infused at a rate of 300 mg/kg/hr or 100 μg/kg/hr, respectively. A control study has been done with the infusion of isotonic saline instead of sodium oleate.

Results of pancreatic secretions were analyzed by multiple analysis of variance (MANOVA) with repeated measures with respect to treatments and time. We analyzed differences among four treatments using Duncan's multiple range test. Differences were considered to be significant at a P level less than 0.05.

Results. *Effects of oleate administered into the duodenum on pancreatic and bile secretion.* Changes in pancreatic juice flow and bicarbonate and protein outputs by the intraduodenal infusion of sodium oleate were all significantly different with respect to time [$F(3,25) = 16.7$ for flow, 7.46 for bicarbonate, and 7.32 for protein, $P < 0.001$], reaching a peak in the period between 60 and 90

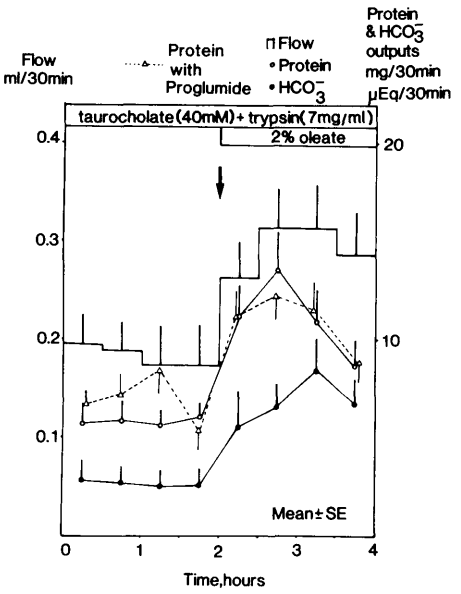

FIG. 1. Pancreatic responses to an intraduodenal infusion of oleate and the effect of proglumide (300 mg/kg/hr). Taurocholate (40 m*M*) containing trypsin (7 mg/ml) was infused at a rate of 1 ml/hr in place of BPJ. Broken lines indicate the protein output with the simultaneous infusion of proglumide. The infusion of sodium oleate into the duodenum significantly increased fluid, bicarbonate, and protein outputs. Proglumide did not affect the pancreatic responses to intraduodenal infusion of sodium oleate. Values are the means ± SE. $n = 5$ without proglumide, and $n = 8$ with proglumide.

min (Fig. 1). The fluid secretion increased 1.8-fold, protein output 2-fold, and bicarbonate output 3-fold at their peak secretions in comparison to respective basal values. F values for control experiments (isotonic saline infused) were 0.19 for flow, 0.97 for bicarbonate, and 0.41 for protein ($P > 0.5$).

Intravenous infusion of proglumide (300 mg/kg/hr) with intraduodenal infusion of oleate significantly increased fluid, bicarbonate, and protein outputs [$F = 5.89$ for fluid, 8.68 for bicarbonate, and 8.99 for protein, $P < 0.001$]. Changes in protein outputs in response to proglumide are shown in Fig. 1. There was no significant interaction effect (proglumide by time) by multiple range test.

Atropine is known to inhibit basal protein and fluid secretions (19). Changes in fluid, bicarbonate, and protein outputs were significantly different with respect to time (Fig. 2), F values being 6.42 for fluid, 7.0 for bicarbonate, and 5.32 for protein output; $P < 0.002$. There were significant differences by MANOVA among animals given different treatments in fluid, bicarbonate, and protein outputs [$F(3,25) = 6.97$ for fluid, 7.05 for bicarbonate, and 6.66 for protein output, $P < 0.002$]. Atropine significantly lowered pancreatic responses to oleate by multiple range test at each time point (oleate, oleate + proglumide > oleate + atropine, saline).

In contrast to pancreatic secretion, bile secretion in terms of its volume and bicarbonate output was unaffected by the intraduodenal infusion of sodium oleate, but bile salt output tended to decrease in the 90–120 min collection period (Fig. 3).

Effects of sodium oleate in the ileum on pancreatic and bile secretion. In contrast to the effects of oleate in the duodenum, the intraileal infusion of oleate did not affect pancreatic secretion to any significant extent. However, bile salt output into the bile decreased significantly although decreases in the volume of bile and bicarbonate output were not statistically significant (Fig. 4).

