Endogenous neurotensin facilitates enterohepatic bile acid circulation by enhancing intestinal uptake in rats

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Gui, Xianyong, Paul R. Dobner, and Robert E. Carraway. Endogenous neurotensin facilitates enterohepatic bile acid circulation by enhancing intestinal uptake in rats. Am J Physiol Gastrointest Liver Physiol 281: G1413–G1422, 2001.—Initial studies on the digestive hormone neurotensin (NT) showing that intestinal NT mRNA expression and blood levels were altered in rats fed chow containing bile acid (BA) and the BA chelator cholestyramine led us to investigate the role of NT in the enterohepatic circulation of BA. In fasted, anesthetized rats with common bile ducts cannulated for bile collection, intravenous NT infusion (10 pmol·kg−1·min−1) enhanced BA output relative to control over 3 h in animals administered donor bile into the duodenum (30 μl/min). This suggested that the effect of NT was on the return of BA from the intestine to the liver, which is rate determining in the normal process. In rats prepared as described above and administered [3H]taurocholate ([3H]TC; 5 mM, 1 ml) duodenally, NT infusion (3–10 pmol·kg−1·min−1) increased the [3H]TC recovery rate in bile approximately twofold, whereas sulfated CCK-8 (12–50 pmol·kg−1·min−1) had no effect. To investigate the roles of endogenous NT and CCK, we administered [3H]TC into the rat duodenum or lower jejunum and tested the effect of the NT antagonist SR-48692 (2 nmol·kg−1·min−1) or CCK-A antagonist loglumide (100 nmol·kg−1·min−1). SR-48692 reduced the [3H]TC recovery rate by ≥50% and ≥24% in the duodenum and jejunum, respectively, whereas loglumide had no effect. These results suggest that NT or a similar peptide is an endogenous regulator of enterohepatic BA cycling, which acts by enhancing BA uptake in the intestine.

cholecystokinin; intestinal absorption

THE ENTEROHEPATIC CIRCULATION (EHC) of bile acids (BA), an efficient and dynamic process of hepatic BA excretion and intestinal BA uptake, plays central roles in the digestion and absorption of lipids and in cholesterol metabolism (32, 33, 51). In animals and humans, the EHC recycles conjugated BA 5–15 times per day, depending on the fat content of the meal (32). Thus it seems reasonable to propose that some intestinal factors, released during fat ingestion, are important regulators of this complex process. Given that the long-term mechanisms are already defined through which BA directly regulates its synthesis in liver and uptake in intestine by feedback effects on gene expression via the farnesoid nuclear receptor (42), we have focused our attention on short-term regulation (i.e., that occurring during a single meal). In regard to short-term effects, it is already well established that a number of enteric peptides contribute to BA cycling by acutely enhancing the delivery of bile constituents in response to a fat stimulus (32, 51). For example, CCK, by promoting gallbladder contraction, initiates BA delivery to the intestine (52), and secretin, by stimulating ductal secretion of bicarbonate and water, increases bile flow (39). Although numerous studies have examined the effects of these and other hormones on bile and BA delivery (48), there is a paucity of work on the short-term regulation of intestinal BA uptake (32). Given that the rate-determining or regulatory step in BA cycling is intestinal uptake (32) and that the intestinal BA uptake rate increases with eating and declines with fasting (12, 35, 38, 44), the mechanisms involved in regulating BA return from intestine to liver appear to warrant further investigation.

Neurotensin (NT), a tridecapeptide existing predominantly in the small intestine, is closely associated with fat intake and utilization. Ingestion of fat is the strongest stimulus for NT release in animals and humans (15, 16, 19, 27, 43, 47, 49), and circulating NT stimulates pancreatic secretion (7, 18, 34), slows intestinal transit (29), and promotes intestinal absorption of fatty acid (5). However, the possible effects of NT on BA cycling have not received much attention, except for the stimulatory action of NT on gallbladder contraction in some species (20, 26, 56). Because many of the effects of NT in the gastrointestinal tract complement those of CCK (14), we hypothesized that NT might stimulate the EHC by enhancing intestinal BA uptake, and we (24) recently demonstrated a stimulatory effect of exogenously infused NT on the absorption of [3H]taurocholate ([3H]TC) from the intestinal lumen of biliary fistula rats.

