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Influence of Gastrointestinal Peptides on Bile Acid Transport by Isolated Rat Hepatocytes[1] (42617)

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Abstract. While numerous effects of gut peptides on gastric, pancreatic, and intestinal secretion have been described, there has been little investigation of the influence of these peptides on hepatic function. In the present studies, effects of vasoactive intestinal peptide (VIP), somatostatin, thyrotropin-releasing hormone (TRH), and bombesin on taurocholate transport by isolated rat hepatocytes have been examined. Somatostatin, TRH, and bombesin in incubation media produced no change from control incubations with regard to either uptake of taurocholate by hepatocytes or efflux of bile acid from preloaded cells. However, incubation of hepatocytes with VIP produced a significant decrease in taurocholate uptake (1.34 ± 0.13 versus 1.73 ± 0.16 nmole $\cdot$ min$^{-1} \cdot 10^6$ cells^{-1}, $P < 0.001$). Studies with verapamil, a calcium-channel blocking agent, and theophylline, an inhibitor of cAMP catabolism, failed to provide evidence for transmembrane Ca^{2+} flux or alteration in intracellular levels of cAMP, respectively, as mechanisms for the observed inhibition of hepatocyte taurocholate uptake by VIP. These data, coupled with both clinical and other basic observations, suggest that VIP may play a significant role in the regulation of hepatic bile secretion.

Gastrointestinal peptides such as somatostatin, vasoactive intestinal peptide (VIP), thyrotropin-releasing hormone (TRH), and bombesin have been shown to have significant effects on secretion by the stomach, pancreas, and small intestine (1–3). More recently, the possibility that these peptides might also alter hepatic bile secretion has begun to receive consideration. Ricci and Fevery found that a continuous intravenous infusion of somatostatin in rats produced a significant decrease in the bile salt independent fraction of hepatic bile secretion and noted that biliary bile acid output was decreased (4). Makhlouf *et al.* (5) demonstrated that intravenous bolus injections of VIP in dogs produced a twofold increase in biliary flow but did not assess changes in bile acid secretion. Two clinical reports suggest that VIP may effect bile secretion and bile acid transport in humans (6, 7). Low concentrations of bile acids were noted in gallbladder bile from two patients with watery diarrhea associated with non-β islet cell pancreatic tumors, and injection of tumor extracts from these two patients into dogs caused an increase in biliary secretion (6). Increased volume of gallbladder bile which had a low bile acid concentration was also noted in a later report of a similar patient with "pancreatic cholera" and high concentrations of VIP in both systemic blood and pancreatic tumor extract (7). Finally, bombesin has been found to increase the choleresis produced by intraduodenal acid infusion in dogs, but no data were given for the direct effects of bombesin alone on hepatic bile production and biliary bile acid output (8).

Although these investigations point to a role for some of the more recently identified gastrointestinal peptides in the regulation of hepatic bile secretion, there has been little study of possible mechanisms by which these peptides might produce their choleretic/anticholeretic effects. Results from several of the aforementioned studies suggest that the changes in bile flow may involve alterations in hepatic bile acid transport. Therefore we examined the effects of somatostatin, VIP, TRH, and bombesin on transport of taurocholate by isolated rat hepatocytes.

Materials and Methods. Somatostatin, vasoactive intestinal peptide, thyrotropin-releasing hormone, and bombesin were purchased from Peninsula Laboratories, Inc.

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(Belmont, CA). Sodium taurocholate was obtained from Calbiochem (La Jolla, CA). [^{3}H]taurocholate (sp act 6.6 Ci/mmole) and [*carboxyl*-^{14}C]inulin (sp act 2.5 mCi/g) were purchased from New England Nuclear (Boston, MA). Bovine serum albumin (fraction V), EGTA, β-D(+) glucose, and theophylline were obtained from Sigma Chemical Co. (St. Louis, MO). Verapamil was purchased from Knoll Pharmaceutical Company (Whippany, NJ).

