Effect of Intraduodenal Bile and Taurodeoxycholate on Exocrine Pancreatic Secretion and on Plasma Levels of Vasoactive Intestinal Polypeptide and Somatostatin in Man

Rudolf L. Riepl, Fritz Fiedler, Johannes Teufel, and Peter Lehnert

Medical Clinic, Klinikum Innenstadt, University of Munich, Munich, Germany

Summary: Intraduodenal (i.d.) application of bile or Na-taurodeoxycholate (TDC) dose dependently enhances basal exocrine pancreatic secretion. The hydrokinetic effect is mediated at least in part by secretin. This study should show, whether vasoactive intestinal polypeptide (VIP), a partial agonist of secretin, may also be involved in the mediation of the hydrokinetic effect. Furthermore, plasma concentrations of somatostatin-like immunoreactivity (SLI) were measured in order to check whether this counterregulating hormone is also released by bile and TDC. Twenty investigations were carried out on 10 fasting healthy volunteers provided with a double-lumen Dreiling tube. Bile and TDC were intraduodenally applied in doses of 2–6 g and 200–600 mg, respectively, at 65-min intervals. Plasma samples were withdrawn at defined intervals for radioimmunological determination of VIP and SLI. Duodenal juice was collected in 10-min fractions and analyzed for volume, pH, bicarbonate, lipase, trypsin, and amylase. I.d. application of bile or TDC dose dependently stimulated hydrokinetic and ecbolic pancreatic secretion. Bile exerted a slightly stronger effect than TDC. Pancreatic response was simultaneously accompanied by a significant increase of plasma VIP and SLI concentrations. The effect of bile on integrated plasma VIP and SLI concentrations seems to be dose dependent; the effect of TDC on integrated SLI, too. For the increase of integrated plasma VIP concentrations after TDC no dose-response relation could be established. We conclude that VIP may be a further mediator of bile-induced volume and bicarbonate secretion. The release of plasma SLI indicates that inhibitory mechanisms concomitantly are triggered by i.d. bile and TDC, as already shown during digestion for the intestinal phase of pancreatic secretion. **Key Words:** Bile—Duodenum—Pancreatic secretion—Somatostatin—Taurodeoxycholate—Vasoactive intestinal polypeptide.

Intraduodenal (i.d.) application of bile or Na-taurodeoxycholate (TDC) dose dependently enhances basal (1) and secretin-stimulated (2) hydrokinetic and ecbolic pancreatic secretion in man. The pancreatic response to i.d. bile (1,3,4) or bile salts (1,5,6) is accompanied by a release of secretin, the most important hormonal mediator of pancreatic volume and bicarbonate secretion (7–9).

Vasoactive intestinal polypeptide (VIP) acts as a

weak hydrokinetic stimulus (10) in man. Burhol et al. (11) were able to show in man, that i.d. application of bile induces an increase of pancreatic hydrokinetic function and a release of VIP as well. In this investigation, however, only a single, high dose (6 g) of bile was applied. Moreover, Chayvialle et al. (12) were not able to demonstrate a release of VIP in both portal and peripheral plasma after i.d. bile (3 g) in dogs. Therefore, one purpose of the present study was to investigate in man, whether VIP is dose dependently released by physiological loads of bile or TDC, a very effective bile salt with regard to pancreatic secretion (2).

The intestinal phase of pancreatic secretion represents a net sum resulting from the responses to stimulatory and inhibitory mechanisms, as well (13). As we previously showed in man (1), i.d. ap-

plication of bile or TDC dose dependently increases the plasma levels of pancreatic polypeptide (PP), an inhibitor of pancreatic secretion (14). In dogs, Chayvialle et al. (12) observed a bile-induced release of somatostatin, a potent inhibitor of exocrine pancreatic function (15,16). On the contrary, Burhol et al. (17) did not find an augmentation of plasma somatostatin by bile in man. Therefore, another aim of our study was to reexamine the influence of bile and TDC on the release of somatostatin-like immunoreactivity (SLI) in man.

METHODS

Experimental procedure

Twenty experiments were carried out on 10 fasting young healthy subjects. Full written consent was obtained from each subject, and the research protocol was approved by the Ethics Committee at the Medical Faculty.

