Hypotonicity-induced increases in duodenal mucosal permeability facilitates adjustment of luminal osmolarity

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Nylander, Olof, Liselotte Pihl, and Michael Perry. Hypotonicity-induced increases in duodenal mucosal permeability facilitates adjustment of luminal osmolarity. Am J Physiol Gastrointest Liver Physiol 285: G360–G370, 2003—The integrated response to hypotonic NaCl solutions (100, 50, 25, and 0 mM NaCl) in proximal duodenum of anesthetized rats was examined. Luminal alkalinization, fluid flux, duodenal contractions, blood-to-lumen clearance of 51Cr-labeled EDTA (mucosal permeability), and perfusate osmolality were studied in the absence and presence of the cyclooxygenase inhibitor indomethacin. In response to hypotonic solutions net fluid absorption, increases in permeability and perfusate osmolality were markedly higher in indomethacin-treated animals than in controls, and these effects were diminished by the nicotinic-receptor antagonist hexamethonium. Infusion of iloprost, a stable PG12 analog, to indomethacin-treated animals markedly reduced the hypotonicity-induced increase in mucosal permeability and diminished the rise in perfusate osmolality. Lowering the NaCl concentration in the perfusion solution but maintaining isotonicity with mannitol had no effect on mucosal permeability. Very good linear correlations were obtained between the degree of luminal hypotonicity and the increase in permeability and perfusate osmolality. It is concluded that luminal hypotonicity increases duodenal mucosal permeability. The hypotonicity-induced increase in permeability modulated by prostaglandins and nicotinic receptors fulfills the function of increasing blood-to-lumen transport of Na+ facilitating adjustment of luminal osmolarity.

absorption; 51Cr-labeled EDTA; cyclooxygenase; motility; secretion

AFTER WATER INTAKE, THE DUODENAL mucosa is often exposed to a fluid osmolality considerably lower than that of blood. This constitutes a potential threat to the duodenal epithelial integrity and maintenance of the intracellular environment (15). Indeed, perfusion with water has been shown to induce mucosal damage in the distal small intestine but not in the stomach or the duodenum (6). Hence the epithelial in the upper gastrointestinal tract possess unique properties that enable them to confront an extreme hypotonic luminal environment without being damaged.

One important function of the duodenum is to adjust luminal osmolality to that of blood plasma. Basically, there are two mechanisms by which the duodenal epithelium regulates luminal hypotonicity, i.e., absorption of water and secretion of solutes (18, 25). In “leaky” epithelia, such as the duodenum, transepithelial movement of solutes or water is not restricted to the transcellular pathway but can also occur through paracellular pathways (2, 29, 30). The tight junction is the main structure restricting solutes and ions from moving freely across the epithelium by the paracellular route. There is increasing evidence that tight junction permeability is subjected to physiological regulation (1, 14). For instance, luminal glucose as well as neural mechanisms have been shown to modulate rat intestinal mucosal permeability (19, 21, 24, 27).

Laparotomy and handling of the intestine induces inhibition of gastrointestinal motility, referred to as postoperative ileus (4), by a mechanism involving endogenous prostaglandins (23, 28). This, in turn, constitutes a problem when performing experiments in anesthetized animals subjected to abdominal surgery, because an exaggerated production of prostaglandins not only inhibits motility but may affect other physiological functions as well. Support for this is the finding that the administration of the nonselective cyclooxygenase (COX) inhibitor indomethacin to rats subjected to an abdominal operation induces duodenal motility and stimulates duodenal mucosal bicarbonate secretion. Furthermore, these indomethacin-induced responses are blocked by the nicotinic-receptor antagonist hexamethonium, indicating that COX-inhibition increase motility and secretion by a neural mechanism (26).

The aim of the present investigation was to examine the integrated response to hypotonic NaCl solutions in proximal duodenum of anesthetized rats with focus on mucosal permeability and its role in the adjustment of luminal osmolality. Because of their demonstrated affect on duodenal motility and secretion, we also investigated the involvement of endogenous prostaglandins...
and nicotinic receptors in the effects of hypotonic solutions on duodenal function. The preparation was the rat duodenum, perfused via the lumen with hypotonic fluid. Measurements were made of mucosal permeability, Na and fluid fluxes, perfusate osmolality, duodenal contractions and bicarbonate secretion in the absence and presence of indomethacin and/or the nicotinic-receptor antagonist hexamethonium.

**MATERIALS AND METHODS**

**Surgical Procedure**

Male F1 hybrids of Lewis-Dark Agouti rats (Biomedical Center, Uppsala, Sweden), weighing 200–300 g, were fasted overnight but had free access to water. The animals were maintained under constant conditions (12:12-h light-dark cycle; temperature, 21°C). The surgical procedures have been described before (10), and a summary is provided here. Rats were anesthetized with thiobutabarbital sodium salt (Inactin; 125 mg/kg body wt ip) and a cannula was inserted through the pylorus and secured by a ligature 2 cm distal to the pylorus for the collection of duodenal fluid. A PE-320 cannula was inserted into the common bile duct very close to its entrance into duodenum to prevent pancreatic juice from entering the duodenum. Silicone tubing was introduced into the mouth, guided into the stomach and through the pylorus and secured by a ligature 2–5 mm distal to the pylorus. A PE-320 cannula was inserted into the duodenum ~3 cm distal to the pylorus and secured by ligatures. The proximal duodenal cannula was connected to a peristaltic pump, and the segment was perfused with isotonic saline (150 mM NaCl, pH 5–6). To complete the surgery, the abdominal cavity was closed with sutures. After surgery, 1 h was allowed for cardiovascular, respiratory, and gastrointestinal functions to stabilize before experiments were commenced. The Uppsala University Ethics Committee for Animal Experiments approved all protocols.