Non-fasted male Sprague–Dawley rats (BioLab, Inc., St. Paul, MN) were used in all experiments. Isolated hepatocytes were prepared according to a modification of the method of Seglen (9). After cannulation of the portal vein, the liver was initially perfused with 200 ml of nonrecirculating modified calcium-free Hanks' buffer containing 1.0 g/dl bovine serum albumin and 5 mmole/liter EGTA continuously infused with 95% O_2–5% CO_2. Subsequently 100 ml of magnesium-free modified Hanks' buffer containing 0.05% collagenase (Millipore Corp., Freehold, NJ) and 5 mmole/liter calcium was recirculated through the liver for 15 min. The liver was then minced gently with forceps, and noncellular debris was removed by passage of cells through 100 μ*M* nylon mesh; cells were washed three times with Hanks' buffer containing 1.0 g/dl albumin and resedimented by centrifugation for 2 min at 200*g*. Hepatocyte viability was routinely assessed by trypan blue exclusion; mean viability for study cell preparations was 96 ± 3%. Hepatocyte O_2 consumption was measured in selected experiments using a Chemical Microsensor Model 1201 (Transidyne General Corp., Ann Arbor, MI).

Somatostatin, TRH, and VIP were dissolved in Hanks' buffer, pH 7.4, containing 1.0 g/dl albumin while bombesin was dissolved in 1% acetic acid. For uptake studies, hepatocytes (3×10^6 viable cells/ml) in Hanks' buffer were preincubated in glass counting vials for 10 min under air at 37°C in a shaking water bath; study hormones or other study drugs were added to hepatocytes at the beginning of the incubation period. Tritiated taurocholate (2 mg/ml; sp act 400–800 dpm/nmole) was then added to the incubation mixture (total vol 2 ml); cells (150-μl samples) were placed in 500 μl polyethylene centrifuge tubes and separated from incubation media by centrifugation (7000*g* for 5 sec) through 200 μl of silicone oil (d, 1.05; Aldrich Chemical Co., Milwaukee, WI) into 3 *N* KOH (50 μl). Radioactivity in cell pellets and supernatant was determined by liquid scintillation counting; correction for adherent incubation medium was made by separate determinations of [*carboxyl*-^{14}C]-inulin in cell pellets. Initial studies characterizing taurocholate transport in this model revealed that uptake was linear for 60 sec; a K_m of 38.9 ± 5.8 μ*M* was obtained (n = 6). Subsequent uptake studies were performed at an incubation taurocholate concentration of 186 μ*M* and samples were obtained at 15, 30, 45, and 60 sec following addition of bile acid. Efflux from hepatocytes was determined by incubation of 4 ml of liver cells (3×10^6/ml) in buffer containing 186 μ*M* [^{3}H]-taurocholate for 3 min at 37°C. Cells were then washed twice with fresh buffer (temperature 4°C), centrifuged for 30 sec at 200*g*, and resuspended in 2 ml of fresh buffer at 37°C. Hormones or other study drugs were added to the cell pellet immediately prior to resuspension. Efflux of radioactivity from cells into the fresh buffer was monitored in samples of supernatant at 15, 30, 45, 60, and 90 sec and was linear over this time period; no [^{3}H]taurocholate efflux from hepatocytes occurred when cell incubations were kept at 4°C.

[^{3}H]Taurocholate uptake was calculated from the slope of the linear regression line obtained by plotting amount of radionuclide taken up into cells (y axis) versus time (x axis). Fractional efflux constants were determined from linear regression lines of radioactivity accumulating in supernatant versus time (10, 11). Significance of differences in uptake and efflux between various experimental groups and concomitantly run control groups were examined using Student's paired t test (12).

Results. Effects of the various study gut peptides on taurocholate uptake by isolated rat hepatocytes are shown in Fig. 1 and Table I. No significant difference in taurocholate uptake was seen between hepatocytes preincubated with somatostatin, bombesin, or TRH and concomitantly studied control cells. However, a significant decrease in