A double-lumen Dreiling tube was advanced to the ligament of Treitz under fluoroscopic control enabling continuous and separate aspiration of gastric and duodenal juice. Details have been described elsewhere (1,2). On one day, standardized dried cattle bile (kindly supplied by Kali-Chemie, Hannover, FRG), dissolved in 20 ml H_2O, was applied i.d. in doses of 2 (twice), 4, and 6 g (310, 639, and 937 mosmol/L; pH 7.45, 7.18, and 7.04, respectively) at 65-min intervals. On another day, chromatographically pure TDC (Calbiochem-Behring, FRG), again dissolved in 20 ml H_2O, was used in doses of 200 (twice), 400, and 600 mg (19.0, 38.5, and 57.5 mmol/L corresponding to 33.0, 50.0, and 67.0 mosmol/L; pH 7.9, 8.1, and 8.1, respectively). The first stimulus in each series—2 g bile or 200 mg TDC, respectively—was given in order to "wash out" preformed zymogens from the pancreatic ducts.

The bile preparation used in this study contained 10% TDC based on weight. In order to compare the effects of total bile and TDC, respectively, doses of TDC were administered corresponding to the amounts applied with bile (e.g., 2 g bile contain 200 mg TDC). Duodenal juice was collected in 10-min fractions and analyzed for volume, pH, bicarbonate, lipase, trypsin, and amylase. The pH values of the duodenal aspirate were within the neutral range after all i.d. stimuli. The determination of pancreatic enzymes and bicarbonate was performed according to the recommendation of the Multi-Centre-Study of the European Pancreatic Club (18). Blood samples for radioimmunological determination of

VIP and SLI were drawn from a peripheral vein in ice-chilled 10-ml syringes (containing 37.1 μmol K_2-ethylenediaminetetraacetic acid (EDTA) and 5000 KIU aprotinin) before, at, and 5, 10, 15, 25, 45, and 65 min after i.d. application of bile or TDC. The samples were immediately centrifuged at 4°C, the plasma stored at −20°C.

Radioimmunoassay procedures

Before the assays plasma samples were extracted with acetone. The supernatant was decanted and dried in a vacuum centrifuge (SpeedVac). The dried product was reconstructed to the original volume with assay buffer containing 0.1 M sodium citrate buffer (pH 6.5), 0.07 mmol/L bovine serum albumin, 3.08 mmol/L NaN_3, 500 KIU/ml aprotinin, and 50 μl/L polyoxyethylenesorbitan monolaurate (Tween 20).

VIP

Our VIP-antiserum (VI2X, K_{eff} = 1.0 × 10^{11} L/mol) showed no cross-reactivity with VIP_{1-12}, other peptides of the secretin-glucagon family, and somatostatin-14. [^{125}I]VIP (81.4 MBq/nmol; NEN, Dreieich, FRG) was used as tracer. The standard (400 μl, synthetic VIP; Sigma, Munich, FRG) and the sample (400 μl) were incubated with 100 μl of a VIP antiserum/anti-rabbit γ-globulin antiserum precipitate (dilution of VIP antiserum 1:54,000), and 100 μl of the tracer (≈1 fmol) in assay buffer. After 96 h in incubation bound and free label were separated by centrifugation at 4°C, the supernatant removed by suction, and the precipitate counted in an automatic γ-counter as described previously (1). The detection limit of the assay was 1 pmol/L with 95% confidence limit. Recovery of known amounts of VIP (3.9, 7.1, and 35.4 pmol/L) in human plasma was 50 ± 3% ($\overline{X}$ ± SD) at each concentration. The coefficient of variation for the within-assay precision was 13.3, 13.4, and 9.9% at 3.0, 15.7, and 51.3 pmol/L, respectively, and for the between-assay precision 15.3 and 13.9% at 12.4 and 27.4 pmol/L, respectively.

Somatostatin

Our antiserum against somatostatin-14 (SO4VIII, K_{eff} 1.1 × 10^{11} L/mol) showed a cross-reactivity of 80% with somatostatin-28 and none with gastrin-17(I), gastrin-34(I), sulfated cholecystokinin (CCK)-8 and CCK-33, peptides of the secretin-glucagon family, PP, peptide YY (PYY), and neuropeptide Y (NPY). [^{125}I]-Tyr$_1$-somatostatin-14 (81.4 MBq/nmol; NEN, Dreieich, FRG) was used