**Measurement of Duodenal Contraction**

Measurement of the intraluminal pressure assessed duodenal wall contractions. The inlet perfusion cannula was connected via a T tube to a pressure transducer, and intraluminal pressure was recorded on a polygraph. The outlet cannula was positioned at the same level as the inlet cannula. Duodenal contractions were quantified by measuring that fraction of time occupied by contractions (FCT) in 10-min periods. A minimum of two consecutive upward deflections of at least 2 mmHg above baseline was defined as a motor response.

**Measurement of Luminal Alkalization**

The rate of luminal alkalization was determined by back titration of the perfusate to pH 5.00 with 50 mM HCl under continuous gassing (100% N2) by using pH stat equipment (Radiometer, Copenhagen, Denmark). The pH electrode was routinely calibrated with a standard buffer before the start of the titration. The rate of luminal alkalization was expressed as micromoles of base secreted per centimeter of intestine per hour.

**Measurement of Mucosal Permeability**

After completion of surgery, 51Cr-EDTA was administered as an intravenous bolus of 75 μCi followed by a constant infusion at a rate of 50 μCi (in 1.0 ml) per hour. One hour was permitted for tissue equilibration of the 51Cr-EDTA. Three to four blood samples (0.2 ml each) were collected at regular time intervals during the experiment, and the blood volume loss was compensated by injection of 7% albumin solution. After centrifugation, 50 μl of the plasma were removed for measurements of radioactivity. The luminal perfusate and the plasma were analyzed for 51Cr activity in a gamma counter (model 1282, Compugamma CS, PerkinElmer Life Science, Wallac, Upplands Väsby, Sweden). If necessary, a linear regression analysis of the plasma samples was made to obtain a corresponding plasma value for each perfusate sample. The clearance of 51Cr-labeled EDTA from blood to lumen was calculated as described previously and expressed as milliliters per minute and per 100 g wet tissue weight (10). Mean increase in 51Cr-labeled EDTA clearance in response to perfusion with hypotonic solutions for 30 min was determined by the difference between the mean of the two last 10-min hypotonic clearance values and the mean of the two 10-min isotonic saline perfusates obtained before start of the hypotonic perfusion.

**Measurement of Fluid Flux**

The difference in weight of collection vials with and without perfusate was used to measure flow over a 10-min interval. The reproducibility of the peristaltic pump (model Minipuls 3; Gilson, Villiers, Le Bel, France) was checked by collecting perfusate every 10 min for 180 min at nine separate occasions. The mean of the three first samples (4.33 ± 0.07 g) did not differ from the mean of the last three samples (4.31 ± 0.06 g). Perfusate volumes were determined after correction for density for each hypotonic solution. The density of isotonic saline was arbitrarily set to 1.0, and the other solutions were adjusted in relationship to the saline. Hence perfusate weights collected during perfusion with 100, 50, and 25 mM NaCl, H2O, 25 mM NaCl + 250 mM mannitol, and 25 mM NaCl + 50 mM mannitol were multiplied by 1.003, 1.007, 1.010, 1.013, 1.016, and 1.004, respectively. The duodenum was perfused (~0.4 or ~0.1 ml/min) with isotonic saline, and perfusate was collected every 10 min. The net fluid flux across the duodenal mucosa was determined by subtracting the mean of two or three 10-min luminal perfusates from the peristaltic pump volume/10 min and expressed as milliliters of fluid per gram wet tissue weight per hour.

**Measurement of Perfusate Osmolality and Na+ Concentration**

The osmolality of perfusate collected from the duodenum and that of the perfusion solution was determined by means of freezing-point depression by using a micro-osmometer (model 3MO; Advanced Instruments, Needham Heights, MA). The concentration of sodium ([Na+]) in perfusate and perfusion solution was determined by flame photometry (model FLM3; Radiometer). After a 30-min period of isotonic saline perfusion, the duodenum was perfused with 50 mM NaCl for 30 min and perfusate was collected in 10-min samples. The first 10-min hypotonic sample was discarded.

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The mean osmolarity and the mean \([\text{Na}^+]\) of the two last hypotonic 10-min samples were determined. The osmolarity or \([\text{Na}^+]\) value of the perfusion solution was subtracted from the perfusate value yielding the change in osmolarity or \([\text{Na}^+]\) per 10 min. The mean osmolarity and mean \([\text{Na}^+]\) of the 50 mM NaCl solution was 95 ± 1 mosmol/kg H2O and 46 ± 1 mM, respectively \((n = 21)\). The net change in perfusate osmolality and \([\text{Na}^+]\) is expressed as milliosmols per kilogram per H2O per centimeter intestine per hour and millimolar per centimeter intestine per hour, respectively.

**Measurement of Sodium Flux**

Net sodium flux, in response to perfusion of the duodenum with 50 mM NaCl was determined by subtracting the amount of \(\text{Na}^+\) in the perfusate (perfusion volume \(10 \text{ min}^{-1}\cdot[\text{Na}^+]\) in perfusate) from that pumped into the duodenum (peristaltic pump volume \(10 \text{ min}^{-1}\cdot[\text{Na}^+]\) in perfusion solution) and expressed as micromoles per centimeter intestine and per hour.

