Neural control of intestinal ion transport and paracellular permeability is altered by nutritional status

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Hayden, Ursula L., and Hannah V. Carey. Neural control of intestinal ion transport and paracellular permeability is altered by nutritional status. Am J Physiol Regulatory Integrative Comp Physiol 278: R1589–R1594, 2000.—This study examined the effect of fasting on the neural control of ion transport and paracellular permeability in piglet jejunum. Muscle-stripped tissues from fed or 48-h fasted piglets were mounted in Ussing chambers. Neural blockade with tetrodotoxin (TTX) or antagonists of muscarinic or nicotinic receptors caused reductions in basal short-circuit current that were approximately threefold greater in fasted piglets. The TTX-induced reduction in short-circuit current in fasted piglets was due to a decrease in residual ion flux and was abolished in the absence of HCO₃⁻. Intestinal paracellular permeability, as indicated by tissue conductance (Gₜ) and fluxes of inulin and mannitol, was significantly increased by fasting. TTX increased inulin flux and Gₜ in fed but not fasted piglets. In fasted piglets, carbachol reduced Gₜ by 29% and mannitol flux by 27% but had no effect on these parameters in the fed state. We conclude that fasting enhances enteric neural control of basal ion transport and increases paracellular permeability in the fed state, and cholinergic stimulation restores fasting-induced elevations in paracellular permeability to fed levels.

fasting; epithelia; enteric nerves; secretion

THE ABSENCE OF LUMINAL NUTRITION has marked effects on intestinal epithelial function. Fasts as short as 24–72 h increase basal ion secretion, enhance ion transport responses to a variety of secretory and absorptive agonists, and increase paracellular permeability to ions and larger solutes (6, 10, 16, 30, 37). These effects are also observed in malnourished individuals, particularly neonates (5, 34), and in models of intestinal bypass (8, 9) and total parenteral nutrition (32). Thus adequate luminal nutrition plays an important role in preserving intestinal barrier function and minimizing fluid and electrolyte losses during pathological states such as secretory diarrhea.

Although well documented, the mechanisms responsible for the higher incidence of intestinal epithelial dysfunction secondary to fasting and malnutrition are still poorly understood (16). One mechanism that has been suggested is altered regulation of the intestinal epithelium by the enteric nervous system (29, 30). It is now well established that enteric neurons participate in nearly all aspects of gastrointestinal function, including epithelial transport, motility, mucosal immune defense, release of gut peptides from enterodendocrine cells, and mucosal blood flow (15). Enteric sensory fibers can respond to luminal and systemic stimuli and activate complex reflex pathways that regulate epithelial function (14). The presence or absence of specific nutrients within the intestinal lumen may be one signal that activates such reflexes (13, 36), although details of specific transport processes and their neurochemical mediators are lacking. Even less is known of the potential role of the enteric nervous system in regulating intestinal paracellular permeability (2, 33) and whether nutritional status alters that control. Thus, in this study, we used a model of short-term fasting in piglets (10) to better understand how nutritional status affects the neural control of basal ion transport and permeability in the small intestine.

MATERIALS AND METHODS

Animals. Cross-bred piglets were removed from the sow at day 10 and placed in individual pens equipped with an automatic feeding device. All piglets were allowed free access to a milk replacer diet (Milk Specialties, Dunde, IL) until day 21. At day 21, piglets were randomly assigned into two groups. “Fed” piglets continued drinking milk replacer until day 23 and “fasted” piglets were allowed water only between days 21 and 23. On day 23, piglets were killed via electrocution, a procedure approved by the University of Wisconsin Animal Care and Use Committee. Segments of proximal jejunum were harvested and immediately placed into ice-cold buffer solution, and adjacent segments were used for Ussing chamber studies.