In this study, we extend our (24) previous work by comparing the effects of NT and sulfated CCK-8 (sCCK-8) on hepatic BA output and intestinal [3H]TC uptake in biliary fistula rats. In addition, we investi-
gate the role(s) of the endogenous hormones by examining the effects of the NT antagonist SR-48692 (25) and the CCK-A antagonist lorglumide (33).

MATERIALS AND METHODS

Materials

NT, sCCK-8, and TC (sodium taurocholate) were from Sigma (St. Louis, MO), and [3H(G)]TC was from New England Nuclear (Boston, MA). Sanofi Recherche (Montpellier, France) supplied the NT antagonist SR-48692. Stocks of NT (1 mM), sCCK-8 (1 mM), and SR-48692 (1 mM, DMSO) were at −20°C, whereas diluted solutions were prepared daily in 37°C sterile 0.85% NaCl, 0.1% BSA. Solutions of BA were prepared daily in 37°C sterile PBS (138 mM NaCl, 3 mM KCl, 6 mM Na2HPO4, and 6 mM KH2PO4, pH 7.0).

Animals and Surgery

Male Sprague-Dawley rats (225–300 g, Taconic Farms, Germantown, NY) were housed in the University of Massachusetts Medical School Animal Facility and given rat chow and water ad libitum. All protocols were approved by the institutional animal care and use committee. Rats used for bile flow study were fasted and anesthetized with ketamine-xylazine (80:10, mg/kg ip; 20:2, mg·kg−1·h−1). The right jugular vein was catheterized, and the bile duct was cannulated using PE-10 tubing. For infusion of donor bile and for delivery of [3H]TC to intestine, PE-10 tubing was superglued into the duodenum and jejunum. Bile flow was stabilized for 20 min before study, and animals with abnormally low flow rates (<100 μl/10 min) were not used in the study.

Infusion of NT

The doses of NT (3–10 pmol·kg−1·min−1) were chosen to mimic plasma NT levels observed in animals and humans after ingestion of fatty foods (15, 16, 19, 27, 43, 47, 49). In our (24) prior study, we established that during infusion of 10 pmol·kg−1·min−1 NT via a cannulated jugular vein, hepatic-portal plasma levels of NT rose from ≈25 pmol to plateau at ≈58 pmol within 20 min. Thus NT infusion began 20 min before bile collection or injection of [3H]TC.

Protocols

Feeding experiments. Adult rats were fasted for 48 h and given 5 g of powdered rat chow (Purina), some of which contained 5% (wt/wt) cholestyramine or 2% (wt/wt) TC and 0.5% (wt/wt) sodium cholate. After 4 h, when all food was consumed, rats were anesthetized, hepatic portal blood was collected into EDTA, and plasma was isolated and stored at −20°C. Plasma was extracted with acid-acetone before RIA for NT using antisera HCS8, which displayed a coefficient of variation of 7.7% (8, 9). To complement this short-term feeding experiment, a second study was performed in which rats were given the same formulated chow (15 g/day) for 5 days. In a third study, fasted rats were given the formulated foods overnight, and tissues were harvested 16 h later for measurement of NT mRNA in 20-cm segments of small intestine. Total RNA was prepared using the guanidinium isothiocyanate extraction method, and NT mRNA was quantitated as described previously (6, 54). Briefly, RNA (20 μg) from either the proximal or distal small intestine of individual rats was mixed with rat 32P-labeled NT and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antisense probes (3 × 106 cpm of each probe), ethanol precipitated, dried, dissolved in 30 μl hybridization buffer, and incubated at 42°C for 24 h. The samples were subsequently digested with nucleases P1 (20 μg/ml) and T1 (1 μg/ml), digested with proteinase K, phenol extracted, ethanol precipitated, dried, and analyzed on sequencing gels. Autoradiograms were scanned and protection products were quantitated using a Bio-Rad Fluor-S MultiImager and MultiAnalyt software. Sense NT mRNA was transcribed from pGEM-RNT (2), linearized with BamH I using SP6 RNA polymerase, and analyzed as described above to generate standard curves. Rat 32P-labeled NT antisense probe was transcribed from EcoR I-linearized prNT4 (2) using T7 RNA polymerase. A rat GAPDH cDNA fragment (nt 882–1016) was amplified by RT-PCR from total rat brain RNA using the following primers: 5′-TTTGGATCTCAACAGAACTCCATTCTC-3′ and 5′-TTTGGATCTACGTAGGCACTGACCTCCAC-3′. The PCR fragment was digested with EcoR I and BamH I and subcloned into plBscript KS(+) (Stratagene) digested with the same enzymes to create prGAPDH. The plasmid was linearized with EcoR I and transcribed with T7 RNA polymerase to generate 32P-labeled antisense probe.

