Involvement of Mast Cells in Basal and Neurotensin-induced Intestinal Absorption of Taurocholate in Rats

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Running Title: Neurotensin and mast cells on bile acid uptake

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ABSTRACT

Neurotensin (NT), a hormone released from intestine by ingested fat, facilitates lipid digestion by stimulating pancreatic secretion and slowing the movement of chyme. In addition, NT can contract the gall bladder and enhance the enterohepatic circulation of bile acids (EHC) to promote micelle formation. Our recent finding that NT enhanced and NT antagonist inhibited \(^3\)H-taurocholate (\(^3\)H-TC) absorption from proximal rat small intestine indicated a role for endogenous NT in the regulation of EHC. Here, we postulate the involvement of intestinal mast cells in the TC uptake process and in the stimulatory effect of NT. In anesthetized rats with the bile duct cannulated for bile collection, infusion of NT (10 pmol/kg/min) enhanced the \(^3\)H-TC recovery rate from duodenojejunum by 2.2-fold. This response was abolished by pretreatment with mast cell stabilizers (cromoglycate, doxantrazole) and inhibitors of mast cell mediators (diphenhydramine, metergoline, zileuton). In contrast, mast cell degranulators (compound 48/80, substance P) and mast cell mediators (histamine, LTC\(_4\)) reproduced the effect of NT. L-NAME enhanced and L-arginine inhibited basal and NT-induced TC uptake, consistent with the known inhibitory effect of NO on mast cell reactivity. These results argue that basal and NT-stimulated TC uptake in rat jejunum are similarly dependent on mast cells, are largely mediated by release of mast cell mediators and are subject to regulation by NO.

Key Words: neurotensin; bile acid; enterohepatic circulation; mast cell; nitric oxide
INTRODUCTION

Bile acids (BA) play a central role in lipid digestion and absorption in animals and humans by forming mixed micelles with monoglycerides and fatty acids (30). The enterohepatic circulation (EHC) enhances the efficiency of fat digestion in animals and humans by recycling the conjugated BA pool 5-15 times per day, depending on the fat content of the diet (29). The uptake of conjugated BAs in the intestine is the rate-determining step for this process, since hepatic uptake and secretion occur about 10 times faster (31). The gall bladder serves primarily to store the BA pool between meals, and it empties rather rapidly with the ingestion of food. Presumably, BA cycling occurs most readily when the demand for BA exceeds that present in the gall bladder. Thus, it seems likely that the EHC is regulated at the level of the intestine, especially in animals lacking a gall bladder (e.g., rats and horses) but also in those possessing one (e.g., cats, dogs and humans).

The regulation of intestinal BA uptake is not well understood. Not only is there controversy regarding the primary site (41) and the mechanism (2, 58) of conjugated BA absorption but there is uncertainty as to whether hormonal factors control this process (31). However, our recent studies in the rat (25, 26) argue that neurotensin (NT), a peptide stored in mucosal enteroendocrine cells (60) and released by the ingestion of fat (21, 22), is a strong candidate regulator of intestinal BA uptake. It is well accepted that NT operates hormonally to optimize the digestion of fat by slowing the movement of chyme (28) and stimulating pancreatic enzyme secretion (3). A role for NT in the regulation of BA cycling would be a logical extension of these findings. Support for the notion that NT relates to BA function derives from our finding that intestinal NT mRNA expression and blood levels of NT are
acutely affected by altering intestinal [BA] during feeding and by changing the distribution of the BA pool in models of cholestasis and biliary diversion (26). Also consistent is the pharmacologic similarity between NT and cholecystokinin, which includes effects on pancreatic secretion, gall bladder contractility, gut motility, anorexia and neuroleptic activity (4, 7).

The evidence linking NT with intestinal BA cycling can be summarized as follows. NT secretion and conjugated BA absorption follow a similar pattern, increasing with eating and decreasing with fasting (17). Infusion of NT into fasted rats, at doses giving near physiologic blood levels, specifically enhanced intestinal absorption of taurocholate (TC), the primary conjugated BA, without altering that for cholic acid, its unconjugated counterpart (25). More importantly, infusion of NT antagonist SR48692 inhibited intestinal TC absorption, suggesting a role for endogenous NT in this process (26). The effect of NT was more evident in proximal than in distal intestine, and it appeared to involve carrier-mediated absorption rather than active transport. Although the distal intestine, which is endowed with a specific Na⁺-BA cotransporter, has been classically regarded as the primary site of BA absorption (31), work indicates that > 60% of the TC secreted into rat (41) and pig (33) intestine is absorbed before it reaches the ileum. Thus, the work on NT is consistent with this new school of thought which contends that proximal intestine plays a major role in BA cycling and that distal intestine functions mostly to ensure that any remaining BA is not lost into the colon. With this model in mind, NT appears to be a good candidate regulator for BA cycling.