as tracer. The standard (300 μl, synthetic somatostatin-14; Sigma, Munich, FRG) and the sample (300 μl) were incubated with 100 μl of a somatostatin antiserum/anti-rabbit γ-globulin antiserum precipitate (dilution of somatostatin antiserum 1:280,000), and 100 μl of the tracer (≈1 fmol) in assay buffer. Incubation and separation procedures were identical to those of VIP. The detection limit of the assay was 1 pmol/L with 95% confidence limit. Recovery of synthetic somatostatin-14 (2.6, 11.5, and 56.9 pmol/L) in human plasma was 57 ± 4% ($\bar{x}$ ± SD) at each concentration. The intra-assay coefficient of variation was 14.8 and 7.8% at 2.7 and 15.4 pmol/L, respectively; the interassay coefficient of variation was 11.1 and 7% at 12.6 and 64.1 pmol/L, respectively.

Gel chromatography studies showed that VIP immunoreactivity in extracted plasma, synthetic VIP, and [^{125}I]-VIP eluted at identical positions. After i.d. application of 6 g bile, somatostatin-like immunoreactivity in extracted plasma is eluted at two peaks corresponding to somatostatin-14 and somatostatin-28 (40 and 60% of total SLI, respectively). Because of this heterogeneity the term somatostatin-like immunoreactivity is used.

Statistical methods

All data were evaluated by the Friedman two-way analysis of variance; $p \leq 0.05$ was considered statistically significant. The value before the second application of 2 g bile or 200 mg TDC, respectively, was used as "basal." It proved to be statistically not different from the other prestimulatory values. In all figures values are presented as $\bar{x}$ ± SEM. Integrated VIP and SLI values were calculated as the areas under the concentration curves for 10 min after application of each i.d. stimulus.

RESULTS

Plasma VIP concentrations significantly increased after all doses of bile and after 200 and 600 mg of TDC (Figs. 1 and 2, top). Integrated plasma VIP was significantly enhanced by 4 and 6 g of bile and by 200 mg of TDC (Fig. 3, top). Integrated VIP was significantly higher after 6 g of bile as compared with 2 and 4 g.

Plasma SLI concentrations were significantly raised by all doses of bile and TDC (Figs. 1 and 2, bottom). Integrated SLI significantly and dose dependently rose after all doses of bile and after 200 and 400 mg of TDC; after 600 mg of TDC no further
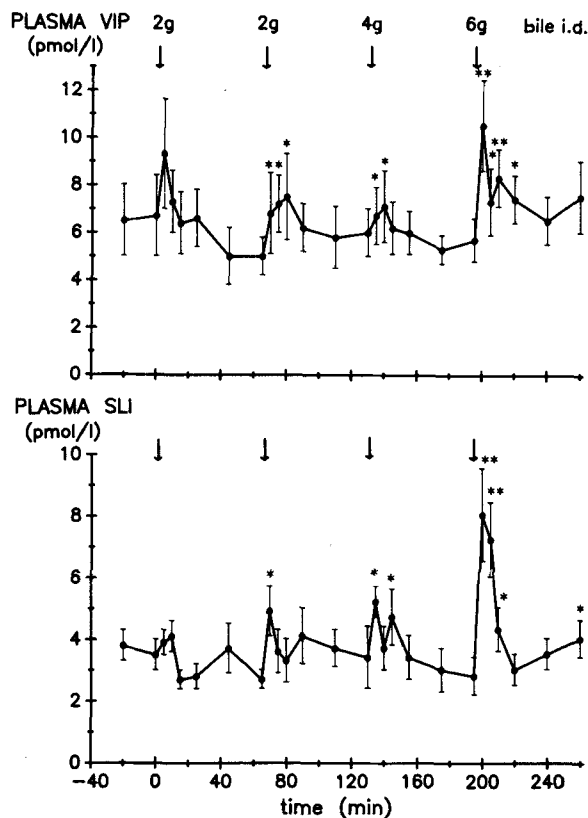

FIG. 1. Effect of 2, 2, 4, and 6 g of bile in the duodenum on the kinetics of plasma VIP (top) and SLI (bottom) concentrations (n = 10). A single asterisk indicates $p \leq 0.05$ and two asterisks $p \leq$ 0.01 compared with the basal value preceding the second i.d. stimulus (also in Figs. 2–5).

increase of SLI was observed as compared with 400 mg (Fig. 3, bottom).