Effect of NT on bile flow and BA output. Bile was collected every 10 min for 150 min in biliary fistula rats receiving saline or NT (10 pmol·kg−1·min−1) by intravenous infusion in the jugular vein. The effects of NT on bile flow and BA output were measured in rats in which bile was not returned to the intestine and in rats in which donor bile was administered into the duodenum at the basal excretion rate (30 μl/min).

Effects of NT and sCCK on [3H]TC uptake. One milliliter of 5 mM TC containing [3H]TC (=1 μCi) was infused (1.57 ml/min) into the duodenum (2 cm distal to pylorus) of biliary fistula rats. The rate of intestinal uptake of [3H]TC was measured by monitoring the appearance of radioactivity (%dose) in bile during intravenous infusion of NT (3–10 pmol·kg−1·min−1), sCCK (12–50 pmol·kg−1·min−1), or NT plus sCCK. Biliary concentration and output of total BA were also measured in some animals.

Effect of NT on hepatobiliary transport of [3H]TC from blood. One milliliter of [3H]TC (1 μCi, 5 mM) was infused (1.57 ml/min) into the jugular vein of biliary fistula rats. The rates of hepatic uptake and transport of [3H]TC into bile were measured during infusion of saline or NT (10 pmol·kg−1·min−1).

Effect of NT antagonist and CCK antagonist on [3H]TC uptake. Biliary fistula rats were infused via the jugular vein with SR-48692 (2 nmol·kg−1·min−1), lorglumide (100 nmol·kg−1·min−1), or vehicle. After 10 min, 1 ml [3H]TC (5 mM, 5 μCi) was injected into the duodenum or lower jejunum (25 cm distal to Treitz’s ligament), and [3H]TC uptake into bile was measured.

Effect of NT antagonist on uptake of [3H]TC administered with lipid into intestine. Biliary fistula rats were infused with SR-48692 (2 nmol·kg−1·min−1), lorglumide (100 nmol·kg−1·min−1), or vehicle. After 10 min, a mixture of 3 ml 20% Intralipid and 1 ml [3H]TC (5 mM, 5 μCi) was administered by bolus injection into the duodenum (1.57 ml/min). The appearance of [3H]TC in bile was monitored.

Effect of NT antagonist on BA uptake from bile infused with lipid into intestine. Biliary fistula rats were infused with SR-48692 (2 nmol·kg−1·min−1) or vehicle. After 10 min, a mixture of rat bile (6 ml) with 20% Intralipid (9 ml) was infused into the duodenum (75 μl/min) while bile flow and BA output were measured.

Simultaneous measurement of intestinal transit and [3H]TC uptake. Intestinal transit was measured by adding [3H]sodium chromate (0.5 μCi) to the [3H]TC solution administered into the duodenum in rats infused with saline or NT (10 pmol·kg−1·min−1). Uptake of [3H]TC into bile was
measured as described above for 100 min. Rats were then killed, and the small intestine was cut into 10 equal segments. The radioactivity of 51Cr was measured in each segment with its contents using a gamma counter. The percentage of radioactivity in each segment was calculated, and the geometric center of 51Cr distribution was determined (46).

