Alteration of Colonic Absorption by Long-Chain Unsaturated Fatty Acids

Influence of Hydroxylation and Degree of Unsaturation

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Unabsorbed dietary unsaturated fatty acids may cause diarrhea in patients with steatorrhea, but their ability to cause colonic fluid secretion is not known. The present study investigated the effect of several dietary long-chain unsaturated fatty acids on colonic absorption and morphology in the rat colon in vivo.

The fatty acids tested induced concentration-dependent net water secretion. The ability of these fatty acids to induce net water secretion varied as follows: linolenic acid (18:3) > linoleic acid (18:2), ricinoleic acid (18:1 OH) > oleic acid (18:1), palmitoleic acid (16:1). Net absorption of sodium and chloride were decreased in fatty acid perfusions. Muco sal activity of sodium potassium adenosine triphosphate and adenylyl cyclase were not significantly altered by fatty acids. Epithelial cell damage was noted and correlated with the ability of the fatty acid to induce fluid secretion. Unsaturated fatty acids induce epithelial cell damage and fluid secretion in the colon, their effect being related to the degree of unsaturation.

Key words: Colon; fatty acids; malabsorption; secretion

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Malabsorption of water by the colon contributes significantly to diarrhea in tropical sprue, a malabsorption syndrome endemic in southern India (1). Water malabsorption in these patients correlates with the fecal levels and output of unsaturated free fatty acids (2). Long-chain hydroxy fatty acids have been incriminated in the genesis of colonic water malabsorption and diarrhea in patients with steatorrhea (3-5). Hydroxy fatty acids have also been shown experimentally to induce colonic fluid secretion (6-9). However, not all patients with steatorrhea have increased fecal levels of hydroxy fatty acids (10, 11). Fecal hydroxy fatty acid levels are not elevated in patients with tropical sprue, whereas large amounts of free unsaturated fatty acids are found in the feces of these patients (11). Limited studies have demonstrated that oleic acid (a mono-unsaturated fatty acid) decreases colonic fluid absorption in vivo (6), but the effects of other unsaturated fatty acids on colonic absorption are largely unknown. The present studies were designed to determine the effect of common dietary long-chain unsaturated fatty acids on absorption, sodium potassium adenosine triphosphatase (Na,K-ATPase) activity, adenylyl cyclase activity, and mucosal morphology in the rat colon in vivo.

MATERIALS AND METHODS

Perfusion studies

Adult white rats of the local inbred strain (200 to 250 g) were starved overnight and then anesthetized with sodium pentobarbital (60 mg/kg intraperitoneally), after which a tracheostomy was performed. After laparotomy, a polyvinyl catheter was introduced into the colon just beyond the cecum. The colon was replaced and the abdominal cavity closed. The colon was flushed with 10 ml of Krebs–Henseleit solution and then perfused continuously at 10 ml/h by means of an LKB peristaltic pump. The colonic effluent was collected through a catheter placed in the rectum. After an hour, collections of effluent were made at 1-h time spans for 3 h. The rats were maintained at 37°C during the procedure. The animals were killed at the end of 3 h, the colon removed, its length measured, and tissue taken from the proximal colon for histology. The mucosa was scraped from the remaining colon and immediately frozen in liquid nitrogen for Na,K-ATPase and adenylyl cyclase assays.

Solutions

Krebs–Henseleit saline (Na,140 mmol/l; K, 5 mmol/l; Ca, 1.2 mmol/l; Mg, 1.2 mmol/l; Cl, 112 mmol/l; HCO3, 25 mmol/l; HPO4 2- 2.4 mmol/l; H2PO4, 0.2 mmol/l; osmolality, 300 mosmol/l) containing polyethylene glycol (PEG 4000, 4 g/l) was used for all perfusions. Sodium taurocholate (3 mmol/l) was added to control solutions and for perfusions with fatty acids. The following fatty acids (concentrations) were used for perfusion: oleic acid (C18:1) (2, 3, and 5 mmol/l), linoleic acid (C18:2) (1, 2, 3, 5 mmol/l), linoleic
acid (C18:3) (1, 2 mmol/l), palmitoleic acid (C16:1) (2, 3, 5 mmol/l), and ricinoleic acid (C18:1 OH) (1, 2 mmol/l).