A detailed study regarding the effects of NT on intestinal uptake of model compounds known to permeate the epithelium via specific routes gave results consistent with an effect of NT on paracellular permeability and/or vascular permeability (25). Various physiologic and
pathologic conditions, such as food digestion (13), food allergy (52) and inflammation (23) are known to alter epithelial and vascular permeability. Mast cell activation occurs in many of these conditions and mediators released from mast cells play a prominent role in initiating the permeability change (39). Given that NT is a potent activator of mast cells (8, 55) and that mast cells contribute to its effects on vascular permeability (9) and its involvement in stress-induced reactions (11) and gut inflammation (12), it seems reasonable to propose that the effect of NT on intestinal TC absorption involves mast cells.

The present study aims to determine whether the enhancing effect of NT on intestinal TC absorption is mast cell dependent and whether it can be reproduced by mast cell mediators. We test the effects of mast cell stabilizers and stimulants, as well as antagonists that specifically block the formation of or actions of histamine, serotonin, leukotriene and nitric oxide (NO). Our results implicate mast cells not only in the response to NT, but also in the normal TC uptake process.
MATERIALS AND METHODS

Materials

Sodium chromoglycate, doxantrazole, diphenhydramine, cimetidine, metergoline, indomethacin, NT, TC (sodium salt), prostaglandin E₂ (PGE₂), substance P, L-arginine, N⁵-Nitro-L-arginine methyl ester hydrochloride (L-NAME) and buffer salts were purchased from Sigma-Aldrich (St Louis, MO). ³H-TC was obtained from New England Nuclear (Boston, MA). Leukotriene C₄ (LTC₄) was from Cayman Chemical Co (Ann Arbor, MI). Sanofi Recherche (Montpellier, France) supplied SR48692. Stocks of NT (1mM) and SR48692 (1mM, DMSO) were at -20°C, and dilutions were made daily in 0.85% NaCl, 0.1% BSA. Solutions of TC (5.0mM) were prepared daily in PBS (138mM NaCl, 3mM KCl, 6mM Na₂HPO₄ and 6mM KH₂PO₄) and ³H-TC (2.5 µCi/ml) was added. Sodium cromoglycate, cimetidine, metergoline and indomethacin were dissolved in DMSO and diluted into saline, 0.1% BSA, giving final [DMSO] < 5%. Doxantrazole was dissolved in 0.5% NaHCO₃, 0.85% NaCl and was diluted into saline-BSA before use. Zileuton, obtained from Abbott Labs (Chicago IL), was suspended in 1% methylcellulose, which served as the control.

Animals

Male Sprague-Dawley rats (200-300g, Taconic Farms, Germantown, NY) were housed in University of Massachusetts Medical School (UMMS) animal facility and given rat chow and water ad libitum. All protocols were approved by the UMMS animal care and use committee.
Experimental Procedures

Intestinal uptake of $^3$H-TC was measured in biliary fistula rats as described by us (25, 26). The rate of appearance of $^3$H-TC in bile was equated with the rate of intestinal uptake since this is the rate-determining step (26). Briefly, rats were fasted 24 hr and anesthetized with ketamine-xylazine (60mg:10mg/kg ip). The right jugular vein was catheterized for NT infusion, the bile duct was cannulated for bile collection and PE-50 tubing was cemented into the duodenum for infusion of $^3$H-TC into the duodenojejunum. After surgery, the animals were kept warm with a heat lamp and bile flow was stabilized for 20 min before experiments. Bile was collected into Eppendorf tubes using a fraction collector at 10-20 min intervals for 120-180 min as indicated. Bile volume was determined gravimetrically, assuming specific gravity of 1.0. To assess the $^3$H-TC uptake rate, 1.0 ml of $^3$H-TC (5 mM) was infused (40µl/sec) into the duodenojejunum and biliary recovery of $^3$H-TC was quantified by determining radioactivity in collected bile (50µl) using a Beckman LS 3801 liquid scintillation counter with quench correction.