Tissue preparation. Jejunal tissues were stripped of external muscle layers, a procedure that removes the myenteric but not submucosal plexus. Tissues were mounted in Ussing chambers that were equipped to measure transmural potential difference (PD) and short-circuit current (Isc), a measure of active ion transport. Total tissue conductance (Gt) was calculated from PD and Isc, values using Ohm's law. The area of tissue exposed in the flux chambers was 1.13 cm². The Krebs buffer bathing mucosal and serosal sides of the tissues contained (in mM) 148.5 Na⁺, 6.3 K⁺, 139.7 Cl⁻, 0.3 H₂PO₄⁻, 1.3 HPO₄²⁻, 19.6 HCO₃⁻, 3.0 Ca²⁺, and 0.7 Mg²⁺. D-Glucose (11.5 mM) or mannitol (11.5 mM) was present in serosal and mucosal solutions, respectively. Tissues were bathed with 10 ml of solution by recirculation from a reservoir maintained at 39°C (porcine core temperature). Solutions were gassed with a 95% O₂-5% CO₂ mixture and maintained at pH 7.4. Drugs

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were added to serosal bathing solutions and subsequent changes in basal electrical parameters (I_sc, PD, G_t) were recorded 10 min later.

Effect of neural blockade on transepithelial ion fluxes. 22Na (3 μCi) and 36Cl (7 μCi) were added to mucosal or serosal bathing solutions, and 30 min were allowed to achieve isotopic steady state. Mucosal-to-serosal and serosal-to-mucosal fluxes of Na+ and Cl− were obtained by sampling fluid from “hot” sides at 10-min intervals. Opposing unidirectional fluxes were paired on the basis of similar G_t (within 20%), and the difference between them represented the net ion flux for that tissue pair. For basal fluxes, two 0.5-ml fluid samples were taken from the sides opposite isotope addition and replaced with Krebs solution that was prewarmed and oxygenated. Ten minutes after the second sample was taken, tetrodotoxin (TTX; 0.5 μM) or vehicle (Krebs solution) was added to serosal solutions. Three additional samples were taken at 10-min intervals, and the calculated flux values were averaged to produce one post-TTX value per tissue pair. At the termination of the experiment, 0.5-ml samples were taken from the “hot” sides. Samples were assayed in gamma and liquid scintillation counters. After correcting for the efficiency of counting 22Na in the two counters, the 22Na activity was subtracted from the combined activity of the two isotopes to yield the 36Cl activity. Because the effect of TTX on I_sc changed as a function of time during a flux period, in these experiments, I_sc was measured as the integrated area under I_sc traces from chart recordings generated during each 10-min flux period. Integrated I_sc values were then averaged to derive the mean integrated I_sc before and after addition of TTX. The residual ion flux (J_R) represents that portion of the I_sc not accounted for by net Na+ and Cl− fluxes and was calculated from the equation J_R = integrated I_sc − J_Na + J_Cl.

Inulin and mannitol fluxes. Unidirectional serosal-to-mucosal fluxes of inulin (TTX studies) or mannitol (carbachol studies) were measured in tissues from fed and fasted piglets. Changes in transepithelial flux of these hydrophilic solutes reflect changes in the permeability of the paracellular pathway (25, 26). [3H]inulin (1 μCi/ml) or [3H]mannitol (1 μCi/ml) was added to serosal solutions 30 min before the sampling periods. Bathing solutions contained 10 mM unlabeled inulin or mannitol on mucosal and serosal sides. Fluid samples (0.5 ml) were taken from the mucosal solutions at 10-min intervals. After the first two samples were withdrawn, TTX (0.5 μM) or carbachol (10 mM) was added to serosal solutions, and post-drug fluxes were calculated from samples taken 10 and 20 min thereafter. Subsequent procedures were similar to those described for ion flux studies. Unidirectional solute fluxes are expressed as nanomoles per square centimeter per hour, and at least four tissues were studied per piglet.