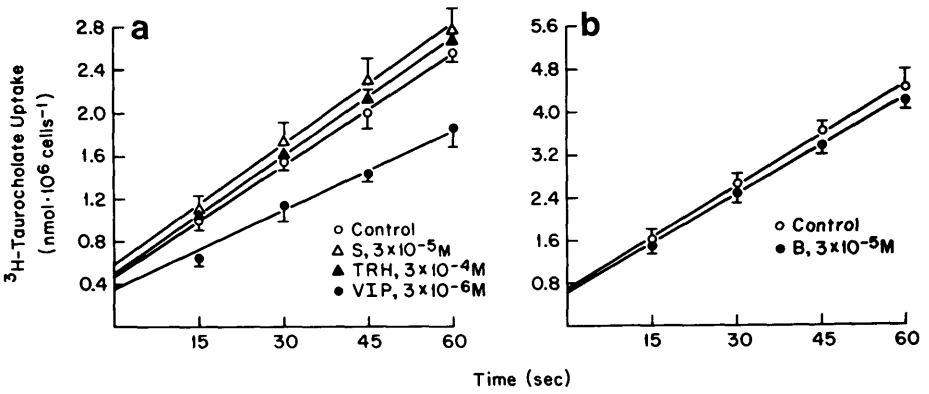

FIG. 1. Uptake of [^{3}H]taurocholate by isolated rat hepatocytes in the presence of gastrointestinal peptides. S, somatostatin; TRH, thyrotropin-releasing hormone; VIP, vasoactive intestinal peptide; B, bombesin. All data were obtained concomitantly using cells from a single liver preparation. Each point represents the mean value ± SEM at that time point for three separate cell incubations. Only the regular incubation buffer used for suspending VIP, TRH, and S was added to control cells (a). A comparable volume of 1% acetic acid, as was used for suspension of bombesin, was added to control incubations for studies with this hormone (b). Taurocholate uptake values calculated from the composite linear regression lines were S, 2.23; TRH, 2.29; VIP, 1.42 versus the control value of 2.16 nmol · min^{-1} · 10^6 cells^{-1}; for B, uptake was 3.46 versus a concomitant control rate of 3.71 nmol · min^{-1} · 10^6 cells^{-1}.

[^{3}H]taurocholate uptake was seen when hepatocytes were preincubated with VIP, 3 × 10^{-6} *M*. A dose–response curve showing changes in taurocholate uptake by hepatocytes incubated with concentrations of VIP ranging from 3 × 10^{-10} to 1.5 × 10^{-5} *M* is shown in Fig. 2. All concentrations of VIP studied, including the lowest concentration of 3 × 10^{-10} *M*, caused a significant inhibition of [^{3}H]taurocholate uptake as compared to concomitantly run control experiments. Magnitude of reduction in taurocholate uptake roughly paralleled VIP concentration in incubation media.

The influence of VIP, somatostatin, TRH, and bombesin on efflux of [^{3}H]taurocholate from rat hepatocytes is shown in Table II. As opposed to its effect on bile acid uptake, VIP did not produce any significant change in bile acid movement out of hepatocytes. Addition of bombesin, TRH or somatostatin to fresh incubation buffer containing hepato-

TABLE I. EFFECTS OF GASTROINTESTINAL HORMONES ON TAUROCHOLATE UPTAKE BY ISOLATED RAT HEPATOCYTES

Hormone[a]	[^{3}H]Taurocholate uptake (nmol · min^{-1} · 10^6 cells^{-1})[b]	Statistical significance[c]
Somatostatin, 3 × 10^{-5} *M* (*n* = 17)	1.44 ± 0.18 (1.40 ± 0.12)	NS
VIP, 3 × 10^{-6} *M* (*n* = 17)	1.34 ± 0.13 (1.73 ± 0.16)	0.001
Bombesin, 3 × 10^{-5} *M* (*n* = 12)	2.64 ± 0.23 (2.55 ± 0.24)	NS
TRH, 3 × 10^{-4} *M* (*n* = 14)	1.54 ± 0.18 (1.48 ± 0.15)	NS

[a] Study hormone concentrations examined were as follows: somatostatin 3 × 10^{-7}–3 × 10^{-5} *M*; VIP 3 × 10^{-10}–1.5 × 10^{-5} *M*; bombesin 3 × 10^{-7}–3 × 10^{-5} *M*; TRH 3 × 10^{-6}–3 × 10^{-4} *M*; except in the case of VIP, data are for the highest hormone concentration studied.

[b] Mean values ± SEM for experimental cells with values for concomitantly run control cells in parentheses.

[c] NS, experimental uptake not significantly different from control.

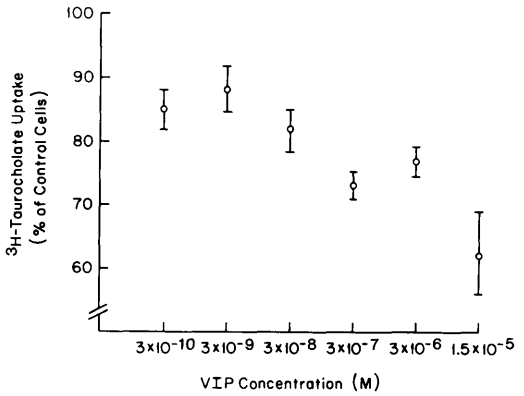

FIG. 2. Effect of varying concentrations of VIP on [^{3}H]taurocholate uptake by isolated rat hepatocytes. Each point represents the uptake seen for 5–18 separate determinations in cells incubated with VIP expressed as the mean percentage ± SEM of concomitantly studied control cells. Mean values for all experimental groups were significantly reduced as compared to controls at the $P < 0.02$ level or greater.