Parallel to the peptide release, volume, bicarbonate, lipase, trypsin, and amylase secretion significantly increased after 4 and 6 g bile and after all doses of TDC (Fig. 4; Table 1). After 2 g bile the increase was significant only for lipase. Volume secretion and bicarbonate output showed a dose-dependent augmentation after both i.d. stimuli, whereas enzyme output did not further increase after the highest dose of bile and TDC, respectively (Fig. 5; Table 1). Comparing the hydrokinetic and ecbolic effect of bile and TDC, a tendency to higher values was found after bile, reaching significance, however, only for bicarbonate after the respective highest dose (Fig. 4; Table 1).

DISCUSSION

The present study shows, that i.d. application of bile or TDC enhances plasma VIP and SLI levels
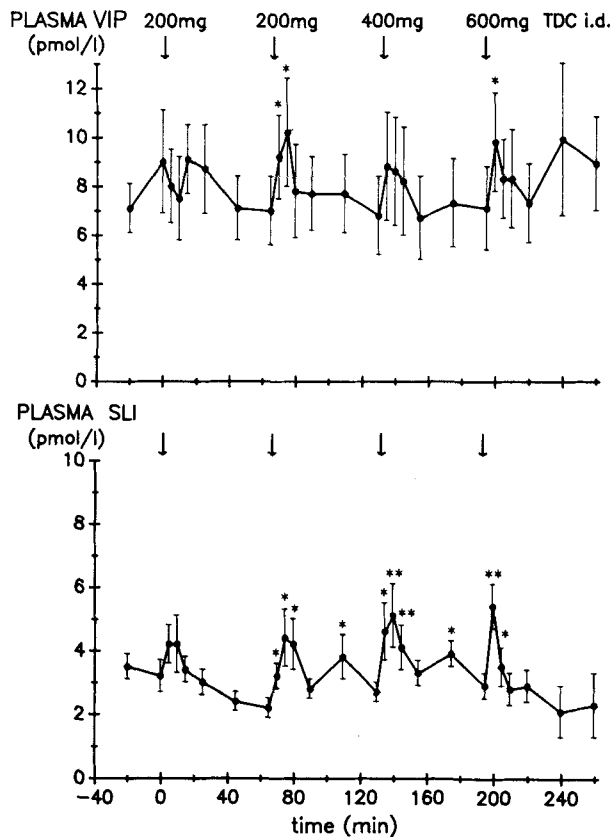
FIG. 2. Effect of 200, 200, 400, and 600 mg of TDC in the duodenum on the kinetics of plasma VIP (top) and SLI (bottom) concentrations (n = 10).

simultaneously with an increase of hydrokinetic and ecbolic pancreatic secretion.

The results are in accordance with our previous findings (1,2) that physiologic amounts of i.d. bile and TDC dose dependently stimulate volume, bicarbonate, lipase, trypsin, and amylase secretion in man (Figs. 4, 5; Table 1). The applied bile solution contained 10% TDC. In order to compare the effects of total bile and TDC, respectively, corresponding amounts of this bile salt were used. On this basis, TDC showed a slightly lower pancreatic response as compared with bile, reaching significance, however, only for bicarbonate after 600 mg TDC vs. 6 g bile. This observation is in agreement with another study showing that the effect of TDC can further be enhanced by combination with other bile salts (19).

The stimulatory effect of bile salts does not depend on their action as detergents (20) or their acidity (21). Moreover, the pH of the solutions applied was kept neutral or slightly alkaline to exclude stimulation of pancreatic secretion by acid equivalents.

Furthermore, the osmolality of the applied solutions cannot account for the stimulatory effect of bile and TDC, because i.d. infusion of a 20% solution of mannitol (1,097 mosmol/kg) in man caused only a slight enhancement of volume secretion but exerted no effect on bicarbonate and enzyme output (22). Others (23,24) found no or even an inhibitory effect on pancreatic secretion by raising the osmolality of i.d. applied solutions in dogs.

The method of repeated stimulus application was chosen, because intraindividual responses in experiments on different days vary considerably. Intervals of 65 min between stimulus applications were established to allow pancreatic secretion and peptide concentrations to return to prestimulatory values.