Discussion. The intraduodenal infusion of sodium oleate significantly increased fluid, bicarbonate, and protein outputs from the

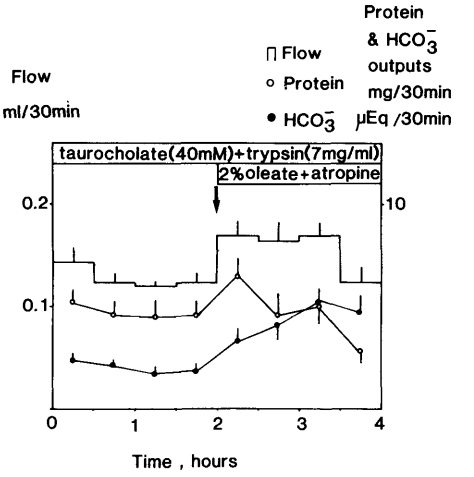

FIG. 2. Pancreatic responses to an intraduodenal infusion of oleate with 100 μg/kg/hr of atropine. $n = 6$. Fluid and bicarbonate outputs significantly increased by oleate even with the simultaneous infusion of atropine.

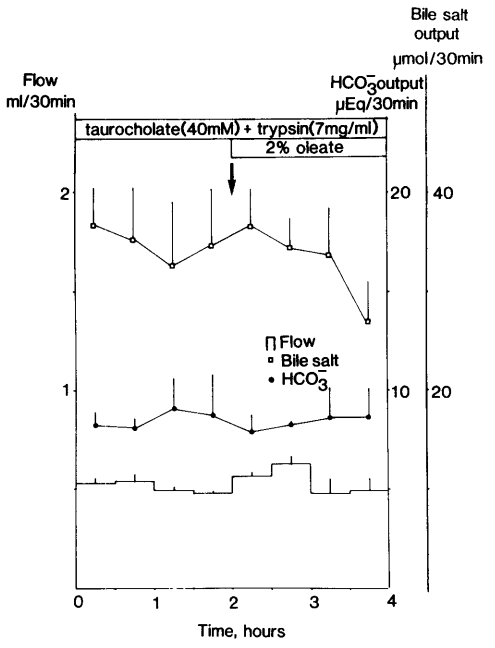

FIG. 3. The bile flow, bile salt, and bicarbonate outputs in response to an intraduodenal infusion of sodium oleate. Sodium oleate infused into the duodenum did not affect bile excretion. [$F(1,11) = 0.33$, $p > 0.9$] with respect to time for bile salt excretion by MANOVA. Values were obtained from the same experiments as shown in Fig. 1.

pancreas in the present study. The magnitude of the increase was similar to that in the previous report (20). Proglumide (300 mg/kg/hr) was reported to inhibit the effect of endogenously released CCK on the pancreas (21, 22). In the present results, however, proglumide did not inhibit protein secretion stimulated by sodium oleate in the duodenum. Radioimmunoassay for rat CCK has not been established, so that the plasma CCK concentration was not measured, but it has been recently reported that oleic acid did not produce a significant increase in plasma CCK concentration by a CCK bioassay in the rat (23). Therefore, it is unlikely that CCK released by sodium oleate in the duodenum totally accounts for the increase of the pancreatic secretion, even if oleate in the duodenum could slightly increase CCK release. Increases in fluid and bicarbonate outputs induced by the intraduodenal administration of oleate were atropine resistant, although the effect of oleate on protein output was partially inhibited by atropine. It appears that oleate in the duodenum of the rat stimulates the pancreas by a cholinergic reflex as seen in dogs (6). Furthermore, these observations indicate the possibility of secretin release by the intraluminally administered oleate in the rat as has been shown in the dog (5). Fukuoka *et al.* reported a conflicting observation in the anesthesized rat, that atropine had no effect on oleate-stimulated pancreatic secretion while proglumide, in conjunction with scopolamine, inhibited the increase in oleate-stimulated amylase release (24). The difference between our observation and that of Fukuoka *et al.* may most likely be attributed to the presence and absence of anesthesia. Pancreatic secretions are known to be markedly affected by anesthesia (25).