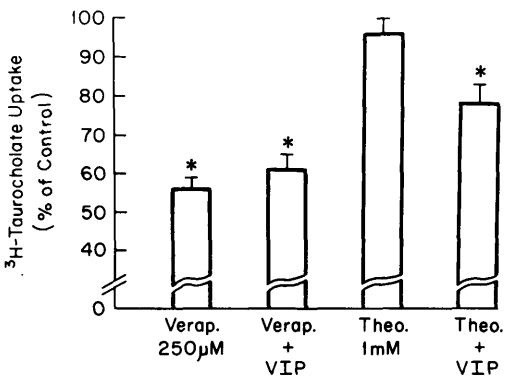

FIG. 3. Effects of verapamil and theophylline, alone and in combination with VIP, on [^{3}H]taurocholate uptake by isolated hepatocytes. VIP concentration was 3×10^{-6} *M*. Each bar shows experimental uptake expressed as mean percentage ± SEM of uptake by concomitantly studied control cells ($n = 15$ and 9 for the verapamil and theophylline groups, respectively). *Values significantly different from controls at the $P < 0.05$ or greater level.

cytes preloaded with [^{3}H]taurocholate also produced no significant effect on bile acid efflux from cells.

Many actions of gastrointestinal peptides on other target organs of the alimentary tract are postulated to involve changes in transmembrane Ca^{2+} flux or intracellular levels of adenosine 3′, 5′-cyclic monophosphate (13, 14). Therefore the effects of verapamil, a calcium channel-blocking agent, and theophylline, a phosphodiesterase inhibitor, on VIP-induced changes in bile acid uptake were assessed (Fig. 3). Incubation of hepatocytes with verapamil, 250 μ*M*, produced a marked decrease in [^{3}H]taurocholate uptake when compared to uptake by control cells (1.26 ± 0.09 versus 2.21 ± 0.07 nmole · min^{-1} · 10^6 cells^{-1}, respectively; $n = 15$, $P < 0.001$); concomitant incubation of cells with both verapamil and VIP, 3×10^{-6} *M*, had no additional inhibitory effect on bile acid uptake (1.35 ± 0.09 nmole · min^{-1} · 10^{-6} cells^{-1}). No sig-

TABLE II. INFLUENCE OF GASTROINTESTINAL HORMONES ON EFFLUX OF [^{3}H]TAUROCHOLATE FROM ISOLATED RAT HEPATOCYTES

Hormone[a]	Fractional efflux of [^{3}H]taurocholate (min^{-1})[b]	Statistical significance[c]
Somatostatin, 6×10^{-6} *M* ($n = 11$)	0.172 ± 0.010 (0.179 ± 0.017)	NS
VIP, 3×10^{-6} *M* ($n = 10$)	0.230 ± 0.013 (0.235 ± 0.012)	NS
Bombesin, 1.5×10^{-5} *M* ($n = 12$)	0.195 ± 0.013 (0.186 ± 0.010)	NS
TRH, 3×10^{-5} *M* ($n = 8$)	0.158 ± 0.016 (0.151 ± 0.012)	NS

[a] Hormone concentrations selected for efflux studies approximated the highest hormone concentration examined in uptake experiments.

[b] Mean values ± SEM for experimental cells with values for concomitantly run control cell incubations in parentheses.

[c] NS, experimental efflux not significantly different from controls.

TABLE III. EFFECTS OF VERAPAMIL AND THEOPHYLLINE ON BILE ACID EFFLUX FROM ISOLATED RAT HEPATOCYTES

Treatment	Fractional efflux of [^{3}H]taurocholate[a] (min^{-1})	Statistical significance[b]
Controls (n = 7)	0.197 ± 0.011	
Verapamil, 250 μM (n = 8)	0.140 ± 0.009	<0.005
Theophylline, 1 mM (n = 6)	0.207 ± 0.021	NS

[a] Means ± SEM.
[b] NS, not significantly different from controls.

nificant change in [^{3}H]taurocholate uptake by isolated hepatocytes was seen when cells were incubated with 1 m*M* theophylline, and the decrease in bile acid uptake seen in cell preparations incubated concomitantly with both theophylline and VIP closely approximated that seen when VIP alone was added to incubation buffer (Fig. 3). Similar to their effects on bile acid uptake, verapamil caused a decreased rate of efflux of [^{3}H]taurocholate from liver cells preloaded with bile acid while addition of theophylline produced no change from controls in efflux rate (Table III).