Saline or NT (10pmol/kg/min) was given by constant intravenous infusion via the cannulated jugular vein at 40µl/min. This dose of NT was chosen to mimic plasma levels of NT observed in animals and humans after ingestion of fatty foods (25). Hepatic portal plasma levels of NT in rats infused with this dose of NT rose from $\approx 25$pM to plateau at $\approx 55$pM within 20 min (25). In our experiments, NT infusion began 20 min before injection of $^3$H-TC to allow blood levels of NT to plateau. To test the effects of the following drugs on the response to NT, animals were pretreated by an initial iv injection 10-20 min prior to NT infusion, followed by an ip injection 40 min later at the following doses: cromoglycate (1 mg/kg iv; 10 mg/kg ip), doxantrasole (10 mg/kg), diphenhydramine (5 mg/kg iv; 10 mg/kg
ip), cimetidine (10 mg/kg), metergoline (2 mg/kg) and indomethacin (10 mg/kg). Zileuton (100 mg/kg) was given orally by gavage 1 hr before NT infusion. SR48692 (1 mg/kg) was given ip 20 min in advance when the endogenous NT is expected to be antagonized. For each drug, the dose and method of delivery were determined by choosing from the literature conditions shown to be most effective.

**Data Expression and Statistical Analysis**

TC uptake was plotted in time as the *cumulative*) \textsuperscript{3}H-TC output expressed as percentage of the administered dose. Data were plotted as mean ± SE, and the Student’s *t*-test was used for statistical comparisons with *p*<0.05 indicating a significant difference.
RESULTS

**Stabilizers of Mast Cells Blocked the Effect of NT on $^{3}$H-TC Uptake**

In agreement with our earlier findings (25, 26), intravenous infusion of biliary fistula rats with a near physiologic dose of NT (10pmol/kg/min) caused a 2.4-fold increase in the rate of recovery of $^{3}$H-TC from the jejunum (Fig 1A). TC recovery (% dose) was linear over the 3-hr time period and the uptake rate (Table 1) increased from $\approx 0.10\% / \text{min (control)}$ to $\approx 0.24\% / \text{min (NT)}$. To test the involvement of mast cells in this response, we examined the effects of mast cell stabilizers, sodium cromoglycate and doxantrazole, on the response to NT. Pretreatment of rats with cromoglycate (1mg/kg) or with doxantrazole (10mg/kg) 10 min prior to testing, abolished the effect of NT, giving TC recovery rates that were similar to that for saline-injected controls (Fig 1A).

**Activators of Mast Cells Mimicked the Effect of NT on $^{3}$H-TC Uptake**

Intravenous infusion of the mast cell degranulator, compound 40/80 (500pmol/kg/min), into biliary fistula rats, enhanced the $^{3}$H-TC uptake rate by 2.5-fold (Fig 1B; Table 1). A similar response (Fig 1C; Table 1) was obtained by infusing substance P (400pmol/kg/min), an inflammatory neuropeptide shown to increase mucosal permeability (23). However, substance P was less potent than NT, since it was ineffective at a dose of 10pmol/kg/min (Fig 1C). Pretreating the rats with mast cell stabilizer, cromoglycate or doxantrazole, greatly inhibited the response to substance P (Fig 1D), showing that it was mast cell-mediated.

**Diphenhydramine Blocked the Effect of NT on $^{3}$H-TC Uptake**
Since rat mast cells release histamine in response to NT in vitro (8) and in vivo (9), we tested the effects of antihistamines on the $^3$H-TC response to NT. Pretreatment of rats with H$_1$-histamine receptor antagonist, diphenhydramine (5 mg/kg) given 40 min prior to testing, abolished the TC uptake response to NT (Fig 2A). In contrast, the H$_2$-histamine receptor antagonist, cimetidine (10mg/kg) given similarly, did not alter the effect of NT (Fig 2A). These data indicated that histamine, acting via H$_1$ histamine receptors, was a potential mediator of the response to NT.