**Experimental Protocol**

**Perfusion with hypotonic NaCl solutions.** The experiments started with a 30-min period of isotonic saline perfusion, at a rate of \(~0.4 \text{ ml/min}\), and this was followed by perfusion for 30 min with either 100, 50, or 25 mM NaCl, or in some experiments, with ultrapure water. Then the duodenum was perfused with isotonic saline for 40 min. Subsequently, the nonselective COX inhibitor indomethacin was injected intravenously at a dose of 5 mg/kg, and the duodenum was perfused with isotonic saline for another 50 min. After that, the duodenum was once again perfused with the same hypotonic solution for 30 min, and the experiment was terminated after another 30-min period of isotonic saline perfusion.

**Hexamethonium.** The use of hexamethonium, a nicotinic receptor antagonist, elucidated the involvement of these receptors in the physiological responses to hypotonic solutions. In hexamethonium-treated rats, the experimental protocol was the same as that described above, except that the animals were given hexamethonium 20 min before start of perfusate collection. Hexamethonium was given as an intravenous bolus at a dose of 20 mg/kg followed by a constant intravenous infusion at a rate of 20 mg·kg\(^{-1}\)·h\(^{-1}\) throughout the experiment.

**Low-dose indomethacin.** In another series of experiments animals were given indomethacin as an intravenous bolus at a dose of 0.5 mg/kg 30 min before start of perfusate collection. The duodenum was perfused with isotonic saline for 30 min and subsequently with 50 mM NaCl for 30 min. The experiment was terminated by perfusion with isotonic saline for 30 min.

**Perfusion with mannitol solutions.** Animals were pretreated with indomethacin at a dose of 5 mg/kg iv 20–30 min before start of perfusate collection. The duodenum was perfused with isotonic saline for 30 min and then with a solution containing 25 mM NaCl + 250 mM mannitol (315 mosmol/kg H2O) for 30 min. The experiment was terminated by perfusion with isotonic saline for 30 min.

In the second series of experiments, the protocol was the same as above except that the duodenum was perfused with a hypotonic solution (25 mM NaCl + 50 mM mannitol, 99 mosmol/kg H2O) instead of the isotonic mannitol solution described above.

**Perfusate osmolality and \([\text{Na}^+]\).** In four separate series of experiments, the perfusion rate was set to 0.1 ml/min to be able to assess changes in perfusate osmolality and \([\text{Na}^+]\). Hexamethonium (20 mg/kg + 20 mg·kg\(^{-1}\)·h\(^{-1}\)) or indomethacin (5 mg/kg) was administered intravenously 30 min before start of perfusate collection. The duodenum was perfused with isotonic saline for 30 min and subsequently with 50 mM NaCl for 30 min. The experiment was terminated by isotonic saline perfusion for 30 min.

E**ffects of iloprost, a stable synthetic PGF\(_2\) analog, were also tested. The duodenum was perfused with isotonic saline for 50 min and subsequently with 50 mM NaCl for 30 min. The experiment was terminated by isotonic saline perfusion for 30 min. Twenty minutes after commencement of perfusate collection and during the perfusion with isotonic saline, iloprost was administered as an intravenous infusion at a rate of 15 \(\mu\text{g}·\text{kg}^{-1}·\text{h}^{-1}\) throughout the experiment.**

In indomethacin + iloprost-treated animals, indomethacin was given intravenously at a dose of 5 mg/kg 30 min before start of perfusate collection. The duodenum was perfused with isotonic saline for 50 min and subsequently with 50 mM NaCl for 30 min. The experiment was terminated by isotonic saline perfusion for 30 min. Twenty minutes after commencement of perfusate collection and during the perfusion with isotonic saline, iloprost was administered as an intravenous infusion at a rate of 15 \(\mu\text{g}·\text{kg}^{-1}·\text{h}^{-1}\) throughout the experiment.

**Chemicals**

Bovine albumin and hexamethonium chloride were obtained from Sigma (St. Louis, MO). Indomethacin (Confortid) and \(^{51}\text{Cr}\) labeled EDTA were purchased from Dumex, Copenhagen, Denmark, and PerkinElmer Life Sciences Boston, MA, respectively. Inactin was obtained from RBI, Natick, MA, and iloprost was a kind gift from Schering, Berlin/Bergkamen, Germany.

**Statistics**

Values are expressed as means ± SE. The statistical significance of data was tested by analysis of variance with contrast (Fisher’s protected least significant difference test) with comparison of results obtained before, during, and after exposure to hypotonic solutions (repeated measures) or of differences among groups of animals (nonrepeated measures). \(P < 0.05\) was considered significant.

**RESULTS**

**Controls**

The perfusion rate \( (~0.4 \text{ ml/min} \) ), intestinal wet weight, and intestinal length did not differ among the four hypotonic groups (data not shown). Figures 1 and 2 demonstrate the effects of luminal perfusion of the duodenum with 100, 50, 25, and 0 mM NaCl on \(^{51}\text{Cr}\) labeled EDTA clearance, luminal alkalination, and duodenal wall contractions (FCT). During the control period \((0–30 \text{ min})\), there were few duodenal contractions and luminal alkalization was stable. Net fluid flux during the perfusion with isotonic saline varied somewhat among the groups (Table 1). Pooling the net fluid flux data, obtained during the perfusion with isotonic saline, from the 100, 50, 25, and 0 mM NaCl groups yielded a mean of \(0.13 ± 0.22 \text{ ml·g}^{-1}·\text{h}^{-1}\) \((n = 32)\), i.e., a value not different from zero. Perfusion with 50 mM NaCl, but not 100, 25, or 0 mM NaCl, caused a small and reversible decrease \((P < 0.05)\) in luminal alkalization. None of the hypotonic solutions affected FCT. All hypotonic solutions induced net fluid absorp-
tion (P < 0.05), and after cessation of the perfusion with the hypotonic solution, net fluid flux returned to near control values (Table 1). No correlation (P = 0.39, \( r^2 = 0.03 \), and \( n = 32 \)) was found between the degree of luminal hypotonicity (the concentration of NaCl in the perfusion solution) and net fluid flux.

