Effect of serotonin treatment on intestinal transport in the rabbit

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Donowitz, Mark, Alan N. Charney, and J. Michael Heffernan. Effect of serotonin treatment on intestinal transport in the rabbit. Am. J. Physiol. 232(1): E85-E94, 1977 or Am. J. Physiol.: Endocrinol. Metab. Gastrointest. Physiol. 1(2): E85-E94, 1977.—The hormone serotonin (5-hydroxytryptamine) has been implicated as the cause of the diarrhea seen in many patients with the carcinoid syndrome. To determine whether serotonin is an intestinal secretagogue, the effect of serotonin on intestinal water and electrolyte transport was evaluated in the rabbit. Two weeks of daily subcutaneous injection of serotonin suspended in oil resulted in a blood serotonin level elevated to twice that of controls. Intestinal transport was studied in vivo by a perfusion technique. Serotonin treatment resulted in ileal secretion and decreased mid jejunal absorption of water and electrolytes but did not effect water absorption in the proximal jejunum or colon. Intestinal absorption of D-glucose and the amino acid L-tryptophan and transport was studied in vivo by a perfusion technique. Serotonin treatment resulted in ileal secretion and decreased mid jejunal absorption of water and electrolytes but did not effect water absorption in the proximal jejunum or colon. Intestinal absorption of D-glucose and the amino acid L-tryptophan and glucose-dependent water and electrolyte absorption were normal in serotonin-treated animals. Serotonin-induced ileal secretion was reversed by methysergide, a peripheral antagonist of serotonin action. No alterations in intestinal histology or permeability occurred in serotonin-treated animals. Serotonin-induced intestinal secretion was not associated with alterations in the activities of intestinal mucosal adenylate cyclase, cyclic nucleotide phosphodiesterase, or Na-K-ATPase.

METHODS

Male albino New Zealand rabbits weighing 1.5-2.5 kg were maintained on a standard rabbit chow diet with free access to water. One group of animals received daily subcutaneous injections for 1, 4, or 14 days with 5-hydroxytryptamin creatinine sulfate (serotonin) (Sigma Chemical Co., St. Louis) suspended in peanut oil, 52 µmol/kg. Several of the animals treated with serotonin for 14 days also were treated with the serotonin antagonist methysergide bimaleate (Sansert, supplied in powdered form by Ms. K. D. Roska, Sandoz, Inc., E. Hanover, N. J.), 0.008 µmol/kg, sc, every 12 h during days 13 and 14 (4 doses). A second group of rabbits was injected subcutaneously daily for 14 days with the major urinary metabolite of serotonin, 5-hydroxyindoleacetic acid (5-HIAA) (Sigma) suspended in peanut oil, 52 µmol/kg. Controls were injected subcutaneously daily with creatinine sulfate (Sigma), suspended in peanut oil, 52 µmol/kg.

Transport studies. Fifteen to nineteen hours after the last injection, each animal was anesthetized with sodium pentobarbital (30 mg/kg), and four intestinal loops, each 20 cm in length, were constructed: a proximal jejunal loop began 2-3 cm distal to the ligament of Treitz; a midjejunal loop began just proximal to the sixth arterial branch of the mesentery (approximately 30 cm distal to the ligament of Treitz); an ileal loop began 35 cm proximal to the ileoappendiceal mesenteric attachment; and an ascending colonic loop began 15 cm distal to the cecal-colonic junction. Loops were washed with warmed saline, cannulated at both ends, and returned to the abdomen. Net transport of water, electrolytes, and glucose was then measured in these loops by a modification of the in vivo perfusion technique previously described by us (7). The loops were perfused at a constant temperature (37°C) and rate (0.5 ml/min) with a peristaltic pump (model 1203, Harvard Apparatus Co., Millis, Mass.). Body temperature was maintained at 37°C with a thermocouple-controlled heating lamp. The perfusion study consisted of an initial 150 min during which the animals were perfused with a Ringer-II solution equilibrated with 95% O₂-5% CO₂, containing 140 mM Na, 5.2 mM K, 119.8 mM Cl, 25 mM HCO₃, 1.2

E85
placed in 10% formalin, stained with hematoxylin and eosin, and alcian blue-periodic acid Schiff, and examined by one of the authors. Animals with macroscopic evidence of chronic enteritis were not studied, and data from animals with microscopic evidence of chronic enteritis were discarded. In preliminary studies, the perfusion studies (including the perfusate containing L-tryptophan) did not affect intestinal histology.