**Histamine Mimicked the Effect of NT on $^3$H-TC Uptake**

Histamine appears to be rapidly destroyed in the blood circulation of rats, since its half-life is estimated at <1 min (49). When infused into the femoral vein of bile fistula rats, histamine (275 nmol/kg/min) did not alter $^3$H-TC uptake (data not shown). However, when given into the mesenteric artery of the intestine, histamine (275 nmol/kg/min) enhanced the rate of $^3$H-TC uptake by 2.5-fold (Fig 2B; Table 1), reproducing the effect of NT. These data indicated that histamine was a potential mediator of the effect of NT, but only when given locally or released locally (i.e., from mast cells within the intestine, not from distant mast cells). Consistent with this, blood levels of histamine were not elevated during NT infusion into these rats, in that [histamine] was <50 nM prior to and after 5, 15 and 60 min of 10pmol/kg/min NT infusion.

**Metergoline Reduced the Effect of NT on $^3$H-TC Uptake**

Since NT also stimulates the release of serotonin from rat mast cells, we tested the effect of metergoline, a type 1 serotonin receptor antagonist (9), on the $^3$H-TC uptake response to
NT. Pretreatment of rats with metergoline (2 mg/kg), 20 min prior to testing, did not alter basal \(^{3}\)H-TC uptake; however, it significantly inhibited (\(\geq 50\%\) reduction) the response to NT (Fig 2C). These data indicated that serotonin participated in the TC uptake response to NT.

**Zileuton Reduced the Effect of NT on \(^{3}\)H-TC Uptake**

When given intravenously to rats, NT stimulates mast cell-dependent leukotriene formation, presumably by enhancing 5-lipoxygenase (5-LOX) activity (9). To investigate the involvement of 5-LOX in the \(^{3}\)H-TC uptake response to NT, we tested the effect of zileuton, an orally active 5-LOX inhibitor (10). In rats pretreated with zileuton (100 mg/kg) 60 min prior to testing, the effect of NT on \(^{3}\)H-TC uptake was abolished (Fig 2D).

**Leukotriene Mimicked the Effect of NT on \(^{3}\)H-TC Uptake**

Bolus injection of the leukotriene LTC\(_4\) (60\(\mu\)g/kg) produced an enhancement of \(^{3}\)H-TC uptake (Fig 3A) that was similar to that produced by NT (Table 1). When infused at a lower dose (10\(\mu\)g/kg/min), LTC\(_4\) did not alter \(^{3}\)H-TC uptake and did not enhance the response to NT (Fig 3A). These data suggested that the TC uptake response to NT involved the release of LTC\(_4\) formed by the action of 5-LOX.

**Indomethacin Did Not Alter the Effect of NT on \(^{3}\)H-TC Uptake**

To investigate the involvement of prostaglandins in the \(^{3}\)H-TC response to NT, we tested the effect of indomethacin, a cyclooxygenase inhibitor (27). NT was as effective in rats pretreated with indomethacin (10mg/kg) 30 min prior to testing, as it was in control rats (Fig 3B), indicating that prostaglandins were not essential participants.
**PGE₂ Enhanced the Effect of NT on ³H-TC Uptake**

By increasing blood flow, vasodilatory prostaglandins such as PGE₂ can enhance permeability responses to leukotrienes. Since our results implicated LTC₄ in the ³H-TC uptake response to NT, we tested the effect of PGE₂ on the response to NT. Although PGE₂ (2µg/kg/min) by itself had little effect on ³H-TC uptake (Fig 3B; Table 1), PGE₂ enhanced the response to NT (10pmol/kg/min) ≅2-fold throughout the time course (Fig 3B). These data were consistent with the participation of LTC₄ in the TC uptake response to NT and indicated that the response could be potentiated by PGE₂.

**L-NAME Enhanced ³H-TC Uptake by an Effect not Involving type 1 NT Receptor (NTR1)**

Since nitric oxide synthase (NOS) can participate in reactions involving changes in intestinal permeability (38), we tested the effects of agents known to alter its action. We found that the NOS inhibitor L-NAME (40mg/kg), given 20 min prior to testing, enhanced ³H-TC uptake, producing an effect equivalent to that of NT (Fig 4A; Table 1). NTR1 antagonist SR48692 (1mg/kg, ip), given 20min before testing, inhibited the response to NT (data not shown) but had little effect on the response to L-NAME (Fig 4B), indicating that L-NAME was not acting via NTR1. These data were consistent with other work showing that L-NAME can enhance intestinal epithelial permeability by disrupting the stabilizing effects of NO on mast cells (36).