Previously, Burhol et al. (11) were able to demonstrate an increase of plasma VIP concentrations after i.d. application of a single dose of 6 g bile in man. Our data confirm this observation and show that 2 and 4 g i.d. bile also significantly enhance plasma VIP concentrations (Fig. 1). Furthermore, integrated plasma VIP was significantly higher after 6 g bile as compared with 2 and 4 g bile, probably
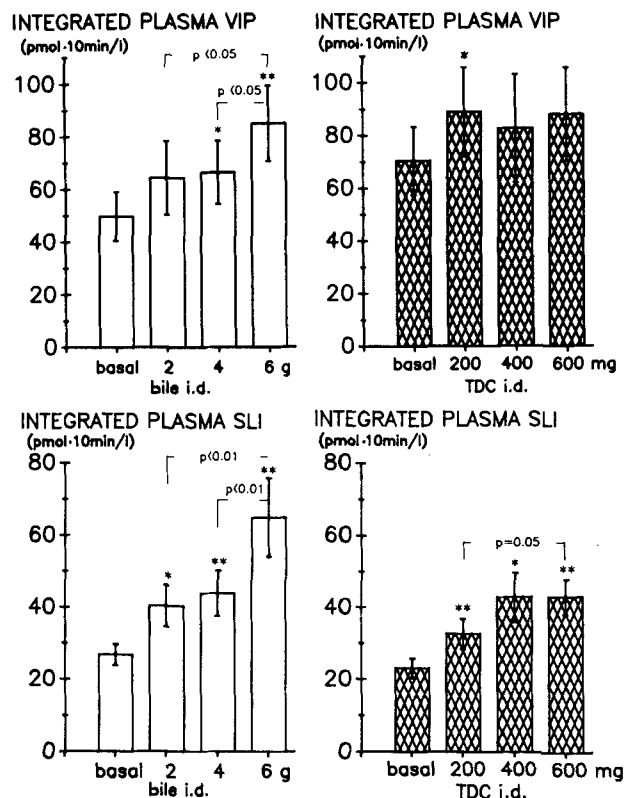

FIG. 3. Dose–response plot of i.d. bile (left) and TDC (right) on integrated plasma VIP (top) and SLI (bottom) (n = 10).
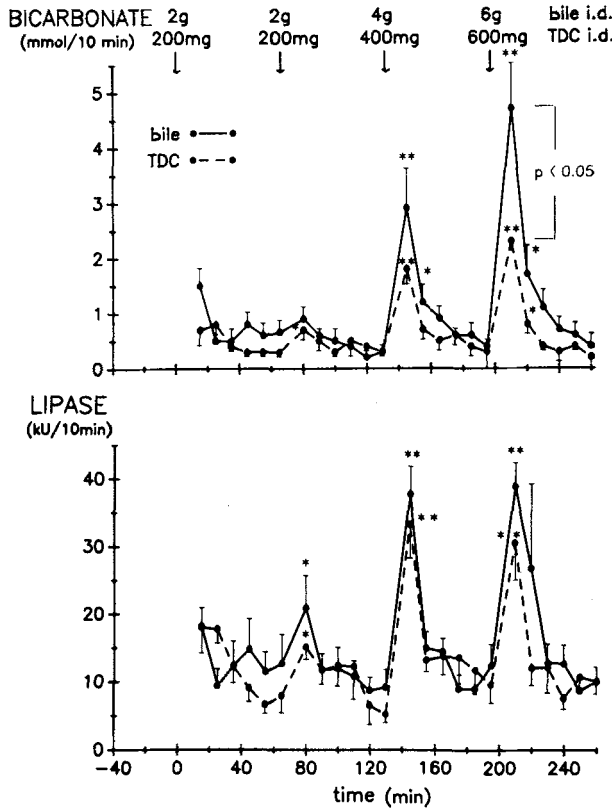
FIG. 4. Effect of 2, 2, 4, and 6 g of bile (●——●) and of 200, 200, 400, and 600 mg of TDC (●---●) on pancreatic bicarbonate (top) and lipase (bottom) secretion rate ($n = 10$). Lipase is representative for all enzymes measured.
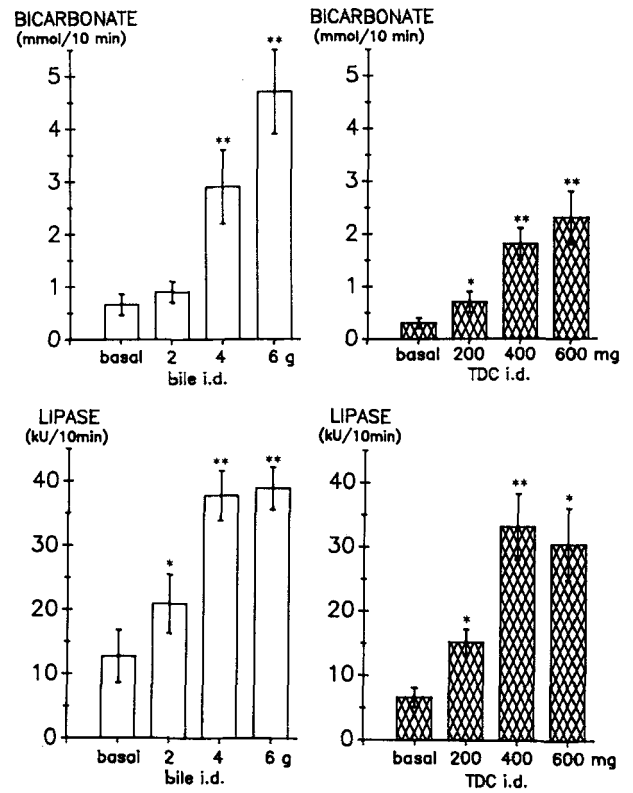