Data analysis and statistics. For nonflux experiments, changes in I_sc evoked by drugs were calculated by subtracting the basal I_sc before stimulation from the maximal or minimal I_sc achieved after drug addition. Data are expressed as means ± SE in microamperes of current per square centimeter of serosal area exposed in the flux chambers (μA/cm²). For each treatment, at least two tissues per piglet were averaged to derive one mean value per animal, and sample sizes reflect number of piglets. Differences between groups were analyzed by paired or unpaired Student’s t-tests or one-way analysis of variance followed by the Scheffé’s test for post hoc analysis of differences among groups. A probability value of P < 0.05 was considered statistically significant.

RESULTS

Piglet body weights. The 48-h fast significantly reduced body weights from 7.0 ± 0.3 kg in 20 fed piglets to 6.2 ± 0.3 kg in 22 fasted piglets (P < 0.05). Apart from the reduction in body weight, fasting had no effect on the overall health of the piglets.

Basal ion transport. Inhibition of tonic neural activity with TTX, which prevents action potential-dependent neurotransmitter release, significantly reduced basal I_sc in fed and fasted piglets, with a substantially greater effect in tissues from fasted animals (Fig. 1). To determine effects of cholinergic pathways on basal ion transport, we used atropine, a muscarinic receptor antagonist, and mecamylamine, a nicotinic receptor antagonist. Both caused small reductions in basal I_sc in the fed piglets and had significantly greater effects in fasted animals (Fig. 1). Addition of a control solution (Krebs buffer) had no effect on basal I_sc over the same time period in either fed or fasted piglets (Fig. 1).

Flux experiments were carried out to identify the ionic mechanisms underlying the effects of TTX on basal I_sc. There were no changes in any ion fluxes or electrical parameters in control tissues from fed or fasted piglets treated with Krebs buffer (data not shown). Ion fluxes before and after addition of TTX are shown in Table 1. In fed piglets, TTX caused a small but significant decrease in basal I_sc, which was consistent with its effect in the separate set of tissues shown in Fig. 1. It should be noted that the TTX-induced changes in I_sc in the two data sets are not directly comparable because of differences in how I_sc values were calculated. The values in Fig. 1 were calculated as the difference between basal and post-TTX I_sc readings, whereas I_sc values in flux experiments represented integrated areas within current traces obtained from chart recordings during the two 10-min flux periods (see MATERIALS AND METHODS).

In fed piglets, TTX increased mucosal-to-serosal fluxes of Na+ and Cl−. Although serosal-to-mucosal fluxes of Na+ and Cl− also tended to increase after TTX, the effects were not significant. There were no significant changes in net fluxes of Na+ or Cl− nor in J_R after TTX administration. TTX induced a significant increase in G_t in fed piglets (Table 1) that was not observed in vehicle-treated tissues (data not shown).

In fasted piglets, TTX significantly reduced basal I_sc but had no effects on unidirectional (or net) fluxes of Na+ and Cl−. The effects of the muscarinic antagonist atropine were similar to those of TTX, although mecamylamine, a nicotinic antagonist, was without effect on I_sc. These changes in I_sc were observed only in tissues from fasted piglets (Table 1).

Fig. 1. Decreases in basal short-circuit current (I_sc) evoked by Krebs buffer (CON), tetrodotoxin (TTX; 0.5 μM), mecamylamine (MEC; 10 μM), and atropine (ATR; 10 μM) in fed (open bars) and fasted (hatched bars) piglet jejunum. Data are means ± SE. Numbers of piglets (fed, fasted) are CON: 15, 16; TTX: 16, 13; MEC: 6, 8; ATR: 7, 7. **P < 0.01, ***P < 0.001 compared with fed values.
Table 1. Transepithelial ion fluxes and tissue conductance in piglet jejunum in the presence and absence of TTX