**L-NAME Enhanced and L-Arginine Inhibited the Effect of NT on ³H-TC Uptake**
The effect of NT was enhanced by L-NAME (Fig 4C; 2-fold increase). In contrast, NOS substrate L-arginine (200mg/kg), given 20 min prior to testing, inhibited the effect of NT (Fig 4D; 90% decrease). These results suggested that NO production acted negatively on the response to NT, which is consistent with prior work showing that NO donors inhibit mast cell reactivity in vivo (53).

*Mast cell-Stabilizers and L-Arginine Reduced Basal $^{3}$H-TC Uptake Rate*  

The basal $^{3}$H-TC uptake rate in saline-infused rats was reduced by >50% in animals given cromoglycate, doxantrazole or L-arginine (Fig 5; Table 1). Since each of these agents exerted a stabilizing effect on mast cells, these results argue strongly that at least 50% of the basal TC uptake rate was attributable to mast cell-derived activity.
DISCUSSION

This paper extends our earlier finding (25, 26) – that physiologic doses of NT enhance intestinal absorption of TC, the primary conjugated BA in rats - by demonstrating the involvement of mast cells and mast cell mediators in this response. Mast cell stabilizers not only blocked the stimulatory effect of NT but they also diminished the basal rate of TC uptake, implicating mast cells in the physiologic process. In addition, the effect of NT was inhibited by preventing mast cell mediators (histamine, serotonin and LTC₄) from acting and it was reproduced by infusion of these mediators. Since intestinal uptake of BA is rate-determining for operation of the EHC, these findings suggest that the NT-mast cell axis could play an important role in regulating the availability of BA to promote lipid digestion and absorption. Although the involvement of mast cells in intestinal inflammation and pathologic conditions is well established (27), there is little information on their participation in BA physiology (31). Here, we suggest that intestinal mast cells regulate epithelial and/or vascular permeability to promote the physiologic absorption and recycling of BA.

Some NT Effects are Mediated by Mast Cells

The idea that mast cells mediate some of the effects of NT has a strong foundation (7). Bolus injection of a large quantity of NT (≥4nmol/kg) into rats produces an anaphylactic reaction involving the release of mast cell derived histamine and leukotrienes (9). The ensuing hypotension, increased vascular permeability and cyanosis can be prevented by prior treatment with cromoglycate to stabilize mast cells (9) or with compound 48/80 to deplete mast cell mediators (8). Other effects of NT that can be blocked by mast cell stabilizers and antihistaminic agents include vasoconstriction (37) hypothermia (34) and contraction of
gastric smooth muscle (43). The ability of NT to bind to and to degranulate isolated mast cells has been demonstrated (8), and the existence of NTR1 on mast cells has been confirmed by mRNA and protein analyses (44, 20). In fact, NT is the only mast cell secretagogue for which a specific G-protein-coupled receptor has been identified in mast cells.

**NT and Mast Cells Interact in the Intestinal Mucosa**

In the small intestine, NT is primarily localized to epithelial endocrine cells, although it is also present in neurons of the mucosa, submucosa and muscularis (50). Endocrine and/or paracrine release of NT occurs postprandially (51) and is best stimulated by fatty acids (21), BA (16) and hormones (18). The intestinal mucosa is highly enriched with mast cells, which are often closely opposed to and interacting with enteric neurons (59) and vascular elements (38). As an integral part of the nerve-endocrine-immune network, mast cells participate in aspects of inflammation and they also contribute to physiologic regulation. Examples for the latter include the involvement of mast cells in cholecystokinin-induced disruption of the intestinal migrating motor complex (32) and in distention-induced and substance P-induced intestinal secretion (19). Some evidence attests to the importance of NT-mast cell interactions in inflammation. For example, pretreatment of rats with NTR1 antagonist SR48692 inhibits stress-induced (11) and toxin A-induced (12) intestinal mast cell activation and the associated changes in intestinal permeability and secretion of PGE$_2$ and mucin. These actions could involve both direct and indirect effects of NT on mast cells since NT stimulates enteric nerves to secrete acetylcholine and substance P (7), which enhance the release of mast cell mediators (40, 42), and mast cell mediators further stimulate enteric neurons (15, 45). The close
relationship between NT and mast cells in pathophysiologic situations suggests that their interaction may also be utilized physiologically.