FIG. 5. Dose–response plot of i.d. bile (left) and TDC (right) on pancreatic bicarbonate (top) and lipase (bottom) output ($n = 10$).

indicating a dose–response relation (Fig. 3). An enhancement of VIP levels secondary to an acid release into the duodenum (25) was prevented by continuous aspiration of gastric juice and documented by the neutral pH of the duodenal aspirate. Furthermore, it is unlikely that the osmolality of the bile solutions is responsible for the VIP release, since Schaffalitzky de Muckadell et al. (25) found no in-

crease of peripheral VIP levels by i.d. perfusion with hypertonic glucose solutions in humans and hypertonic saline and glucose solutions in pigs. Thus, a nonspecific effect of bile may be ruled out. In contrast to the results in man, Chayvialle et al. (12) noted no change in systemic and only an insignificant rise in portal plasma VIP concentrations after i.d. application of 3 g bile in dogs. This discrepancy may be due to species differences and/or the different study design (26). In the latter experi-

TABLE 1. *Effect of i.d. bile and TDC on volume and bicarbonate secretion and on lipase, trypsin, and amylase output* (n = 10)[a]

	Bile (g)				TDC (mg)			
	Basal	2	4	6	Basal	200	400	600
Volume (ml/10 min)	18.2 ± 3.5	33.8 ± 5.5	63.4 ± 5.3**	83.2 ± 6.0**	10.3 ± 1.4	32.7 ± 4.7*	47.2 ± 6.3**	61 ± 9.3**
Bicarbonate (mmol/10 min)	0.6 ± 0.2	0.9 ± 0.2	2.9 ± 0.7**	4.7 ± 0.8**	0.3 ± 0.1	0.7 ± 0.2*	1.8 ± 0.3**	2.3 ± 0.5**
Lipase (kU/10 min)	12.7 ± 4.1	20.9 ± 4.6*	37.7 ± 3.9**	38.9 ± 3.3**	6.6 ± 1.5	15.2 ± 2.0*	33.2 ± 5.1**	30.5 ± 5.6**
Trypsin (kU/10 min)	0.6 ± 0.2	1.2 ± 0.2	2.6 ± 0.4**	2.3 ± 0.2**	0.2 ± 0.9	1.1 ± 0.3*	2.2 ± 0.4**	1.9 ± 0.4**
Amylase (kU/10 min)	4.2 ± 1.5	9.1 ± 2.6	12.2 ± 2.4**	12.2 ± 1.9**	2.8 ± 0.8	5.6 ± 1.1*	9.9 ± 2.3*	10.6 ± 2.5*

[a] A single asterisk indicates $p \leq 0.05$ and two asterisks $p \leq 0.01$ compared with the basal value.

ments, pancreatic juice was not withdrawn from the duodenum.

TDC, i.d. applied in a physiological dose range, significantly enhanced plasma VIP levels (Fig. 2). Integrated plasma VIP, however, increased significantly only after 200 mg of TDC and showed no further increase even at the highest dose of TDC used. As VIP was measured in peripheral, not in portal plasma, peaks of VIP release were only small and short lasting. Therefore, intraindividual variations might have contributed to the missing dose dependence.