Enzyme assays. Mucosa was obtained from perfused intestinal segments by scraping with a glass slide. One part of the mucosa was homogenized with an iced sintered glass homogenizer in a solution containing 75 mM tris(hydroxymethyl)aminomethane and 25 mM MgCl₂ (pH 7.6). The whole homogenate was assayed for adenylate cyclase activity by the method of Krishna, Weiss, and Brodie (30) with minor modifications as previously described (8). All samples were assayed within 1 min of removal of the intestinal segment from the animal, and all animals were alive at the time the intestinal segments were removed. Approximately 50 µg of homogenate protein were incubated for 10 min at 37°C in a solution containing 1.5 mM ATP, 1 µCi (alpha-³²P) ATP (New England Nuclear), 10 mM MgCl₂, 10 mM theophylline, 30 mM tris(hydroxymethyl)aminomethane, 5 mM phospho(enol)pyruvate, 50 µg/ml pyruvate kinase, and 20 µg/ml myokinase. After passage over columns of Bio-Rad (AG50 W-X4) resin (Bio-Rad Laboratories, Richmond, Calif.), and alumina, the radioactive 3',5'-cyclic adenosine monophosphate (cAMP) in the eluate was measured in a Beckman beta counter. Appropriate corrections were made for incubations run without enzyme and for the incomplete recovery of cAMP. Results were expressed as picomoles cAMP formed per milligram protein per 10 min.

Simultaneously, another part of the mucosa was homogenized with a Teflon pestle and iced glass homogenizer in a solution containing 130 mM NaCl, 5 mM Na₂EDTA, 30 mM imidazole, and 2.4 mM sodium deoxycytolate (pH 6.8). The membrane-rich pellet obtained after successive centrifugations at 770 x g and 10,000 x g for 10 min at 0°C (41) was assayed for Na-K-ATPase and Mg-ATPase activities as previously described (7, 8). Approximately 100 µg of pellet protein were incubated for 15 min at 37°C in a solution containing 100 mM NaCl, 20 mM KCl, 10 mM imidazole, 5.4 mM MgCl₂, and 5.4 mM disodium ATP (grade 2, Sigma). The inorganic phosphate (P₁) liberated was measured spectrophotometrically, and results were expressed as micromoles of inorganic phosphate hydrolyzed per milligram protein per hour.

In another series of experiments, mucosal scrapings were homogenized with an iced sintered glass homogenizer in 50 mM tris(hydroxymethyl)aminomethane (pH 7.4). The whole homogenate was assayed for cyclic nucleotide phosphodiesterase activity by a modification of the method of Kimberg et al. (28). Approximately 100 µg of homogenate protein were incubated for 10 min at 37°C in a final volume of 0.4 ml containing 50 mM Tris-HCl buffer (pH 7.4), 5 mM MgCl₂, 2 µM cAMP, and 0.16 µCi[³²P]cAMP. The reaction was terminated by placing the samples in a 95°C bath for 3 min. Water was added to bring the final volume to 1 ml. After the addition of 0.2 ml 250 mM ZnSO₄ and 0.25 ml 300 mM
Da(OH)₂, the samples were centrifuged at 3,500 rpm for 20 min, and the radioactive cAMP in 0.5-ml aliquots of supernatant was counted in a Beckman beta counter. Cyclic nucleotide phosphodiesterase activity was defined as the difference between the initial and final concentrations of cAMP in the incubation mixture and was expressed as nanomoles cAMP per milligram protein per 10 min.