In contrast to the intraduodenal infusion, the intraileal infusion of sodium oleate did not affect pancreatic secretion in our study. Laugier and Sarles (26) and Demol and Sarles (27) reported that oleic acid or oleate infused into the ileum inhibited the pancreatic protein output. In Laugier's rat experi-

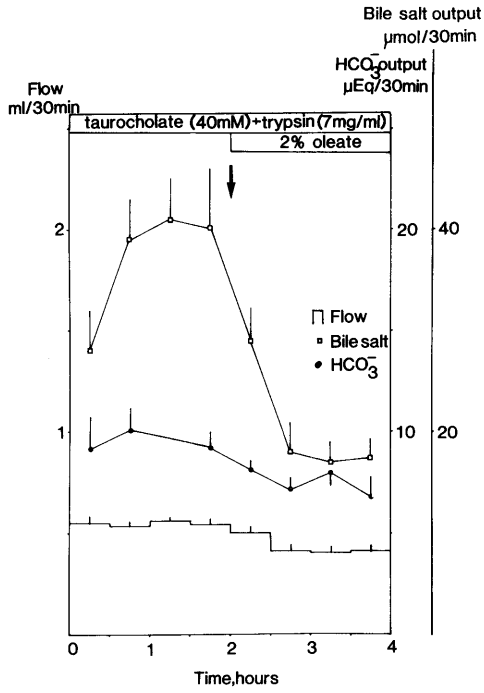

FIG. 4. The bile flow, bile salt, and bicarbonate outputs in response to an intraileal infusion of oleate. Oleate infused into the ileum significantly decreased the bile salt excretion. [$F(1,11) = 0.69$, $P < 0.025$] by MANOVA.

ments, neither the bile nor the pancreatic juice was returned to the duodenum during the experiments, while in our study, BPJ was continuously returned to the intestine between experiments, and even during experiments BPJ was replaced with TC + trypsin. As was previously reported, bile is known to have an important role in maintaining luminal trypsin activities by preventing their autodigestion (28, 29), and the absence of bile decreases trypsin activities in the proximal intestine. A decrease in luminal trypsin activities causes pancreatic hypersecretion, probably by releasing CCK (28, 30). Laugier and Sarles, therefore, examined the effect of oleic acid on secretion stimulated by bile and pancreatic juice diversion and not on basal secretion. Thus, the results of the present study do not exclude the possible release of a hormone in the distal intestine by oleic acid but suggest that such a hormone, if released, does not affect the basal (nonstimulated) pancreatic secretion when BPJ is returned as in the present study. In studies in the cats, the dogs, and humans (8–10), CCK or secretin was continuously infused because of low basal pancreatic secretion in these species.

Sodium oleate infused intraduodenally did not affect the bile secretion. On the other hand, it decreased bile salt output when introduced into the distal ileum. It is well known that the rate of bile secretion depends partly on the amount of bile acids excreted into the bile (the bile salt-dependent component). There are several reports that fatty acids in the intestine prevent bile salt, glucose, and electrolyte absorption *in vitro* (31, 32). It is also possible that the absorption of bile acids in the terminal ileum decreased due to a diminution in the bile salt concentration consequent to dilution by the infusion of the sodium oleate solution. However, it was reported that in rats the active transport system in the ileum did not account for the major bile salt absorption and the luminal bile salt concentration in the terminal ileum was only one-tenth that in the proximal intestine (33). Therefore, the decrease in bile secretion by intraileal administration of oleate may not be totally explained by the decreased bile salt absorption in the ileum. The second possibility is that some hormone(s) possesing an inhibitory effect on bile (and pancreatic secretions) was released from the distal intestine by sodium oleate as suggested previously (8–10, 26, 27).

The factors that turn off pancreatic hypersecretion after food intake remain unknown. If hormones that have an inhibitory effect on pancreas such as pancreotone (9) or PYY (34, 35) were released from the distal intestine or colon by digestive products this would be a reasonable regulatory mechanism. Moreover, it is also reasonable that these hormones do not have any effect on basal (nonstimulated, BPJ returned) pancreatic secretion as observed in the present study.

In conclusion, sodium oleate infused into the duodenum probably stimulates pancreatic secretion by releasing not CCK but probably secretin. Sodium oleate infused into the duodenum has no effect on bile secretion. Sodium oleate introduced into the terminal ileum significantly suppresses the bile salt excretion, whereas pancreatic secretion is not affected. The release of a substance from the distal intestine which inhibits bile after the infusion of sodium oleate is suggested.

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