**Mast Cell Activation is Essential for Basal and NT-stimulated TC Uptake**

Our data show that structurally distinct mast cell degranulators (48/80, substance P and L-NAME) enhanced the rate of TC absorption, reproducing the effect of NT. In contrast, agents purported to stabilize mast cells (cromoglycate, doxantrasole and L-arginine) reduced basal and NT-stimulated TC uptake. These findings support our contention that mast cells participate in TC uptake and mediate the enhancing effect of NT. Tissue mast cells are heterogeneous and intestinal mast cells are more sensitive to the inhibitory effects of cromoglycate than are mast cells from other tissues such as lung and skin (46). The fact that cromoglycate totally blocked the effect of NT on TC-uptake suggests that this response involves the activation of mast cells within the intestine. There are at least two types of mast cells, mucosal-type mast cells (MMC) and connective tissue-type mast cells (CTMC), in the intestine. Although NT (48) and substance P (56) can stimulate both MMC and CTMC, compound 48/80 is relatively specific for CTMC. Similarly, doxantrasole inhibits the activation of both MMC and CTMC, while cromoglycate primarily affects CTMC (47). Our results are most consistent with the involvement of CTMC but do not exclude the participation of MMC in the response to NT. The fact that CTMC are associated with the vasculature suggests that effects on vascular permeability could contribute to NT's effect on $^3$H-TC uptake. The localization of MMC just below the epithelial layer suggests that effects on epithelial permeability (36, 54) could also be important.
Mast Cell-derived Mediators Enhance TC Uptake

The inhibitory effects of pretreating rats with diphenhydramine and zileuton implicate histamine and leukotriene as obligatory participants in the response to NT. The fact that histamine and LTC4 were capable of enhancing TC uptake supports this contention. Metergoline gave partial inhibition of the NT response, suggesting that serotonin could be a minor contributor. In prior work, we showed in rats that NT causes a rapid release of mast cell histamine, followed by leukotriene generation (9). Since histamine stimulates formation of leukotrienes (7), and since LTC4 is a powerful permeability enhancer (27), it seems likely that NT acts by releasing histamine which generates leukotrienes, the final mediators of the response. Prostaglandins are known to potentiate the effects of leukotrienes and here we found that PGE2, which was ineffective alone, enhanced the response to NT. The fact that systemic blood levels of histamine were not elevated during NT infusion argues that the histamine involved in NT-induced TC uptake was produced locally.

Nitric Oxide and Mast Cells in Intestinal Uptake of BA

Whereas excessive NO released by inflammatory cells can damage the intestinal epithelium (24), a low level produced by the intestinal endothelium plays an important role in maintaining the epithelial barrier (1). Thus, NO donors decrease epithelial permeability, whereas inhibitors of NOS have the reverse effect (57). These changes have been attributed to the stabilizing effects of NO on intestinal mast cells (35, 53). Here we found that basal and NT-induced TC uptake were inhibited in animals receiving NO donor L-arginine, whereas they were enhanced in animals that received NOS inhibitor L-NAME. These data support our contention that the enhancement of intestinal TC uptake seen in our experiments is caused by
mast cell-mediated increases in intestinal permeability, and they also illustrate that this process can be modulated by NO.

**Mechanisms Involved in Regulation of TC Uptake**

It is possible that NT altered the lumenal [3H-TC] during our experiments; however, this would not provide an explanation for the response observed since NT stimulates secretion of fluid and mucus (22) which would likely act to diminish the rate of 3H-TC uptake. While active transport of TC occurs in ileum, the primary mechanism operating in jejunum is likely to be carrier-mediated transport (41). NT has little effect on TC uptake in ileum but as shown here, it markedly enhances TC uptake in proximal intestine (25). Since NT also enhances intestinal uptake of substrates absorbed primarily via the paracellular route (3H-mannitol, 51Cr-EDTA; ref 25), it is conceivable that some of its effect on TC uptake involves increased paracellular permeability. Considerable evidence links mast cell activation and mast cell mediators (5, 54) to increases in paracellular permeability observed during development (14) and in pathologic conditions involving stress (62), food allergy (39), enteritis (12) and ischaemia (61). On the other hand, some findings are at odds with the hypothesis that NT enhances TC uptake by increasing intestinal paracellular permeability. In the rat, NT secretion (51) and BA-uptake (31) are enhanced in the fed state, yet intestinal paracellular permeability is diminished as compared to the fasted condition (63). Although it is possible that NT stimulates TC-transport at the level of the epithelium, it seems more likely that its effects on vascular permeability enhance the passage of TC into the circulation. It is commonly thought that passage across the intestinal epithelium is rate-determining for absorption of conjugated BA (30); however, the dependence on vascular permeability has not
been thoroughly examined (30). Since NT and mast cell mediators alter blood flow and dramatically increase vascular permeability to albumin, it seems likely that these effects contribute to the enhancement of TC uptake in the intestine.