Protein concentrations were determined by the method of Lowry, Rosebraugh, Farr, and Randall (32) using standards of rabbit serum albumin. In preliminary studies, neither the subcutaneous injections of creatinine sulfate or peanut oil nor the perfusion studies affected enzyme activity.

Permeability measurements. The change in transmural potential difference (ΔPD) or streaming potential after a change in perfusate from an isosmolar to an hyperosmolar solution was used to estimate intestinal permeability, as previously described (6, 8, 29). Cannulated ileal loops were prepared as described above. PD was measured by bridges of polyethylene tubing (PE-190) filled with a Ringer-HEPES solution containing 15 mM glucose in 3% agar placed adjacent to the mucosal and serosal surface of each loop. The bridges were inserted into half-cells containing saturated KCl and balanced calomel electrodes, and the PD was measured by a high-impedance DC potentiometer (model 801a, Orion Research, Inc., Cambridge, Mass.). Values were recorded every 5 min for 30 min after a 30-min steady state. The relative intestinal permeability of serotonin-treated and control animals was determined by comparing the ΔPD induced by the addition of 100 mM mannitol to the Ringer-HEPES plus 15 mM glucose perfusate (390 vs. 290 mosmol/kg). In preliminary experiments, the use of the ΔPD streaming potential as an electrical measurement of permeability in the rabbit ileum was validated by examining the effect on PD of a Ringer-HEPES perfusate containing 25 mM EDTA, a substance known to increase intestinal permeability (6).

Ileal loop studies. In a separate series of experiments, two doubly ligated 10-cm ileal loops were constructed as previously described (11) in untreated rabbits and in rabbits treated for 14 days with serotonin. After flushing with warmed saline, 1 ml of normal saline was inoculated into one loop and 50 μg of purified cholera enterotoxin (choleraagen) dissolved in 1 ml of normal saline were inoculated into the second loop. The choleraagen (lot 0792, prepared by R. A. Finkelstein, M.D. [16]) was generously provided by C. E. Miller, D. V. M., from The National Institutes of Health, Bethesda, Md. The loops were then returned to the abdomen. After 5 h, the volume of loop contents and loop length were determined and mucosal adenylate cyclase activity of each loop measured, as described above.

Statistical analyses were performed by the Student t test for paired or unpaired data and were two-tailed; linear regression analysis was performed by the method of least squares (48). All results are expressed as mean ± standard error.

RESULTS

Whole-blood serotonin levels (Table 1). Compared to other species, rabbits have high basal levels of blood serotonin (13, 54). In our series, control values were 4.20 ± 0.35 μg/liter (0.024 ± 0.002 μM). Animals injected daily for 2 wk with serotonin, 52 μmol/kg, had blood serotonin levels twice control levels (8.49 ± 0.37 μg/liter, 0.046 ± 0.002 μM) 24 h after the last serotonin injection. There was no overlap in blood serotonin levels in control and serotonin-treated animals (range 1.28–5.80 μg/liter in controls, 6.53–9.54 μg/liter in serotonin-treated animals). In several animals, blood serotonin levels were drawn just prior to, and at 1, 3, 8, 14, 21, 24, 36, 72, and 96 h after the last injection of the 2-wk serotonin treatment course. In control animals, blood serotonin was constant during this time period; in serotonin-treated animals, blood serotonin levels were constant for 24 h after the last injection. Between 24 and 96 h after the last injection of serotonin, blood serotonin levels declined linearly with a T½ of approximately 50 h. Thus, blood serotonin levels were relatively constant during the 300 min of the perfusion study during which no serotonin was injected.