Discussion. Somatostatin, VIP, and bombesin have all been previously reported to affect *in vivo* rates of bile flow in various animal species (4, 5, 8). Results of the present study suggest that alterations in bile flow associated with somatostatin and bombesin administration are not due to changes in the bile acid dependent fraction of bile flow. Such data complement a number of previous observations. Both Hanks *et al.* (15) and Rene *et al.* (16) found that infusions of somatostatin produced decreased bile flow in dogs, but neither group demonstrated any significant change in bile acid output. Kaminski and Deshpande found no change in bile acid output when bombesin was administered in conjunction with intraduodenal hydrochloric acid infusion and concluded that the increased rate of bile flow seen in response to bombesin infusion was likely due to bombesin-enhanced release of secretin rather than to direct effects of bombesin on hepatic or ductular bile flow (8). Data from these studies do not agree with the findings of Ricci and Fevery in the isolated perfused rat liver (4). These investigators found that somatostatin infusion into the perfusate resulted in decreased bile acid output; they did not perform studies to determine whether this decrease was due to impaired bile acid uptake or reduced bile acid secretion. We could not demonstrate any significant effects of somatostatin on either bile acid uptake or bile acid efflux, and differences in the study model, amount of hormone administered, or actual somatostatin preparation may be responsible for this apparent discrepancy. However, most previous investigations and the results reported here support a conclusion that neither somatostatin nor bombesin has an important physiological or pharmacological effect on bile acid transport.

The same cannot be said for VIP which produced a significant reduction in [^{3}H]-taurocholate uptake by isolated hepatocytes at media concentrations as low as 3×10^{-10} *M*. Plasma VIP levels of approximately 1.8×10^{-11} *M* have been found in normal human subjects while a concentration of 3×10^{-10} *M* was measured in plasma of a patient with pancreatic cholera (7). Thus it appears that the lower concentrations of VIP which produced *in vitro* inhibition of hepatocyte bile acid uptake are not far removed from *in vivo* concentrations actually seen in humans. Recent studies by Gewirtz *et al.* (17) suggest that sympathetic neurotransmitters such as norepinephrine and epinephrine play a role in the release of bile acids from liver cells. Since VIP is also present in intrinsic neurons of the gastrointestinal tract (18), even higher concentrations of neural VIP may be available for modulation of bile production. Gallbladder bile volume was very high in the patient with VIP-secreting pancreatic tumor reported by Rambaud (7) which supports the possibility that VIP stimulates bile flow in humans; however, bile acid concentration in the gallbladder bile of the patient was extremely low, which is consistent with an inhibitory effect of VIP on bile acid transport such as was seen in our isolated rat hepatocyte studies. These observations suggest that, if VIP does have a physiological role in regulation of bile production, its effects may be multifactorial.

The mechanism(s) by which VIP exerts its inhibitory effect on hepatocyte bile acid uptake is not clear. A nonspecific detrimental effect of VIP on hepatocyte membrane integrity seems unlikely. Percentage viability of hepatocytes exposed to VIP, 3×10^{-6} *M*, was $104 \pm 4\%$ ($n = 8$) of that for similarly incubated control cells as measured by trypan blue exclusion, and determinations of pellet protein in VIP experiments closely approximated those found in controls (0.499 ± 0.015 versus 0.467 ± 0.099 mg · 0.45×10^6 cells^{-1}, respectively; $n = 6$). Finally, O_2 consumption by control cells and hepatocytes exposed to VIP was very similar (20.5 ± 1.9 versus 21.0 ± 0.4 nmol · min^{-1} · 10^6 cells^{-1}, respectively; $n = 6$).