<table>
<thead>
<tr>
<th></th>
<th>Fed</th>
<th>TTX</th>
</tr>
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<tbody>
<tr>
<td>J Na⁺</td>
<td>5.88±0.43</td>
<td>5.74±0.45</td>
</tr>
<tr>
<td>J Na⁺</td>
<td>0.13±0.57</td>
<td>0.13±0.57</td>
</tr>
<tr>
<td>J Na⁺</td>
<td>5.87±0.41</td>
<td>5.61±0.59</td>
</tr>
<tr>
<td>J Cl⁻</td>
<td>-0.64±0.86</td>
<td>0.17±0.01</td>
</tr>
<tr>
<td>Gt</td>
<td>0.45±24.5</td>
<td>0.61±6.9</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of tissue pairs from 7 fed and 6 fasted piglets. Units are in μeq·cm⁻²·h⁻¹, except for tissue conductance (Gt), which is in mS/cm². Flux samples were taken either before (basal) or after addition of 0.5 µM tetrodotoxin (TTX). J Na⁺ and J Cl⁻ represent absorptions by the mucosal-to-serosal and serosal-to-mucosal flux, respectively; J Na⁺ net, net flux; Gt, integrated short-circuit current (see MATERIALS AND METHODS); J Cl⁻ net, net flux; P values indicate significance of differences between basal and TTX fluxes. NS, P values > 0.05.

Na⁺ or Cl⁻ nor on Gt (Table 1). The sole change in these studies to determine whether cholinergic stimulation could restrict movement of a paracellular marker smaller than inulin. Thus we reasoned that any permeability changes induced by carbachol in these studies would also apply to paracellular movement of larger molecules such as inulin.

Under basal conditions, Gt and mannitol flux were significantly greater in fasted than in fed piglets (Fig. 3, A and B). Addition of 10 µM carbachol to serosal solutions caused a transient increase in basal I sc of 32 ± 3 µA/cm² in fed piglets and 96 ± 3 µA/cm² in fasted piglets (P < 0.05). These changes in I sc reflect increases in active Cl⁻ secretion (10). Gt and mannitol flux were measured 10 and 20 min after addition of carbachol, when I sc had returned to prestimulus levels. Carbachol had no effect on either parameter in fed piglets, but in
enteric neurons. indirectly by release of another neurotransmitter from epithelial muscarinic receptors and not one mediated shown). This indicated a direct effect of carbachol on solutions that were pretreated with atropine (data not showed). The effects of carbachol on paracellular permeability were unchanged in the presence of TTX but were absent in solutions that were pretreated with atropine (data not shown). This indicated a direct effect of carbachol on epithelial muscarinic receptors and not one mediated indirectly by release of another neurotransmitter from enteric neurons.

**DISCUSSION**

Neural control of basal ion transport in fed and fasted piglets. Earlier studies by Nzegwu and Levin (29, 30) suggested that neural influences play a role in the enhanced secretory responses to Escherichia coli heat-stable enterotoxin that are observed after fasting or malnutrition. In the present study we examined whether the altered transport properties of the piglet intestinal epithelium under basal (nonstimulated) conditions are due in part to activity of enteric neurons. We found that blockade of tonic neural activity had different effects on basal $I_{sc}$ depending on nutritional state, because TTX, the muscarinic antagonist atropine, and the nicotinic antagonist mecamylamine all reduced basal $I_{sc}$ to a greater extent in fasted compared with fed animals. The results with the nicotinic ganglionic blocker mecamylamine further indicate that fasting increases the level of synaptic activity in the piglet submucosal plexus, because nicotinic receptors are not found on epithelial cell membranes nor have they been reported on other cells within the mucosa (15). However, because the fall in basal $I_{sc}$ after addition of TTX was much larger than that due to atropine or mecamylamine, other neurotransmitters in addition to acetylcholine are probably involved in the tonic regulation of active ion transport in piglet intestine.