All hypotonic solutions, except 100 mM NaCl, induced a reversible increase in \(^{51}\text{Cr}-\text{EDTA} \) clearance (Figs. 1 and 2) with the greatest increase being observed in response to water (P < 0.05). Regression analysis revealed a fair negative linear correlation (P < 0.05) between the degree of luminal hypotonicity and the mean increase in \(^{51}\text{Cr}-\text{EDTA} \) clearance (Fig. 3A). A weak but significant correlation was found between the hypotonicity-induced net fluid flux and the mean increase in \(^{51}\text{Cr}-\text{EDTA} \) clearance (\( y = 0.14x + 0.14, r^2 = 0.14, P = 0.04 \) and \( n = 32 \)).

**Indomethacin-treated animals.** As demonstrated in Figs. 1 and 2, indomethacin induced or increased duodenal contractions and stimulated luminal alkalinization in all groups. Indomethacin induced a brief increase in fluid secretion (Fig. 4) followed by a decrease to a value not different from control. Changes in fluid flux rates obtained in response to each of the hypotonic solutions were all significantly higher in indomethacin-treated animals than in controls (Table 1). No correlation (P = 0.62, \( r^2 = 0.01 \), and \( n = 32 \)) was obtained between the degree of luminal hypotonicity and net fluid flux. The hypotonic solutions (100, 25, and 0 mM NaCl) did not affect or slightly decreased (50 mM NaCl, P < 0.05) luminal alkalinization. None of the hypotonic solutions affected FCT.

Indomethacin induced a brief increase (P < 0.05) in \(^{51}\text{Cr}-\text{EDTA} \) clearance followed by a decrease to a value not different from controls. All hypotonic solutions induced a reversible increase in \(^{51}\text{Cr}-\text{EDTA} \) clearance. Furthermore, the increase in permeability was markedly larger in the presence of indomethacin than in controls. An excellent negative linear correlation was found between the degree of luminal hypotonicity and the mean increase in \(^{51}\text{Cr}-\text{EDTA} \) clearance (Fig. 3B). No correlation was found between the hypotonicity-induced net fluid flux and the mean increase in \(^{51}\text{Cr}-\text{EDTA} \) clearance (P = 0.68, \( r^2 = 0.01 \), and \( n = 32 \)).
Table 1. Net fluid flux across the duodenal mucosa before, during, and after perfusion with hypotonic saline solutions

<table>
<thead>
<tr>
<th>Treatment Groups, Drug + [NaCl] in the Perfusion Solution, mM</th>
<th>n</th>
<th>Net Fluid Flux, Before Hypo (ml·g⁻¹·h⁻¹)</th>
<th>Δ Fluid Flux, During Hypo (ml·g⁻¹·h⁻¹)</th>
<th>Net Fluid Flux, After Hypo (ml·g⁻¹·h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>7</td>
<td>-1.20 ± 0.25</td>
<td>-2.35 ± 0.36</td>
<td>-0.63 ± 0.18</td>
</tr>
<tr>
<td>Indo + 100</td>
<td>7</td>
<td>-0.93 ± 0.35</td>
<td>-6.02 ± 0.82</td>
<td>-0.30 ± 0.54</td>
</tr>
<tr>
<td>50</td>
<td>10</td>
<td>0.80 ± 0.24</td>
<td>-5.18 ± 0.74</td>
<td>0.71 ± 0.21</td>
</tr>
<tr>
<td>Indo + 50</td>
<td>10</td>
<td>1.43 ± 0.39</td>
<td>-6.88 ± 0.29</td>
<td>0.94 ± 0.68</td>
</tr>
<tr>
<td>25</td>
<td>7</td>
<td>-0.26 ± 0.34</td>
<td>-3.95 ± 0.72</td>
<td>-0.25 ± 0.50</td>
</tr>
<tr>
<td>Indo + 25</td>
<td>7</td>
<td>0.34 ± 0.61</td>
<td>-7.80 ± 0.86</td>
<td>-0.18 ± 0.74</td>
</tr>
<tr>
<td>0</td>
<td>8</td>
<td>-0.03 ± 0.42</td>
<td>-3.43 ± 0.84</td>
<td>0.36 ± 0.35</td>
</tr>
<tr>
<td>Indo + 0</td>
<td>8</td>
<td>0.43 ± 0.31</td>
<td>-4.95 ± 0.68</td>
<td>0.53 ± 0.38</td>
</tr>
<tr>
<td>Hexa + 50</td>
<td>6</td>
<td>0.00 ± 0.22</td>
<td>-2.63 ± 0.46</td>
<td>-0.27 ± 0.13</td>
</tr>
<tr>
<td>Hexa + Indo + 50</td>
<td>6</td>
<td>-0.80 ± 0.23</td>
<td>-3.47 ± 0.31</td>
<td>-1.07 ± 0.19</td>
</tr>
<tr>
<td>Indo 0.5 + 50</td>
<td>8</td>
<td>-0.14 ± 0.40</td>
<td>-6.90 ± 0.50</td>
<td>-0.13 ± 0.28</td>
</tr>
<tr>
<td>Indo +mannitol + 25</td>
<td>6</td>
<td>-0.55 ± 0.60</td>
<td>-7.62 ± 1.11</td>
<td>-0.56 ± 0.52</td>
</tr>
</tbody>
</table>

Values are means ± SE in milliliters per gram per hour; n = number of experiments. The mean net fluid flux values during the perfusion with isotonic saline before and after the exposure of the duodenum to the hypotonic solution are shown. Flux values presented in the column entitled Before Hypo is the mean of the 3 consecutive 10-min values obtained before exposure to hypotonic solutions. Flux values presented in the column entitled After Hypo is the mean of the 2 or 3 consecutive 10-min values obtained 10 min after exposure to the hypotonic solution (see Fig. 4). In the column entitled During Hypo, the change (Δ) in fluid flux in response to the hypotonic solution is shown.