In conclusion, basal and NT-stimulated TC uptake in the rat intestine are similarly dependent on mast cells, are largely mediated by release of mast cell mediators and are subject to regulation by NO. These findings are consistent with the idea that intestinal NT is an endogenous regulator of EHC whose effects are mediated by the activation of intestinal mast cells. Although the mechanism may involve enhanced transport through the epithelium, our data is most consistent with an effect on vascular permeability and blood flow.
ACKNOWLEDGEMENTS:

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REFERENCES


Table 1. Effect of Mast Cell-directed Agents on the Rate of $^3$H-TC Uptake from Rat Duodenojejunum *in vivo*

<table>
<thead>
<tr>
<th>Agent</th>
<th>Dose (pmol/kg/min)</th>
<th>$^3$H-TC Uptake Rate * (% / min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>-</td>
<td>0.10±0.01</td>
</tr>
<tr>
<td>NT</td>
<td>10.</td>
<td>0.24±0.04 ‡</td>
</tr>
<tr>
<td>Compound 48/80</td>
<td>1000.</td>
<td>0.25±0.06 ‡</td>
</tr>
<tr>
<td>Substance P</td>
<td>400.</td>
<td>0.21±0.04 ‡</td>
</tr>
<tr>
<td>Histamine †</td>
<td>2.8 x 10⁵</td>
<td>0.25±0.02 ‡</td>
</tr>
<tr>
<td>LTC4 †</td>
<td>9.6 x 10⁴</td>
<td>0.31±0.03 ‡</td>
</tr>
<tr>
<td>L-NAME</td>
<td>9.3 x 10⁴</td>
<td>0.30±0.04 ‡</td>
</tr>
<tr>
<td>PGE2</td>
<td>5.7 x 10³</td>
<td>0.10±0.02</td>
</tr>
<tr>
<td>L-Arginine</td>
<td>5.7 x 10⁶</td>
<td>0.035±0.01 ‡</td>
</tr>
<tr>
<td>Cromoglycate †</td>
<td>-</td>
<td>0.02±0.003 ‡</td>
</tr>
<tr>
<td>Doxantrazole †</td>
<td>-</td>
<td>0.04±0.008 ‡</td>
</tr>
</tbody>
</table>

* Agents were infused intravenously, $^3$H-TC was injected into the duodenojejunum and the rate of $^3$H-TC uptake was calculated from the slope of the cumulative uptake profile.

† Histamine was given intraarterially and LTC4 was given as a bolus. Dosages of cromoglycate and doxantrazole are given in text.

‡ Results are significantly different from vehicle control (p<0.05).
FIGURE LEGENDS

Fig. 1. The rate of TC uptake from proximal intestine was enhanced by NT (A), compound 48/80 (B) and substance P (C, D), and these effects were inhibited by mast cell stabilizers. Infusion of stimuli began at –20 min, $^3$H-TC was injected at 0 min (arrow), and mast cell stabilizers were given at times shown (arrows). Cumulative recovery (%) of the administered dose of $^3$H-TC is plotted as a function of time (mean±SEM; n=5). A) The enhancing effect of NT (10pmol/kg/min) on TC uptake was abolished (p<0.01) by pretreatment by cromoglycate (1mg/kg) or doxantrasole (10mg/kg). B) Compound 48/80 (1µg/kg/min) enhanced TC uptake (p<0.01). C) Substance P (400pmol/kg/min) enhanced TC uptake (p<0.01) but SP (10pmol/kg/min) had no effect. D) The response to substance P (400pmol/kg/min) was inhibited by cromoglycate (p<0.05) and doxantrazole (p<0.05).