VIP resembles secretin in its primary structure and its ability to stimulate pancreatic volume and bicarbonate secretion (27). VIP is a secretin-like full agonist in cats (27,28), but only a partial agonist in dogs (29), pigs (30), and man (31). The low potency of VIP [1–5% as effective as secretin (32)] has been used as an argument against an important physiological role of VIP as a hormonal stimulant of hydrokinetic pancreatic secretion. Because the existence of VIP-containing endocrine cells of the gut is questioned as well (33), a hormonal action of VIP on the exocrine pancreas is uncertain.

VIP is, however, now generally accepted as a peptidergic neurotransmitter (34,35). This regulatory peptide is localized in vagal nerve fiber endings and in a dense network of peptidergic nerves in the pancreas (36–38). Moreover, VIP-ergic nerves travel along pancreatic ducts capable of water and bicarbonate secretion (39). Electrical stimulation of the vagus nerve in the isolated perfused pig pancreas induces an atropine-resistent enhancement of pancreatic volume and bicarbonate secretion and a release of VIP into the portal effluent as well (39–41).

As VIP is known to be rapidly degraded (34) and extracted from portal blood by the liver (42), only a small fraction escapes to the peripheral venous blood. Consequently, the increase of VIP in peripheral plasma after i.d. bile or TDC apparently corresponds to higher VIP concentrations in the portal vein. Moreover, at the site of VIP release, i.e., the synaptic cleft between the VIP-ergic nerve terminals and the receptors in the pancreas, sufficiently high concentrations of VIP have to be assumed to be able to stimulate water and bicarbonate secretion (40). Therefore, an increase of VIP measured in peripheral plasma may be an expression of an "overspill" phenomenon of VIP from the synaptic cleft into the venous effluent.

It cannot be excluded, that the bile-induced in-

crease of plasma VIP partially originates from VIP-ergic neurons of the myenteric and submucous plexus of the duodenum or upper jejunum. Such a release, however, seems unlikely because very high amounts of bile (10.8 g) applied into the ileum of dogs did not influence peripheral plasma VIP levels (43).

Thus, neuronally released VIP may contribute to the mediation of pancreatic volume and bicarbonate secretion induced by i.d. bile or TDC, although VIP is considered merely as a partial agonist of secretin in man.

Ingestion of a meal evokes not only stimulatory but also inhibitory mechanisms (13), probably to prevent an "overstimulation" of the effector organs (e.g., pancreas) (16,44). Previously, we found a dose-dependent increase of plasma PP levels after i.d. application of bile or TDC (1). The present study shows, that somatostatin is also released (Figs. 1–3). There was observed not only a significant increase of plasma SLI concentrations, but also a dose-dependent augmentation of integrated SLI after bile and 200 and 400 mg of TDC. An acid-induced rise of SLI (17) can be excluded (see above). In contrast, Burhol et al. (17) did not find a significant change of plasma SLI after duodenal infusion of 3 g cattle bile in man. The reason for this discrepancy remains unclear. On the other hand, Chayvialle et al. (12) noted a significant increase of SLI in portal and peripheral plasma after i.d. application of 3 g bile in dogs. Gel chromatographic analysis of plasma SLI released by bile revealed that total SLI consists of approximately 60% somatostatin-28 and 40% somatostatin-14. The release of somatostatin-28 may primarily be due to a direct action of bile on the duodenal D cells, because somatostatin-28 is predominantly released from the proximal segment of the small intestine (45,46). Somatostatin-14 is believed to originate from the stomach and the pancreas (45,46). Because in our experiments a duodenogastric reflux was only rarely observed, a release of somatostatin-14 caused by a direct action of bile on gastric D cells seems unlikely. As SLI is also released by vagal stimulation (17,47), neural mechanisms may be responsible for the bile-induced release of somatostatin-14 from the pancreas. In this context, it is noteworthy that cholinergic mechanisms are activated by bile as indicated by bile-induced PP release (1) and by Forell (22). Gastroenteropancreatic peptides (e.g., secretin) are involved in the mediation of the bile effect on the exocrine pancreas (1). Therefore, additional

release of somatostatin secondary to these peptides (48) warrants discussion.

After the highest dose of bile, the increase of plasma SLI concentrations was comparable to that found postprandially (15,17). Moreover, postprandial plasma levels of SLI are capable of inhibiting exocrine pancreatic secretion (15), especially with respect to the ecbolic output (49). Therefore, the release of somatostatin may—together with that of PP (1)—be responsible for the lack of further increase of enzyme secretion after the highest doses of bile and TDC, respectively.

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