Assays
Sodium and potassium were estimated by flame photometry. PEG was quantitated turbidimetrically (12). Net water and electrolyte movement was calculated in accordance with standard formulas, and expressed as microliters or micromoles per centimeter length of colon per hour of perfusion. Na,K-ATPase activity was measured in mucosal homogenates as described earlier (11), as the difference between total ATPase and ouabain-resistant ATPase activity. Adenyl cyclase activity was also measured. Mucoza, 25-30 mg, was homogenized in 0.4 ml of ice-cold 50 mM Tris-HCl buffer, pH 7.5. Homogenate, 30 µl, was then incubated at room temperature in 300 µl of solution containing Tris-HCl, 40 mM; MgCl₂, 5 mM; bovine serum albumin, 1 mg/ml; 1-methyl-3-isobutyl xanthine, 1 mM; creatine phosphate, 10 mM; creatine phosphokinase, 0.1 mg/ml; and disodium ATP, 4.5 mM. After incubation for 15 min the reaction was stopped by immersing in a boiling water bath for 3 min. Appropriate blanks were obtained by adding homogenate immediately before stopping the reaction. The assay mixture was centrifuged at 2000 rpm for 10 min, and cAMP determined in the supernatant by means of a cyclic adenosine monophosphate assay kit (TRK 432, Amersham, UK). Homogenate protein was estimated by the method of Lowry et al. (13).

Histology
Sections of 4-5 µm, cut from Bouin-fixed, paraffin-embedded specimens, were stained with hematoxylin–cosin and Alcian blue–periodic acid–Schiff and examined by light microscopy.

Statistics
All values are expressed as mean ± standard error. The Student t test was used to assess the significance of differences between means.

RESULTS
Absorption
In comparison with control perfusions (with and without

<table>
<thead>
<tr>
<th>Water</th>
<th>Sodium</th>
<th>Potassium</th>
<th>Chloride</th>
<th>Na,K-ATPase</th>
<th>Adenyl cyclase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>48 ± 12</td>
<td>21.6 ± 5.3</td>
<td>-3.7 ± 0.4</td>
<td>13.6 ± 1.7</td>
<td>1.40 ± 0.27</td>
</tr>
<tr>
<td>3 mM sodium</td>
<td>63 ± 10</td>
<td>18.4 ± 2.8</td>
<td>-2.2 ± 0.5</td>
<td>12.2 ± 2.4</td>
<td>1.12 ± 0.21</td>
</tr>
<tr>
<td>taurocholate</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Palmitoleic acid, 16:1</td>
<td>65 ± 18</td>
<td>19.4 ± 2.5</td>
<td>-1.0 ± 0.2</td>
<td>22.4 ± 3.0</td>
<td>3.20 ± 1.20</td>
</tr>
<tr>
<td>Oleic acid, 18:1</td>
<td>60 ± 5</td>
<td>19.0 ± 7.0</td>
<td>-1.7 ± 0.3</td>
<td>11.3 ± 2.8</td>
<td>2.90 ± 0.80</td>
</tr>
<tr>
<td>Linoleic acid, 18:2</td>
<td>3 ± 10*</td>
<td>1.0 ± 3.0</td>
<td>-2.6 ± 0.3</td>
<td>3.1 ± 1.3*</td>
<td>2.20 ± 0.50</td>
</tr>
<tr>
<td>Linolenic acid, 18:3</td>
<td>-54 ± 35*</td>
<td>10.6 ± 9.01</td>
<td>-3.6 ± 0.8</td>
<td>-8.5 ± 5.01</td>
<td>1.60 ± 0.38</td>
</tr>
<tr>
<td>Ricinoleic acid, 18:1 OH</td>
<td>1 ± 11*</td>
<td>0.0 ± 2.9*</td>
<td>-2.3 ± 0.5</td>
<td>0.8 ± 1.5*</td>
<td>2.80 ± 0.50</td>
</tr>
</tbody>
</table>

All values are mean ± SE. Net water absorption is expressed in µl/cm/h, and net electrolyte absorption in µmol/cm/h. A – sign indicates net secretion into the lumen of the colon. Na,K-ATPase activity is expressed as µmol pi/mg prot/h, and adenyl cyclase as pmol cAMP/mg prot/h. The symbols after each fatty acid denote the chain length, number of double bonds, and presence of a hydroxyl group.