Reductions in basal $I_{sc}$ induced by TTX have been reported in small intestinal tissues of other species (3, 7, 20, 23, 31, 35). In guinea pig ileum, TTX significantly reduced basal $I_{sc}$ only when glucose was absent from the mucosal bathing solution (7, 13). This suggested that in vitro, the chemical composition of the mucosal solution may alter enteric neural activity that influences basal ion transport. Moreover, the nicotinic ganglionic blocker hexamethonium reduced $I_{sc}$ in the absence of mucosal glucose, implicating an intramural reflex pathway in the response (13). In the current study, tissues from both fed and fasted piglets responded to TTX and cholinergic antagonists with reductions in basal $I_{sc}$. The observation that fasting significantly increased those responses suggests an activation or enhancement of a neural reflex pathway that is sensitive to changes in luminal composition. In contrast to the results in guinea pig ileum (13), this effect must have been caused by processes that occurred in vivo, because in the Ussing chamber experiments glucose and other nutrients were absent from mucosal solutions for all tissues. Thus changes in luminal composition in vivo appear to have effects on the expression of neurally mediated ion transport that persist after tissues are harvested.

**Flux experiments were carried out to determine the ionic basis of the change in basal $I_{sc}$ evoked by TTX in piglet intestine. Although TTX reduced basal $I_{sc}$ and increased mucosal-to-serosal $Na^+$ and $Cl^-$ fluxes in fed piglets, there were no significant differences in any net ion fluxes ($Na^+$, $Cl^-$, or $I^R$), probably because of variability in flux values coupled with the relatively small effect of TTX in this group. However, the trend for increased absorption of $Na^+$ and $Cl^-$ after addition of TTX is consistent with the suppression of $Na^+$ and $Cl^-$ absorption by tonic activity of submucosal neurons observed in other species (1, 7, 31, 35).

In fasted piglets, TTX had no effect on unidirectional or net $Na^+$ and $Cl^-$ fluxes. The TTX-induced reduction in the integrated $I_{sc}$ in those tissues was associated with a change in $J^R$ to a significant, negative value. The residual ion flux represents the sum of any active ion movements that are not accountable for by changes in net $Na^+$ or $Cl^-$ transport. Because $K^+$ transport in the jejunum is primarily a passive process, significant changes in $J^R$ are typically attributed to $HCO_3^-$ movement, with a positive value representing $HCO_3^-$ secretion. These results thus provide indirect evidence for a $HCO_3^-$ secretory mechanism (or alternatively, a mechanism that inhibits $HCO_3^-$ absorption) that is regulated by enteric neural activity and unmasked by fasting. Although we did not directly measure changes in
luminal pH induced by TTX in this study, this conclusion is supported by the observation that TTX had no effect on basal $I_{sc}$ when tissues were bathed in HCO$_3$-free solutions. Neurally regulated HCO$_3^-$ transport has been best described for the duodenum (17), although there is evidence for such a mechanism in other intestinal segments. For example, TTX reduced the basal rate of luminal alkalinization in pig jejunal (4) and it significantly reduced J$^R$ in mouse jejunal (35). In addition, application of intraluminal pressure evoked HCO$_3^-$ secretion in rat ileum, an effect that was inhibited by the ganglionic blocker hexamethonium (19).

The physiological significance of a neurally regulated HCO$_3^-$ transport mechanism in the piglet jejunum that is unmasked by fasting is as yet unclear. One possibility, however, is related to the ability of fasting to reduce hormonal stimuli that normally contribute to pancreatic and duodenal HCO$_3^-$ secretion during a meal (22). This may increase the potential for injury to the intestinal mucosal by gastric acid that enters the intestinal lumen under basal conditions or during the contractile phase of the interdigestive migrating motility complex. Thus increased activity of enteric neurons in the fasted state may provide a basal level of HCO$_3^-$ secretion to help protect the mucosa under fasted conditions. Recently, Mellander and Sjövall (28) provided evidence that in the fasted (interdigestive) state in humans, duodenal HCO$_3^-$ absorption may be tonically inhibited by a cholinergic neural pathway. This suggests that release of acetylcholine inhibits HCO$_3^-$ absorption and/or stimulates HCO$_3^-$ secretion in the absence of food in the intestinal lumen. The effect of TTX on J$^R$ in jejunal tissues of fasted piglets may reflect a similar inhibition of cholinergically mediated HCO$_3^-$ transport. Whether the neurally mediated pathway unmasked by fasting represents inhibition of HCO$_3^-$ absorption or stimulation of HCO$_3^-$ secretion will require more detailed studies.