Effect of serotonin treatment on weight gain, stool water, and serum osmolality, electrolytes, and hematocrit (Table 1). Animals treated with serotonin for 14 days gained a similar amount of weight as control animals. Serotonin-treated animals usually had formed stools, and only an occasional animal developed watery or pasty stools; however, as a group, serotonin-treated animals had an increased percentage of stool water (65*5 vs. 52.1 ± 3.0% (9) in controls, P < 0.05). Serotonin-treated animals were not dehydrated com-

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TABLE 1. Effect of serotonin treatment on blood serotonin level, weight gain, serum osmolality, electrolytes, and hematocrit

<table>
<thead>
<tr>
<th>Animal Group</th>
<th>Blood Serotonin μg/liter</th>
<th>Weight Gain kg/2 wk</th>
<th>Serum Osmolality mosmol/kg</th>
<th>Serum Electrolytes mM</th>
<th>Hematocrit %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.20 ± 0.35</td>
<td>0.26 ± 0.05</td>
<td>307 ± 2</td>
<td>Na (9) 139.0 ± 2.1</td>
<td>45.7 ± 1.7</td>
</tr>
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<td></td>
<td></td>
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<td></td>
<td>K (9) 4.9 ± 0.4</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cl (9) 98.0 ± 2.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CO₂ (9) 16.3 ± 3.0</td>
<td></td>
</tr>
<tr>
<td>Serotonin treated</td>
<td>8.49 ± 0.37</td>
<td>0.21 ± 0.04</td>
<td>310 ± 4</td>
<td>Na (9) 140.7 ± 0.7</td>
<td>44.7 ± 2.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>K (9) 4.9 ± 0.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cl (9) 101.0 ± 0.9</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CO₂ (9) 18.0 ± 1.2</td>
<td></td>
</tr>
<tr>
<td>P²</td>
<td>&lt;0.001</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SE. The numbers of animals studied are in parentheses. * Serotonin-treated animals were treated with 52 μmol/kg per day for 14 days. † P values refer to comparison of control and serotonin-treated animals.
pared to the controls, however, as suggested by the similar serum osmolality, serum electrolytes, and hematocrits in control and serotonin-treated animals.

**Effect of serotonin treatment on intestinal water and electrolyte transport (Fig. 1, Table 2).** As shown in Fig. 1, net water absorption in control rabbits was greatest in the midjejunum, but net absorption also occurred in proximal jejunum, ileum, and ascending colon. Net water secretion was not observed in the jejunum or colon of any control animal and occurred in the ileum of only one control animal. The net movement of individual electrolytes and total solute movement in control animals were similar in midjejunum, ileum, and colon (Table 2). In the proximal jejunum, qualitatively similar but quantitatively less Na and total solute absorption occurred. Net K secretion was observed only in the colon.

Serotonin treatment for 14 days markedly affected net water and electrolyte movement in the midjejunum and ileum, but did not alter water or electrolyte transport in the proximal jejunum or colon (Fig. 1, Table 2). The most striking effect of serotonin was in the ileum, where, in contrast to the net absorption of water and Na seen in control animals, net secretion of water and Na was observed. Net ileal secretion occurred in seven of the eight treated animals. Serotonin treatment caused a significant decrease in net water and Na absorption in the midjejunum, although net secretion of water and Na was observed in only two of the six midjejunal segments. The difference in net water movement between control and serotonin-treated animals, however, was similar in the midjejunum (29.9 ± 8.3 μl/min per cm) and ileum (47.4 ± 12.1 μl/min per cm). In both these segments, in addition, serotonin treatment decreased net K and Cl absorption and increased net residual anion secretion.

**Effect of serotonin treatment on intestinal glucose and L-tryptophan transport (Figs. 2–4).** Because intestinal secretagogues may also affect glucose and amino acid absorption (3, 34, 46), glucose and L-tryptophan absorption were measured in control and serotonin-treated animals. In control animals, glucose absorption from an isotonic perfusate containing 15 mM glucose was similar in the midjejunum and ileum (47.4 ± 12.1 μl/min per cm). In both these segments, serotonin treatment for 14 days did not alter the level of glucose absorption but did increase net residual anion secretion.