**Analyses and Statistics**

Bile volume was measured gravimetrically, and BA concentration was measured using the 3-α-hydroxysteroid dehydrogenase assay (Sigma kit). Scintillation counting was performed using a Beckman LS 6500 with quench correction. Biliary BA output (volume \times \text{concentration}) over time was calculated. Bile flow rate and BA output were normalized to reduce variability and expressed as percent initial rate and output, respectively (30 min after cannulation). The rate of intestinal BA uptake was taken as the rate of appearance in bile since intestinal uptake was rate determining. Data are given as means ± SE, and n is the number of independent animals. One-way ANOVA with post hoc Bonferroni multiple comparison test and Student's t-test were used for comparisons.

**RESULTS**

**BA Decreased and BA Chelator Increased NT Blood Levels**

When adult rats were fed for 4 h or 5 days with chow containing BA (TC and cholate), blood levels of NT were decreased by ≈40% (P < 0.05) compared with rats eating chow alone (Fig. 1). On the other hand, blood levels of NT were elevated by ≈80% (P < 0.01) in rats fed chow with the BA chelator cholestyramine (Fig. 1).

**BA Decreased Intestinal NT mRNA Expression**

To complement these findings, a third experiment was performed in which rats were fed overnight, and 16 h later, blood was taken to measure NT and intestines were processed to measure NT mRNA. Blood levels of NT were decreased in rats fed BA chow (70 ± 13% of control, P < 0.05) and increased in rats fed cholestyramine chow (164 ± 26% of control, P < 0.05).

Similar results were obtained for NT mRNA expression (Fig. 2A) quantitated in proximal and distal intestine (Fig. 2B). In the distal intestine, where most NT-containing cells exist, NT mRNA expression was decreased in rats fed BA chow (57% of control, P < 0.01) but not significantly affected in rats fed cholestyramine chow. Similar results were obtained in the proximal intestine (Fig. 2B; BA chow = 51% of control).

**Biliary Fistula Increased and Bile Duct Ligation Decreased NT Release Response**

To complement these findings, biliary fistula and bile duct ligation were employed to manipulate the BA pool and the effects on NT release were determined (Fig. 3). The increment in hepatic-portal NT blood level was measured 30 min after injection of an Intralipid-TC.
mixture into the duodenum of 1) rats treated for 6 h with biliary fistula (model for reduction of the BA pool); 2) rats treated for 6 h with common bile duct ligation (model for expansion of the BA pool); and 3) sham-operated rats (control group). In agreement with the results of the diet studies, NT release increased approximately twofold with the reduction of the BA pool and decreased approximately twofold with the expansion of the BA pool (Fig. 3).

The fact that intestinal NT secretion and mRNA expression were altered by manipulations known to change intestinal BA concentration and total BA pool size suggested that NT, which is released during fat ingestion, might regulate BA secretion and/or enterohepatic BA cycling. To test this, we infused rats with a dose of NT shown by us (24) to mimic the two- to threefold increase in NT blood level seen after fat ingestion.

**NT Enhanced Bile Flow and BA Output Only When EHC is Maintained**

In fasted biliary fistula rats, in which bile bypassed the intestine (i.e., EHC was interrupted), NT infusion (10 pmol·kg⁻¹·min⁻¹) had little effect on bile flow rate and BA output (data not shown). However, in rats similarly prepared but with bile duct administered into the duodenum at the basal excretion rate (i.e., EHC was mimicked), NT significantly enhanced bile flow (Fig. 4A) and BA output (Fig. 4B) compared with control (P < 0.01). The apparent dependence on the presence of bile in the intestine suggested that the effect of NT was on the return of BA from the intestine to the liver, and further experiments described below confirmed this.