The duodenum was perfused with a hypotonic solution for 30 min. In each group, the fluid flux value was determined by subtracting the Before Hypo net fluid flux value from the mean of the 2 last 10-min net fluid flux values obtained during perfusion with the hypotonic solution. The minus sign depicts net absorption. Note that in all experimental groups, irrespective of treatment, the hypotonic solution induced a significant change in net fluid flux. Indomethacin (Indo) was injected at a dose of 5 mg/kg iv, except in the Indo 0.5 + 50 group where the dose was 0.5 mg/kg. Hexa, hexamethonium; [NaCl], NaCl concentration. *P < 0.05 compared with the Before Hypo flux value in the 50 group (independent samples); †P < 0.05 compared with the Δ Fluid Flux value in the 50 group (independent samples); ‡P < 0.05 compared with the Δ Fluid Flux value in the Indo 0+ group (independent samples); ‡‡P < 0.05 compared with the Δ Fluid Flux value in the Indo + 50 group (independent samples).

**Low Dose of Indomethacin**

The administration of indomethacin at 0.5 mg/kg induced fewer duodenal contractions (mean FCT, 0.23 ± 0.04) than in animals treated with 5 mg/kg (mean FCT, 0.35 ± 0.03). The mean rate of luminal alkalinization was 15.2 ± 2.5 and 13.1 ± 2.0 µmol·cm⁻¹·h⁻¹ (not significant) during the perfusion with isotonic saline and 50 mM NaCl, respectively. The increase in fluid absorption, in response to perfusion with 50 mM NaCl, was the same as that in the high-dose group (Table 1). The mean increase in ⁵¹Cr-labeled EDTA clearance in response to 50 mM NaCl was 1.26 ± 0.38 ml·min⁻¹·100 g⁻¹, lower (P < 0.05) than in the high-dose group (2.30 ± 0.21 ml·min⁻¹·100 g⁻¹) but greater (P < 0.05) than in controls (0.32 ± 0.12 ml·min⁻¹·100 g⁻¹).

**Perfusion with Isotonic and Hypotonic Mannitol Solutions**

Experiments were performed in indomethacin-treated animals to elucidate whether the increase in ⁵¹Cr-labeled EDTA clearance was due to reduction of the NaCl concentration ([NaCl]) or osmolality. In Fig. 5, it is shown that perfusion of the duodenum with an isotonic mannitol solution (25 mM NaCl + 250 mM...
mannitol) had virtually no effect on $^{51}$Cr-labeled EDTA clearance (mean increase was $0.03 \pm 0.04$ ml·min$^{-1}$·100 g$^{-1}$). The mean rate of luminal alkalinization during the 30-min control period was $19.2 \pm 1.6$, which decreased ($P < 0.05$) to $11.9 \pm 1.0$ µmol cm$^{-1}$·h$^{-1}$ (the mean of the 2 last 10-min values during the perfusion with the mannitol solution) in response to the isotonic mannitol solution. The mannitol solution did not affect FCT or net fluid flux (data not shown).

Perfusion of the duodenum with a hypotonic mannitol solution (25 mM NaCl + 50 mM mannitol) markedly increased $^{51}$Cr-labeled EDTA clearance (Fig. 5). The mean increase in $^{51}$Cr-labeled EDTA clearance ($1.85 \pm 0.35$ ml·min$^{-1}$·100 g$^{-1}$) was significantly lower ($P < 0.05$) than that in response to 25 mM NaCl ($3.60 \pm 0.45$ ml·min$^{-1}$·100 g$^{-1}$) but not different from that induced by 50 mM NaCl. The hypotonic mannitol solution did not affect FCT but decreased luminal alkalinization ($P < 0.05$). The change in fluid flux was not different from that seen in response to 50 or 25 mM NaCl (Table 1).

Hexamethonium-Treated Animals

Our laboratory has previously shown (26) that hexamethonium, a nicotinic-receptor antagonist, markedly reduces both the indomethacin-induced duodenal motility and the increase in luminal alkalinization. Furthermore, hexamethonium has been shown to decrease small intestinal fluid secretion induced by a variety of secretagogues (13). It was thus of interest to examine whether hexamethonium could affect the hypotonicity-induced increase in mucosal permeability and fluid absorption.

During the perfusion with isotonic saline, $^{51}$Cr-labeled EDTA clearance and luminal alkalinization were lower ($P < 0.05$) in hexamethonium-treated animals than in controls (Fig. 4). No duodenal contractions were observed. Luminal alkalinization decreased ($P < 0.05$) and fluid absorption increased ($P < 0.05$) in response to perfusion with 50 mM NaCl. The hypotonicity-induced fluid absorption was significantly lower compared with controls (Table 1). Perfusion with 50 mM NaCl did not lower ($P < 0.05$) than that in response to 25 mM NaCl (3.60 ± 0.45 ml·min$^{-1}$·100 g$^{-1}$) but not different from that induced by 50 mM NaCl. The hypotonic mannitol solution did not affect FCT but decreased luminal alkalinization ($P < 0.05$). The change in fluid flux was not different from that seen in response to 50 or 25 mM NaCl (Table 1).