A number of GI hormones are thought to mediate their effects through changes in intracellular calcium levels (13), and change in calcium concentration is another parameter which has been reported to affect hepatic bile production and bile acid transport. Reichen *et al.* (19) found that taurocholate excretion by the perfused rat liver was markedly inhibited by the elimination of calcium from the perfusate. When studies were attempted to assess the effects of calcium omission on VIP-induced depression of bile acid uptake in our laboratory, technical problems were encountered. Elimination of calcium from the incubation buffer resulted in a marked reduction in viability of cells incubated in the calcium-free buffer ($27 \pm 2\%$ of that seen for cells similarly incubated in the standard buffer containing calcium, $n = 4$); protein in the cell pellets after centrifugation of cells through silicone oil was also significantly decreased in calcium-free experiments (0.076 ± 0.016 versus 0.548 ± 0.049 mg · 0.45×10^{-6} cells^{-1}, $n = 3$). Since total elimination of calcium from the incubation buffer produced such detrimental effects on hepatocytes, we elected to assess the relationship of calcium to VIP-induced changes in bile acid transport by utilizing the calcium transport blocker, verapamil. The failure to demonstrate an additive effective of verapamil on VIP-induced inhibition of bile acid uptake and the suppression of bile acid efflux by verapamil in contrast to the lack of effect of VIP on this parameter suggest that, while both agents influence hepatocyte bile acid transport, they do so by different mechanisms. Similarly, the failure of theophylline to elicit an effect on hepatocyte bile acid uptake is evidence against a role for cAMP as a mediator of VIP-induced alterations in this aspect of hepatic function.

Edmondson *et al.* (20) recently demonstrated that glucagon produced hyperpolarization of hepatocyte membranes and that this increased membrane potential was accompanied by an increase in taurocholate uptake by isolated rat hepatocytes. VIP has a chemical structure closely related to that of glucagon (21), and it is possible that VIP might act as either an agonist or an antagonist with regard to the membrane parameter(s) upon which glucagon acts to produce increased membrane potential. Examples of both similar and different effects for VIP and glucagon on various body tissues have been reported. While both peptides increase adenylate cyclase activity in liver and fat cell membranes, the binding affinity and maximal effect of the two hormones is markedly different, suggesting that distinct receptors are present for each hormone (21). In rabbit ileal mucosal, VIP produced a fivefold increase in mucosal cAMP level while no change in cAMP content was seen in response to glucagon (14). It is possible that VIP and glucagon may have opposite effects on uptake of bile acids by isolated hepatocytes despite their structural similarities reminiscent of the opposing effects of gastrin and CCK on lower esophageal sphincter pressure (22). Very preliminary results in our laboratory using thiocyanate equilibration (20, 23) do suggest an effect of VIP on membrane potential (R. G. Knodell, unpublished data); however, they also indicate that media taurocholate concentration affects hepatocyte membrane potential and that interaction(s) between bile acids and VIP with regard to their effects on membrane potential are complex and will require detailed study to define.

Until recently, the liver has received little attention as a possible important target organ for gastrointestinal hormones (24). However, identification and characterization of a liver VIP receptor (25) and demonstration of hepatic extraction and degradation of VIP, somatostatin, and CCK (26–28) all imply that

significant *in vivo* gut peptide–liver interactions occur. The inhibitory effect of VIP on bile acid uptake by isolated rat hepatocytes demonstrated in this study suggests that VIP-hepatic bile secretion may represent one such interaction.