**NT but Not CCK-8 Enhanced Intestinal Absorption of [³H/TC**

In biliary fistula rats, NT dose dependently enhanced (up to 5-fold; P ≤ 0.01) the initial absorption rate of [³H/TC from the duodenum into hepatic bile...
The rate enhancement by NT was maximal during the 60- to 80-min collection periods (Fig. 5A). The basal rate of $[^{3}H]$TC recovery (averaged over 3 h) was 0.9% dose/min (Fig. 5B), which was increased 2.3-fold by NT (10 pmol·kg$^{-1}$·min$^{-1}$). In contrast, sCCK-8 infusion at 12 (Fig. 5B) and 50 pmol·kg$^{-1}$·min$^{-1}$ (data not shown) had no effect. Infusion of sCCK-8 (12 pmol·kg$^{-1}$·min$^{-1}$) also did not alter the response to 3 pmol·kg$^{-1}$·min$^{-1}$ NT (Fig. 5C).

**NT Enhanced Intestinal but Not Hepatic Uptake of $[^{3}H]$TC**

The effect of NT was shown to be on the intestine and not on the liver, because NT did not alter the rate of transport of $[^{3}H]$TC from blood into bile (Fig. 5D). Consistent with this, the basal hepatic uptake rate for $[^{3}H]$TC from blood into bile ($\approx$300 nmol/min) was 200 times that from jejunum into bile (compare Fig. 5, D and B), indicating that intestinal uptake was rate determining for operation of the EHC.

**NT Enhanced BA Absorption From Bile Infused into Duodenum**

When bile was administered into the duodenum of biliary fistula rats, total BA output was significantly elevated in NT-infused animals (Fig. 6B, $P < 0.01$) precisely during the time of enhanced intestinal $[^{3}H]$TC uptake (Fig. 6A). These results indicated that the effect of NT was exerted not only on $[^{3}H]$TC but also on a large portion of the intestinal pool of BA returning to the liver.

**NT but Not CCK Antagonist Reduced Absorption Rate for $[^{3}H]$TC From Intestine**

In biliary fistula rats, infusion of the NT antagonist SR-48692 (2 nmol·kg$^{-1}$·min$^{-1}$) reduced the recovery rate for $[^{3}H]$TC from duodenum into bile (Fig. 7A, $P < 0.01$) by $>50\%$, suggesting that endogenous NT regulates intestinal BA uptake. In contrast, the CCK-A antagonist lorglumide (100 nmol·kg$^{-1}$·min$^{-1}$) did not affect the rate of $[^{3}H]$TC absorption from the duode-
num (Fig. 7B). SR-48692 also reduced the rate of [3H]TC absorption from the distal jejunum, where the basal uptake rate was ≈8 times that in duodenum, although the inhibition by SR-48692 was only ≈24% (Fig. 7C, P < 0.01).

**Enhanced Intestinal [3H]TC Uptake in Presence of Lipid**

Because BA uptake is known to be a function of lipid ingestion, we also tested the effect of SR-48692 in the presence of a lipid load. When 3 ml of Intralipid were given with 1 ml [3H]TC into the duodenum of biliary fistula rats, SR-48692 infusion reduced the rate of [3H]TC recovery by ≈20% over 4 h (Fig. 8A, P < 0.01).

**DISCUSSION**

In addition to showing that near-physiological levels of NT infused into fasted biliary fistula rats increased [3H]TC absorption from the intestine, resulting in enhanced hepatic BA output, the present studies demonstrate that the NT antagonist SR-48692 inhibited [3H]TC absorption from the intestine, diminishing hepatic BA output. Together, these results argue that NT or a similar peptide is an endogenous regulator of the EHC, which acts to enhance intestinal BA absorption. Also supporting this notion is the present finding that NT secretion into blood is acutely affected by altering intestinal BA concentration during feeding and by changing the distribution of the BA pool in models of cholestasis and biliary diversion. Although the importance of the EHC in the efficient use of BA to promote digestion and absorption is well recognized, a hormonal regulator of this process has not been identified (31, 32, 51). The fact that ingestion of fat, which can enhance jejunal uptake of BA and increase hepatic bile flow (35, 45, 53), is the strongest stimulus for NT release (15, 19, 27, 43, 47, 49) suggests that intestinal NT could play a role in this process. Established roles (14) for NT in fat digestion include effects on intestinal motility and pancreatic enzyme secretion. Here, we provide evidence that enhancement of enterohepatic BA cycling is yet another mechanism by which NT could facilitate the digestion and absorption of lipids.