Neural control of paracellular permeability in fed and fasted piglets. As we reported previously (10), the 48-h fast significantly increased ionic conductance of the piglet jejunum as well as the transepithelial flux of the inert marker inulin. Inhibition of ongoing neural activity with TTX had no effect on these indicators of paracellular permeability in fasted piglets, but it significantly increased both inulin flux and $G_t$ in fed piglets. The effect of TTX on $G_t$ in fed animals was observed in both the ion- and inulin-flux experiments, which used separate groups of animals. These results therefore suggest that the permeability of the paracellular pathway under fed conditions is regulated, at least in part, by tonic release of one or more neurotransmitters from submucosal neurons. Moreover, this neural input to paracellular permeability is either reduced by short-term fasting or is opposed by other factors that mask its influence.

There are conflicting reports on the effects of neural activity and specific neurohumoral agents on paracellular permeability in intestinal epithelia. Tetrodotoxin had no effect on $G_t$ or $^{51}$Cr-EDTA flux in canine ileum (27) or in rat jejunum (24). However, the neurotoxin significantly increased $G_t$ in porcine distal jejunum (20), in mouse jejunal (12, 35), guinea pig ileum (7), and rabbit ileum (21). Because the effect of TTX on $G_t$ was absent in the fasted piglets in our study, the nutritional state of animals at the time of death might have contributed to the variability in effects of neural blockade on $G_t$ that were reported in these studies.

Our results suggest that one neurotransmitter that may regulate paracellular permeability in piglet jejunum is acetylcholine, because the cholinergic agonist carbachol partially returned the elevated $G_t$ and fully returned the elevated mannitol flux characteristic of fasted piglets to the lower values observed in fed animals. The cholinergic regulation of paracellular permeability in piglet jejunum is clearly influenced by nutritional state, because carbachol had no effect on $G_t$ or mannitol flux in fed animals. Moreover, carbachol has no effect on $G_t$ in piglets that are fasted and then refed for an additional 48 h (Hayden and Carey, personal observations). These results therefore suggest a model in which a TTX-sensitive neural pathway, possibly involving cholinergic receptors, regulates paracellular permeability under normal (fed) conditions.

Other studies have reported reductions in intestinal tissue conductance after cholinergic stimulation. In the proximal jejunum of older, weaned pigs, carbachol evoked a small but significant reduction in $G_t$ (11). Carbachol also reduced $G_t$ in mouse jejunal, an effect that appeared to be due to enteric neural activity because the effect was abolished in mucosal preparations that were devoid of nerve plexuses (35). On the other hand, the muscarinic agonist bethanechol had no effect on $G_t$ and $^{51}$Cr-EDTA flux in rat jejunum (24), and other reports suggest that muscarinic stimulation increases, not decreases, paracellular permeability (2, 18, 33). The reason for these disparate results is not entirely clear, but could be due to several factors, including differences in species, intestinal segment and type of tissue preparation used, and, as illustrated by the present results, nutritional state of the animals at the time of death.

In summary, this study demonstrates that the neural regulation of active ion transport and paracellular permeability in piglet small intestine is altered by nutritional status. Short-term fasting enhances reflex activity within the submucosal plexus that maintains basal ion transport. Enhanced neural activity induced by fasting appears to promote basal HCO$_3^-$ secretion and/or inhibit HCO$_3^-$ absorption. Tonic neural activity modulates paracellular permeability in the fed state, and acetylcholine is a putative neurotransmitter that contributes to this process. These results highlight the importance of luminal nutrition in the maintenance of normal transport and barrier functions of the intestinal epithelium.

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