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Hexamethonium-Treated Animals

Our laboratory has previously shown (26) that hexamethonium, a nicotinic-receptor antagonist, markedly reduces both the indomethacin-induced duodenal motility and the increase in luminal alkalinization. Furthermore, hexamethonium has been shown to decrease small intestinal fluid secretion induced by a variety of secretagogues (13). It was thus of interest to examine whether hexamethonium could affect the hypotonicity-induced increase in mucosal permeability and fluid absorption.

During the perfusion with isotonic saline, $^{51}$Cr-labeled EDTA clearance and luminal alkalinization were lower ($P < 0.05$) in hexamethonium-treated animals than in controls (Fig. 4). No duodenal contractions were observed. Luminal alkalinization decreased ($P < 0.05$) and fluid absorption increased ($P < 0.05$) in response to perfusion with 50 mM NaCl. The hypotonicity-induced fluid absorption was significantly lower compared with controls (Table 1). Perfusion with 50 mM NaCl did not lower ($P < 0.05$) than that in response to 25 mM NaCl (3.60 ± 0.45 ml·min$^{-1}$·100 g$^{-1}$) but not different from that induced by 50 mM NaCl. The hypotonic mannitol solution did not affect FCT but decreased luminal alkalinization ($P < 0.05$). The change in fluid flux was not different from that seen in response to 50 or 25 mM NaCl (Table 1).
affect $^{51}$Cr-labeled EDTA clearance or FCT. Effects of indomethacin on duodenal contractions and luminal alkalinization were both markedly attenuated in hexamethonium-treated animals. Furthermore, the hypotonicity-induced increase in $^{51}$Cr-labeled EDTA clearance (mean net increase was 0.23 ± 0.05 ml·min$^{-1}$·100 g$^{-1}$) and fluid absorption was significantly lower than in animals treated with indomethacin alone (Fig. 4 and Table 1).

**Perfusate Osmolality and [Na$^+$]**

In these series of experiments, the perfusion rate was set to 0.1 ml/min to improve detection of changes in luminal composition. The rationale of using iloprost in these experiments was that this stable PGI$_2$ analog has been shown to abolish indomethacin-induced duodenal contractions and to reduce luminal alkalinization (21). We were thus interested in testing whether iloprost, in the absence and presence of indomethacin, could affect $^{51}$Cr-labeled EDTA clearance as well as net fluid flux. No duodenal contractions, a low rate of luminal alkalinization, and no or a slight increase in $^{51}$Cr-labeled EDTA clearance in response to 50 mM NaCl characterized hexamethonium- or iloprost-treated animals (Fig. 6). In contrast, indomethacin-treated animals exhibited duodenal contractions and a high rate of luminal alkalinization throughout the experiment. Furthermore, when exposed to 50 mM NaCl, these animals responded with an increase in $^{51}$Cr-labeled EDTA clearance of similar magnitude as that obtained in indomethacin-treated animals perfused with an identical hypotonic solution but at a fourfold higher rate.

Infusion of iloprost to animals treated with indomethacin reduced FCT and luminal alkalinization (Fig. 6). Furthermore, during the perfusion of the duodenum with isotonic saline, iloprost changed net fluid flux from a basal value of 0.87 ± 0.37 to −1.29 ± 0.21 ml·g$^{-1}$·h$^{-1}$ ($P < 0.05$). This proabsorptive (or antisecretory) effect of iloprost was not seen in the absence of indomethacin. In indomethacin + iloprost-treated animals, the 50 mM NaCl-induced mean increase in $^{51}$Cr-labeled EDTA clearance was markedly lower ($P < 0.05$) than in animals treated with indomethacin alone.

Figure 7 shows the net increase in perfusate osmolality and [Na$^+$] (A and B) as well as the net flux of sodium and fluid (C and D) during the perfusion of the duodenum with 50 mM NaCl in four different treatment groups. In the presence of hexamethonium or iloprost, there was a net increase in osmolality ($P < 0.05$) and [Na$^+$] ($P < 0.05$) in the perfusate, whereas the net flux of sodium was close to zero. Indomethacin caused an increase (relative to iloprost and hexamethonium) in perfusate osmolality and [Na$^+$] ($P < 0.05$), a greater absorption of fluid (Fig. 7D), and a significant net secretion of Na$^+$ during perfusion with 50 mM NaCl (Fig. 7C). The addition of iloprost to indomethacin-treated animals reduced significantly the indomethacin-induced increase in perfusate osmolality and [Na$^+$]. However, the increase in fluid absorption remained the same as that observed with indomethacin alone (Fig. 7D). Iloprost did, however, cause a significant net absorption of Na$^+$ compared with the net secretion observed with indomethacin alone (Fig. 7C).