**Effects of BA on NT mRNA Expression and Secretion**

It is well established (15, 17) that lipids stimulate intestinal NT secretion. In this study, we show that postprandial blood levels of NT in rats are decreased by feeding chow containing TC and cholic acid, whereas
they are increased by feeding chow containing the BA chelator cholestyramine. We obtained these results in both acute (for 4 to 16 h) and chronic feeding experiments (for 5 days). We supported these results by showing that intestinal NT mRNA expression was diminished in rats fed chow with BA. The fact that cholestyramine did not enhance NT mRNA expression suggests that feeding by itself maximizes this parameter. Overall, our findings indicate that meal-stimulated NT synthesis and secretion are subject to regulation by intestinal BA, and when combined with the finding that NT enhances enterohepatic BA cycling, they suggest that this represents a regulatory feedback loop. Thus NT release is enhanced in the face of falling intestinal BA concentration, and this serves to stimulate enterohepatic BA cycling until the BA concentration rises to a level that inhibits further NT synthesis and release. Consistent with this, Lillienau et al. (40) showed that the rate of ileal BA absorption in rodents was increased by feeding chow with cholestyramine and decreased by feeding TC-enriched chow.

Our findings in biliary fistula and cholestatic rats are also in agreement with this idea. Here, we found that NT release in response to lipid was enhanced in BA-depleted biliary fistula rats, whereas it was diminished in BA-replete cholestatic rats. Although we do not know yet whether it is luminal BA concentration per se that regulates NT release or if it is the blood BA concentration that is sensed, the results in cholestatic rats, in which intestinal BA concentration is low and blood BA concentration is high, suggest that elevated blood BA concentration can inhibit NT release. Consistent with a possible role for NT in the regulation of BA uptake are observations by Sauer et al. (50) showing a reduction of active ileal uptake of [3H]TC and a down-regulation of the ileal Na\textsuperscript{+}-BA transporter in cholestatic rats. Sauer et al. (50) have also proposed that intestinal BA uptake is regulated by systemic BA concentration and that a protective feedback mechanism exists.

Our finding that elevation of the BA pool in rats inhibits postprandial NT release is in agreement with studies in dogs (21) showing that NT release in response to food is inhibited by concomitant administration of either TC or bile. Although bile and BA were found to increase NT release in perfused rat intestine (11), inhibition may not be possible in this system since BA does not accumulate in the vascular perfusate. Our finding that diminishing the BA pool in rats enhanced postprandial NT release is in accord with studies in dogs (21) showing that bile diversion increased NT release in response to triglyceride. A reasonable working hypothesis at this point is that the regulation of postprandial NT release by BA involves increased or decreased feedback inhibition imposed by BA present in hepatic portal blood.

**Bile Flow, BA Output, and Status of BA Cycling**

The initial bile flow rate (220 \(\mu\)l/10 min) and BA output rate (2.8 \(\mu\)mol/10 min) in the rats in the current study are in good agreement with those measured in other studies (12, 30) in fasted adult biliary fistula rats. For 250-g rats, Higgins et al. (30) found bile flow to be...
180 μl/10 min and BA output to be 2.7 μmol/10 min. Dumaswala et al. (12) found bile flow to be 196 μl/10 min and BA output to be 2 μmol/10 min. Because the total BA pool for a 250-g rat is ~50–60 μmol (41), the fistula rats in the current study excreted ≈30% of their BA pool per hour. Therefore, it is not surprising that infusing donor bile into the intestine was necessary to obtain a response to NT. The BA pool was so easily depleted that BA output fell 40–50% over 2–3 h, even when donor bile was infused at the excretion rate. Despite our difficulty in mimicking an intact EHC in the rat fistula model, NT infusion in rats receiving donor bile maintained a 10–20% higher BA output relative to controls for 2 h. The fact that NT enhanced BA output only when sufficient bile was present in the intestine suggested that the effect of NT was on the rate of intestinal BA uptake. This was proven by showing that NT increased [3H]TC uptake from the intestine but not from blood.