1. Pearse AGE, Polak JM, Bloom SR. The new gut hormones. Gastroenterology **72**:746–761, 1977.
2. Mitchenere P, Adrian TE, Kirk RM, Bloom SR. Effect of gut regulatory peptides on intestinal luminal fluid in the rat. Life Sci **29**:1563–1570, 1981.
3. Gullo L, Labo G. Thyrotropin-releasing hormone inhibits pancreatic enzyme secretion in humans. Gastroenterology **80**:735–739, 1981.
4. Ricci GL, Fevery J. Cholestatic action of somatostatin in the rat: Effect on the different fractions of bile secretion. Gastroenterology **81**:552–562, 1981.
5. Makhlouf GM, Said SI, Yau WM. Interplay of vasoactive intestinal peptide (VIP) and synthetic VIP fragments with secretin and octapeptide of cholecystokinin (octa-CCK) on pancreatic and biliary secretion. Gastroenterology **66**:737, 1974. [Abstract]
6. Tompkins RK, Kraft AR, Zollinger RM. Secretin-like choleresis produced by a diarrhenogenic non-beta islet cell tumor of the pancreas. Surgery **66**:131–137, 1969.
7. Rambaud J-C, Modigliani R, Matuchansky C, Bloom S, Said S, Pessayre D, Bernier J-J. Pancreatic cholera: Studies on tumoral secretions and pathophysiology of diarrhea. Gastroenterology **69**: 110–122, 1975.
8. Kaminski DL, Deshpande YG. Effect of somatostatin and bombesin on secretin-stimulated ductular bile flow in dogs. Gastroenterology **85**:1239–1247, 1983.
9. Seglen PO. Preparation of rat liver cells: Effect of Ca^{++} on enzymatic dispersion of isolated, perfused liver. Exp Cell Res **74**:450–454, 1972.
10. Schwarz LR, Burr R, Schwenk M, Pfaff E, Greim H. Uptake of taurocholic acid into isolated rat liver cells. Eur J Biochem **55**:617–623, 1975.
11. Schwarz LR, Schwenk M, Pfaff E, Greim H. Excretion of taurocholate from isolated hepatocytes. Eur J Biochem **71**:369–373, 1976.
12. Snedecor GW, Cochran WG. Statistical Methods. Ames, Iowa State Univ. Press 7th ed., 1980.
13. Schultz I. Messenger role of calcium in function of pancreatic acinar cells. Amer J Physiol **239**:G335–G347, 1980.
14. Schwartz CJ, Kimberg DV, Sherrin HE, Field M, Said SI. Vasoactive intestinal peptide stimulation of adenylate cyclase and active electrolyte secretion in intestinal mucosa. J Clin Invest **54**:536–544, 1974.
15. Hanks JB, Kortz WJ, Anderson DK, Jones RS. Somatostatin suppression of canine fasting bile secretion. Gastroenterology **84**:130–137, 1983.
16. Rene E, Danzinger RG, Hofmann AF, Nakagaki M. Pharmacologic effect of somatostatin on bile formation in the dog. Gastroenterology **84**:120–129, 1983.
17. Gewirtz DA, Randolph JK, Goldman ID. Induction of taurocholate release from isolated rat hepatocytes in suspension by α-adrenergic agents and vasopressin: Implications for control of bile salt secretion. Hepatology **4**:205–212, 1984.
18. Saffouri B, DuVal JW, Arimura A, Makhlouf GM. Effects of vasoactive intestinal peptide and secretin on gastrin and somatostatin secretion in the perfused rat stomach. Gastroenterology **86**:839–842, 1984.
19. Reichen J, Berr F, Le M. Calcium deprivation increases biliary permeability and leads to failure to translocate bile acids in the perfused rat liver. Hepatology **3**:834, 1983. [Abstract]
20. Edmondson JW, Miller BA, Lumeng L. Effect of glucagon on hepatic taurocholate uptake: Relationship to membrane potential. Amer J Physiol **249**:G427–G433, 1985.
21. Desbuquois B, Laudat MH, Laudat P. Vasoactive intestinal polypeptide and glucagon: Stimulation of adenylate cyclase activity via distinct receptors in liver and fat cell membranes. Biochem Biophys Res Commun **53**:1187–1194, 1973.
22. Cohen S, Harris LD. The lower esophageal sphincter. Gastroenterology **63**:1066–1073, 1972.
23. Hoek JB, Nicholls DG, Williamson JR. Determination of the mitochondrial protonmotive force in isolated hepatocytes. J Biol Chem **255**:1458–1464, 1980.
24. Grossman MI. Neural and hormonal regulation of gastrointestinal function: An overview. Annu Rev Physiol **41**:27–33, 1979.
25. Nguyen TD, Williams JA, Gray GM. Liver vasoactive intestinal peptide (VIP) receptor is a membrane glycoprotein. Gastroenterology **88**:1518, 1985 [Abstract]
26. Misbin RI, Wolfe MM, Morris P, Buynitzky SJ, McGuigan JE. Uptake of vasoactive intestinal peptide by rat liver. Amer J Physiol **243**:G103–G111, 1982.
27. Berelowitz M, Kronheim S, Pimstone B, Shapiro B. Somatostatin-like immunoreactivity in rat blood: Characterization, regional differences and responses to oral and intravenous glucose. J Clin Invest **61**:1410–1414, 1978.
28. Doyle JW, Wolfe MM, McGuigan JE. Hepatic clearance of gastrin and cholecystokinin peptides. Gastroenterology **87**:60–68, 1984.

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