*p < 0.05; **p < 0.01 compared with control and taurocholate perfusions.
sodium taurocholate), perfusion with fatty acid-containing solutions decreased net absorption of water in a concentration-dependent manner, resulting in net secretion at higher concentrations (Fig. 1). The effects of 2 mmol/l linoleic acid (18:3), 3 mmol/l linoleic acid (18:2), and 5 mmol/l palmitoleic acid (16:1) were roughly equivalent (Fig. 1). Oleic and palmitoleic acids at a concentration of 2 mmol/l did not alter absorption, whereas ricinoleic and linoleic acids significantly reduced net absorption to zero, and linolenic acid induced net water secretion (Table 1). Colonic absorption of sodium and chloride paralleled water absorption, being significantly diminished in perfusions with 2 mmol/l linoleic acid and ricinoleic acids, and reversed to net secretion with 2 mmol/l linolenic acid (Table 1). No significant differences in potassium fluxes were noted between groups (Table 1).

**Mucosal enzymes**

Sodium potassium ATPase activity was mildly increased in fatty acid-perfused rats but did not differ significantly among the various fatty acids (Table 1). Mucosal adenyl cyclase activity was similarly not significantly altered in perfusions with 2 mmol/l concentrations of the various fatty acids.

**Histology**

Fatty acid concentration-dependent colonocyte damage was present at the end of the 3-h perfusion (Fig. 2). Mild changes were shortening of epithelial cell height and variations in the intensity of staining of luminal epithelial cells. In more advanced changes, there was increased exfoliation of the surface epithelium, and the crypt epithelium did not stain properly. At the highest concentrations the surface epithelium appeared to have entirely sloughed off. Inflammatory cell infiltration was not marked, although occasional animals showed neutrophils in the lamina propria. Histologic damage observed with individual fatty acids at equivalent concentrations paralleled their ability to induce changes in colonic absorption of water (Fig. 3).

**DISCUSSION**

The present studies indicate that the dietary unsaturated fatty acids studied and ricinoleic acid (a hydroxy fatty acid) induce colonic fluid secretion in a dose-dependent manner. Differences in secretory effect were noted between individual fatty acids. Their relative potency in inducing secretion was as follows: oleic acid > palmitoleic acid < linoleic acid > ricinoleic acid < linolenic acid. Oleic and palmitoleic acid are both mono-unsaturated fatty acids; linoleic acid has two double-bonds, and linolenic acid has three double-bonds. These results suggest that the degree of unsaturation of the fatty acids determines the magnitude of colonic secretion. The secretory effect of ricinoleic acid, which has both a \( C = C \) double-bond and a hydroxyl group, is equivalent to that of linoleic acid, which contains two \( C = C \) double-
bonds. Hydroxylation and the degree of unsaturation may therefore independently confer secretory properties on the long-chain fatty acids, with an additive effect.

Epithelial cell damage was a consistent observation in these studies. Similar changes in the colonic epithelium have been described with various laxatives, including ricinolic acid (8), doxyl sodium sulfoxocinate (14), bisacodyl (15, 16), Fleet's enema (16), and dihydroxy bile acids at high concentrations (17). The reduced staining intensity with hematoxylin and eosin of the crypt epithelium, resulting in a 'partially erased' appearance, has also been reported with bisacodyl treatment (16). The degree of epithelial damage induced by these fatty acids correlated with the amount of fluid secretion noted.

There are conflicting data on a role for adenyl cyclase activation in colonic secretion induced by hydroxy fatty acids (18). In the present study activation of adenylate cyclase did not appear to be a major factor in colonic secretion induced by unsaturated fatty acids. Fatty acids may also affect colonic permeability (19) and act as calcium ionophores (20), but the role of such changes was not addressed in this study.

Unsaturated fatty acids inhibit sodium potassium ATPase activity in the rat colon in vitro (11). In the present in vivo studies, short-term perfusion of the rat colon caused a decrease in net water and sodium absorption but did not result in altered sodium potassium ATPase activity. Sodium potassium ATPase is located on the basolateral membrane of the epithelial cells and may not be accessible to inhibition by luminal fatty acids when exposed for short periods. In patients with steatorrhea the colonic epithelium is chronically exposed to free fatty acids. It is possible that in these patients uptake of fatty acids occurs in colonic epithelial cells (21, 22), with incorporation into the cell membrane (23, 24). Under these circumstances, inhibition of sodium potassium ATPase may well be an additional mechanism of altered fluid absorption.

The unsaturated fatty acids tested in this study are normally present in the southern Indian diet and are found in large amounts in the stool of patients with tropical sprue and steatorrhea in southern India, whereas hydroxy fatty acids are not significantly increased in the feces of these patients (11). In these patients fecal water output correlated directly with (and colonic water absorption correlated inversely with) fecal unsaturated fatty acid excretion (2). These findings and the present observations suggest that a diet with a high unsaturated/saturated fatty acid ratio may increase diarrhea in patients with malabsorption.

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