Very good positive linear correlations were obtained between mean increases in $^{51}$Cr-labeled EDTA clearance and both the mean increase in perfusate osmolality ($y = 34.1 + 18.9x$, $r^2 = 0.79$) and the mean increase in perfusate [Na$^+$] ($y = 13.1 + 9.3x$, $r^2 = 0.71$) (Fig. 8). Figure 9 summarizes the effects of hexamethonium, indomethacin, and indomethacin + iloprost on duodenal motor activity, bicarbonate secretion, paracellular permeability, and the net flux of Na$^+$ and fluid during the exposure of the duodenum to a hypotonic solution.
DISCUSSION

In the present investigation, duodenal mucosal permeability was assessed by measuring the passage of $^{51}$Cr-labeled EDTA from blood to lumen. Since $^{51}$Cr-labeled EDTA disappears rapidly from the circulation (16), mainly by renal elimination, a constant intravenous infusion of $^{51}$Cr-labeled EDTA was required to ensure a relatively stable plasma concentration of this tracer during the experiment. Cr-labeled EDTA has to cross three barriers before reaching the duodenal lumen: the vascular endothelium, the interstitium, and the epithelium. With the use of lymph as an index of interstitial concentration Crissinger et al. (5) found that the lymph-to-plasma concentration ratio of $^{51}$Cr-labeled EDTA approached a value of 1 in $\frac{1}{10^2}$ min after intravenous administration of this tracer. Furthermore, based on data in the literature (9) these authors predicted an osmotic reflection coefficient for $^{51}$Cr-labeled EDTA of 0.002, strongly suggesting that intestinal capillaries offer no or negligible hindrance to the diffusion of $^{51}$Cr-labeled EDTA into the interstitium.

Another factor that may affect the plasma clearance of $^{51}$Cr-labeled EDTA is blood flow. However, it has been shown that the blood-to-lumen clearance of $^{51}$Cr-labeled EDTA is blood flow independent (22, 31). Taken together, these data strongly suggest that the rate-limiting barrier for the blood-to-lumen movement of $^{51}$Cr-labeled EDTA is the intestinal epithelium. Furthermore, the hydrophilic properties and the cross-sectional radius of $^{51}$Cr-labeled EDTA strongly suggest that the paracellular pathway constitutes the predominant route of transepithelial passage for this tracer (3, 12).

In this study, a negative linear correlation was obtained between the [NaCl] in the perfusion solution and the blood-to-lumen clearance of $^{51}$Cr-labeled EDTA in untreated and indomethacin-treated animals, with an excellent correlation in the latter group. Furthermore, data suggest that the increase in blood-to-lumen clearance of $^{51}$Cr-labeled EDTA, in response to the various NaCl solutions, is due to the decrease in luminal osmolality rather than reduction of the luminal [NaCl]. Moreover, no change in duodenal blood flow (as assessed with laser-Doppler flowmetry) was obtained in response to luminal perfusion of the duodenum with 50 mM NaCl in indomethacin-treated rats.
Hypotonic luminal environment

NaCl and water

Hexamethonium

Indomethacin

Indomethacin + iloprost

Very low motor activity

High motor activity

Low motor activity

High HCO₃⁻secretion

Low HCO₃⁻secretion

Net secretion of Na⁺

Net absorption of Na⁺

Low paracellular permeability

Low-to-medium paracellular permeability

Low osmolality-adjusting capability

Low-to-medium osmolality-adjusting capability

Fig. 9. A schematic model showing a summary of the physiological responses to a hypotonic luminal environment in animals treated with hexamethonium, indomethacin, and indomethacin + iloprost. In this model, it is hypothesized that the hypotonicity-induced fluid absorption occurs predominately across the villous tip epithelium, whereas the increase in paracellular permeability to Na⁺ takes place in the basal region of the villous. The following scenario is postulated. In hexamethonium-treated animals, the secretion of Na⁺ balances the absorption. The increase in luminal osmolality is mainly due to water absorption. In indomethacin-treated animals, the secretion of Na⁺ is higher than the absorption. Increase in luminal osmolality is due to the combined effect of a high Na⁺/H⁺ ratio (paracellular) and high water absorption. In indomethacin + iloprost-treated animals, the absorption of Na⁺ is higher than the secretion. The increase in luminal osmolality is due to high water absorption.

(unpublished observation, n = 4). It is therefore proposed that luminal hypotonicity, by some mechanism, induces dilatation of the paracellular shunts, thereby increasing the surface area for diffusion of ⁵¹Cr-labeled EDTA across the duodenal epithelium.

Interestingly, the sensitivity as well as the maximal response of the duodenal mucosa to hypotonicity-induced increases in ⁵¹Cr-labeled EDTA clearance was markedly augmented after indomethacin treatment. This effect appears to be dose dependent since the increase in permeability in response to 50 mM NaCl was smaller and also exhibited a much greater variation in animals treated with 0.5 mg/kg than in those treated with a 10-fold higher dose. Furthermore, the stable prostacyclin analog iloprost diminished the hypotonicity-induced increase in mucosal permeability in indomethacin-treated animals. These data thus suggest that endogenous prostaglandins exert a strong inhibitory effect on the hypotonicity-induced increase in mucosal permeability.

A characteristic feature of the hypotonicity-induced changes in ⁵¹Cr-labeled EDTA clearance was the rapidity of the decrease after cessation of perfusion with hypotonic solutions. Already 20 min after cessation of perfusion with the hypotonic salt solution, regardless of the tonicity of the luminal solution used, ⁵¹Cr-labeled EDTA clearance had returned to near control values. For comparison, it is worth mentioning that 5-min exposure of the duodenum to 100 mM HCl in indomethacin-treated rats resulted in substantial increase in ⁵¹Cr-labeled EDTA clearance that was maintained at a high level at least for 55 min after cessation of the acid perfusion (20). It is therefore postulated that the hypotonicity-induced increase in mucosal permeability reflects a physiological response rather than disturbance of mucosal integrity, which is probably the case for high acid concentrations. Furthermore, the increase in permeability in response to 50 mM NaCl was markedly diminished by nicotinic receptor blockade, suggesting neural involvement and thus physiological regulation.