Fig. 2. Antagonists of mast cell mediators inhibited NT-induced TC uptake (A,C,D), whereas histamine mimicked the effect of NT (B). NT infusion (10pmol/kg/min) started at -20min, $^3$H-TC was given at 0 min (arrow), and antagonists were given at times shown (arrows). Cumulative recovery (%) of the administered dose of $^3$H-TC is plotted as a function of time (mean±SEM; n=5). A) The effect of NT was abolished by diphenhydramine (10mg/kg; p<0.01) but was unaffected by cymetidine (10mg/kg). B) Intraarterial infusion of histamine (275nmol/kg/min) enhanced TC uptake (p<0.01). C) Metergoline (2mg/kg) inhibited the TC uptake response to NT by ≅50% (p<0.05). D) Zileuton (100mg/kg) abolished the effect of NT on TC uptake (p<0.01).
Fig. 3. Leukotriene (LTC₄) mimicked the effect of NT (A); prostaglandin (PGE₂) enhanced the response to NT (B); whereas, indomethacin did not alter the response to NT (B). Stimuli were given at –20 min, ³H-TC was given at 0 min (arrow) and indomethacin was given as shown (arrows). Cumulative recovery (%) of the administered dose of ³H-TC is plotted as a function of time (mean±SEM; n=5). A) LTC₄ was given as a bolus (60μg/kg) or as an infusion (10μg/kg/min) in the presence and absence of NT (10pmol/kg/min). The bolus of LTC₄ enhanced TC uptake (p<0.01), whereas infusion of LTC₄ at the lower dose had no effects. B) Infusion of PGE₂ (2μg/kg/min) did not alter TC uptake but enhanced the response to NT (p<0.05). Indomethacin (10mg/kg) did not alter the response to NT.

Fig. 4. NOS inhibitor (L-NAME) increased TC uptake (A); the effect was independent of NTR1 (B) and it enhanced the response to NT (C); in contrast, NOS substrate (L-arginine) inhibited the effect of NT (D). Stimuli were given at –20 min and ³H-TC was given at 0 min (arrow). Cumulative recovery (%) of the administered dose of ³H-TC is plotted as a function of time (mean±SEM; n=6). A) L-NAME (40mg/kg bolus, followed by 25μg/kg/min infusion) enhanced TC uptake (p<0.01). B) Pretreatment with SR48692 (1mg/kg) did not alter the response to L-NAME, although it blocked the response to NT (data not shown). C) L-NAME enhanced the response to 10pmol/kg/min NT (p<0.05). D) L-arginine (200mg/kg bolus, followed by 1mg/kg/min infusion) inhibited the response to 10pmol/kg/min NT (p<0.05).

Fig. 5. Basal TC uptake was inhibited by mast cell stabilizers, cromoglycate and doxantrazole (A) and L-arginine (B). ³H-TC was injected at 0 min (arrow) and mast cell stabilizers were given at times shown (arrows). Cumulative recovery (%) of the administered dose of ³H-TC
is plotted as a function of time (mean±SEM; n = 6).  A) Basal TC uptake was inhibited 48% by doxantrazole (p<0.01) and 73% by cromoglycate (p<0.01).  B) Basal TC uptake was inhibited 54% by L-arginine (p<0.05).
Figure 1
Figure 2
Figure 3

A

**NT or Vehicle or LTC₄ Infusion**

- LTC₄ (bolus)
- LTC₄
- Vehicle + NT
- LTC₄ + NT
- Vehicle

Cumulative ³H-TC Output (%)

B

**NT or Vehicle or PGE₂ Infusion**

- Vehicle + NT
- PGE₂
- NT + PGE₂
- NT + Indomethacin
- Indomethacin / Vehicle

Cumulative ³H-TC Output (%)

Time (mins)
Figure 4

A. L-NAME or Vehicle Infusion

B. L-NAME or L-NAME + SR48692

C. NT or NT+L-NAME Infusion

D. NT or NT + L-Arginine Infusion

Cumulative $^3$H-TC Output (%)

Time (mins)
Figure 5

A

Saline Infusion

- Cromoglycate
- Doxantrazole
- Vehicle

Cumulative $^3$H-TC Output (%)

Time (mins)

B

Saline Infusion

- L-Arginine
- Vehicle

Cumulative $^3$H-TC Output (%)

Time (mins)