**Magnitude of Responses to NT and SR-48692**

Bile flow, hepatic BA output, and [3H]TC absorption from the intestine are all reduced ~50% in fasted rats compared with fed rats (12, 30, 45). In the present study, infusion of NT into fasted rats elevated bile flow ≥10%, increased BA output ≥20%, and enhanced [3H]TC recovery from the intestine approximately two-fold. Thus decreased NT secretion during fasting could contribute significantly to the decrements in these parameters. This idea is further supported by the present finding that infusion of the NT antagonist SR-48692 decreased [3H]TC recovery by ≥50% and ≥25% in the proximal and distal gut, respectively. The finding that SR-48692 infusion decreased the recovery of [3H]TC ≥20% when mixed with lipid suggested that NT regulation may also occur during normal digestion.

**Relationship of NT to CCK**

Fat ingestion is a strong stimulus for the release of intestinal NT and CCK (15, 22, 27, 36, 37, 49), hormones that exhibit some similar biological effects when
given peripherally (e.g., pancreatic secretion, gut motility pattern) and centrally (e.g., anorectic, neuroleptic). An established physiological function of CCK is to relax the sphincter of Oddi and to contract the gall bladder, thereby increasing the delivery of bile to the intestine and initiating the BA cycling process (31, 32, 51). In animals possessing a gallbladder, NT might be regarded as a backup for CCK since it contracts the gallbladder of mammals (20, 26, 56) and birds (Carraway, unpublished data). However, in animals lacking a gall bladder (e.g., rat), the effects of NT and CCK to mobilize the BA pool may be more subtle and complex. Our present finding that CCK and the CCK antagonist lorglumide, unlike NT and the NT antagonist SR-48692, had no effect on the rate of $[^3]$H$\text{TC}$ uptake from the rat intestine argues that the ability of NT to promote intestinal BA absorption is not shared with CCK. Thus this effect of NT in rats (to enhance BA return to liver) compliments that of CCK (to relax the sphincter of Oddi), with the result being a stimulation of enterohepatic BA cycling. If NT exhibits this effect in humans, where BAs are recycled 10–15 times per day and exert important effects on cholesterol handling, then NT could be a key contributor to the efficient digestion and absorption of fats and to human health.

Site and Mechanism of Action

The effect of NT on $[^3]$H$\text{TC}$ recovery was more pronounced in the proximal gut (2.3-fold increase) than in the distal gut (1.3-fold increase), although a significant response was obtained at both sites (24). Thus the enhanced BA uptake is likely to involve passive mechanisms (which are known to predominate in proximal gut) rather than the active $Na^+$-BA cotransporter (localized to distal gut). In addition to passive diffusion, carrier-mediated transport of $[^3]$H$\text{TC}$ occurs in the jejunum, as demonstrated originally by Amelsberg et al. (3, 4) and extended by Abe et al. (1) and Walters et al. (55), who identified the sodium-independent organic anion transporting polypeptide (OATP) in apical brush-border membranes from rat and mouse jejunal enterocytes. Whether NT targets this transporter or other undefined transporters in the proximal intestine is an important question. This possibility is supported by our observation that NT also enhances carrier-mediated uptake of $[^3]$H$\text{glucose}$ and $[^3]$H$\text{leucine}$ (24). The mechanism is not yet defined, but the fact that NT does not significantly affect gut transit (present data) and does not alter the uptake of $[^3]$H$\text{cholic}$ acid and $[^3]$H$\text{antipyrine}$ (24) argues against the involvement of a nonselective action on peristalsis or villus motility. Although NT increases intestinal blood flow and vascular permeability (28), such changes are also not likely to selectively affect the uptake of conjugated BA. Because NT receptors are abundant in the myenteric plexus of the gut, it seems reasonable to suggest that the absorptive effects of NT may involve release of mediators from enteric neurons. The finding by Grune et al. (23) that cAMP enhances BA transport in hepatoctyes suggests that it may be fruitful to investigate cAMP formation in intestinal epithelial cells.

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REFERENCES


44. McClintock C and Shiau YF. Jejunal is more important than terminal ileum for taurocholate absorption in rats. *Am J Gastroenterol* 244: G507–G514, 1983.