Although the [NaCl] used in this study seems physiologically pertinent, at least in humans (8), the rate of perfusion might be higher than during physiological conditions. However, reduction of the perfusion rate from 0.4 to 0.1 ml/min did not change the increase in ⁵¹Cr-labeled EDTA clearance in response to 50 mM NaCl. Hence, an increase in duodenal mucosal permeability is likely to occur even when the rate of discharge of hypotonic fluid from the stomach into the duodenum is as low as 0.1 ml/min.

The duodenal mucosa is considered to be a “leaky” epithelium, and as such, it is supposed to allow for passive transport of both electrolytes and water via paracellular routes. A close relationship between the degree of luminal hypotonicity and net fluid flux was thus anticipated. However, no such correlation was found, at least not for the hypotonic solutions tested in the present study. The reason for this lack of correlation is not known but could hint that the hypotonicity-induced fluid absorption is not entirely governed by the osmotic pressure gradient between lumen and blood but also is influenced by prostaglandins and nicotinic receptors. Support for this are the findings that COX-inhibition enhanced hypotonicity-induced fluid absorption and that hexamethonium reduced this response. However, another alternative also has to be considered. In the present study, it is shown that indomethacin induced, or augmented, duodenal wall contractions. These contractions possibly enhance mixing of the luminal bulk fluid with that in the intervillous space, allowing the hypotonic luminal fluid to reach deeper regions of the mucosa, resulting in a larger surface area for fluid absorption (11, 32). Unfortunately, the data in this study do not allow us to distinguish between the two aforementioned alternatives.

It has been shown in frog small intestine in vitro that fluid absorption is associated with dilatation of the lateral intercellular spaces (17). Expansion of these spaces may thus increase the size and the dimension of the paracellular shunts and allow for increased solute permeability. This might be the mechanism by which luminal hypotonicity increases the interstitium-to-lumen movement of ⁵¹Cr-labeled EDTA. If this is the case, it seems reasonable to predict a close relationship between the hypotonicity-induced fluid flux and the increase in ⁵¹Cr-labeled EDTA clearance. The finding...
of a linear correlation, albeit a weak one, between hypotonicity-induced fluid absorption and increases in $^{51}$Cr-labeled EDTA clearance in controls supports this notion. However, no correlation was obtained between these variables in indomethacin-treated animals. Furthermore, infusion of iloprost to indomethacin-treated animals markedly decreased the hypotonicity-induced increase in $^{51}$Cr-labeled EDTA clearance, but the net fluid flux was the same as in animals treated with indomethacin alone. This lack of correlation between net fluid flux and the blood-to-lumen clearance of $^{51}$Cr-labeled EDTA is in agreement with previous permeability measurements in the rat small intestine in vivo (5, 7). Hence increased fluid absorption per se does not appear to be the causative mechanism for the hypotonicity-induced increase in $^{51}$Cr-labeled EDTA clearance. Instead, it is speculated that luminal hypotonicity is “sensed” by some kind of osmoreceptor and that signals transmitted to increase mucosal permeability. The magnitude of the permeability increase is dependent on the degree of luminal hypotonicity in combination with COX activity and stimulation of hexamethonium-sensitive nerves.

What could be the physiological function of the hypotonicity-induced increase in mucosal permeability? In the present study, it is shown that indomethacin-treated animals demonstrated a better capability to adjust luminal osmolality than did animals treated with hexamethonium or iloprost. This difference in the osmolality-adjusting capability may well be related to mucosal permeability, because correlations were obtained between increases in $^{51}$Cr-labeled EDTA clearance and increases in perfusate osmolality or [Na$^+$]. Furthermore, infusion of iloprost to indomethacin-treated animals markedly reduced the hypotonicity-induced increase in $^{51}$Cr-labeled EDTA clearance and at the same time diminished the rise in perfusate osmolality and transformed the duodenum from a net Na$^+$-secretory to a net Na$^+$-absorbing organ. Conceivably, the hypotonicity-induced increase in permeability might fulfill the function of boosting interstitium-to-lumen movement of Na$^+$,facilitating adjustment of luminal osmolality.

In indomethacin + iloprost-treated animals, the osmolality-adjusting capability was somewhat between indomethacin- and hexamethonium-treated animals. It should be noted, however, that within the indomethacin + iloprost group, there were two animals that showed a moderate increase (~1 ml/min $^{-1}$100 g$^{-1}$) in $^{51}$Cr-labeled EDTA clearance when exposed to 50 mM NaCl, whereas the increase was minimal in the other three animals. Excluding those animals with moderate increases in permeability diminished the increase in perfusate osmolality from 51.1 ± 7.6 to 39.0 ± 3.0 mosmol/kgH$_2$O·cm$^{-1}$·h$^{-1}$, i.e., a value not different from that in hexamethonium-treated animals. Hence the reason why indomethacin + iloprost-treated animals showed a somewhat better capability to adjust luminal osmolality than did hexamethonium-treated animals possibly relates to the fact that iloprost failed to abolish the hypotonicity-induced increase in $^{51}$Cr-labeled EDTA clearance in the two experiments referred to above.

In conclusion, luminal hypotonicity increases mucosal permeability, thereby increasing the pore area for diffusion of Na$^+$ across the duodenal epithelium. This mechanism possibly facilitates the osmolality-adjusting capability of the duodenal mucosa. We can also conclude that endogenous prostaglandins, on balance, reduce, whereas activation of nicotinic receptors augments, the hypotonicity-induced increase in permeability, implicating physiological regulation of this process.

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DISCLOSURES

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REFERENCES