**Table 2. Effect of serotonin treatment on net intestinal electrolyte movement**

<table>
<thead>
<tr>
<th>Intestinal Segment</th>
<th>Animal Group</th>
<th>n</th>
<th>Na</th>
<th>K</th>
<th>Cl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>μeq/20 min per cm</td>
<td>μeq/20 min per cm</td>
<td>μeq/20 min per cm</td>
</tr>
<tr>
<td>Proximal jejunum</td>
<td>Control</td>
<td>5</td>
<td>1.98 ± 0.71</td>
<td>0.22 ± 0.02</td>
<td>4.32 ± 1.36</td>
</tr>
<tr>
<td></td>
<td>Serotonin</td>
<td>5</td>
<td>2.10 ± 0.62</td>
<td>0.11 ± 0.08</td>
<td>4.62 ± 1.94</td>
</tr>
<tr>
<td>Midjejunum</td>
<td>Control</td>
<td>7</td>
<td>5.67 ± 1.40</td>
<td>0.26 ± 0.06</td>
<td>7.00 ± 1.50</td>
</tr>
<tr>
<td></td>
<td>Serotonin</td>
<td>6</td>
<td>0.28 ± 0.74</td>
<td>0.15 ± 0.18</td>
<td>2.91 ± 0.72</td>
</tr>
<tr>
<td>Ileum</td>
<td>Control</td>
<td>9</td>
<td>4.10 ± 1.91</td>
<td>0.20 ± 0.06</td>
<td>6.60 ± 1.38</td>
</tr>
<tr>
<td></td>
<td>Serotonin</td>
<td>8</td>
<td>-3.31 ± 1.45</td>
<td>-0.01 ± 0.07</td>
<td>1.85 ± 0.91</td>
</tr>
<tr>
<td>Colon</td>
<td>Control</td>
<td>6</td>
<td>5.91 ± 1.28</td>
<td>-1.01 ± 0.17</td>
<td>6.42 ± 0.47</td>
</tr>
<tr>
<td></td>
<td>Serotonin</td>
<td>6</td>
<td>5.25 ± 2.73</td>
<td>-0.97 ± 0.34</td>
<td>7.86 ± 0.87</td>
</tr>
</tbody>
</table>

Values are means ± SE. Serotonin-treated animals were treated with 50 μmol/kg per day for 14 days. *P* values refer to comparisons of control and serotonin-treated animals for each intestinal segment. A positive sign indicates net absorption from the lumen; a negative sign indicates net secretion into the lumen.

**Fig. 1.** Effect of serotonin treatment on net intestinal water movement. Values were obtained with an isotonic Ringer-HCO₃ perfusate. Results are expressed as mean ± SE. Values above line indicate net absorption; below line net secretion. Number in bar indicates number of animals studied. *P* values refer to comparisons of control and serotonin-treated animals.

**Fig. 2.** Effect of serotonin treatment on net intestinal glucose movement. Values were obtained with a Ringer-HCO₃ perfusate containing 15 mM glucose. Results are expressed as mean ± SE. Values above line indicate net absorption; below line net secretion. Number in bar indicates number of animals studied. *P* values refer to comparisons of control and serotonin-treated animals.

**Fig. 3.** Difference in net water movement: serotonin-treated minus control. Values were obtained with a Ringer-HCO₃ perfusate containing 15 mM glucose. Results are expressed as mean ± SE. Values above line indicate net absorption; below line net secretion. Number in bar indicates number of animals studied. *P* values refer to comparisons of control and serotonin-treated animals.

**Fig. 4.** Effect of glucose on water and electrolyte transport in serotonin-treated animals actually converted the net ileal secretion of water and Na to net absorption. However, the level of net water and Na absorption attained in the presence of glucose did
EFFECT OF SEROTONIN TREATMENT ON INTESTINAL TRANSPORT

**FIG. 2.** Effect of serotonin treatment on intestinal D-glucose (15 mM) and L-tryptophan (5 mM) absorption. Results are expressed as mean ± SE. Number in bar indicates number of animals studied. NS, comparisons of control and serotonin-treated animals.

**FIG. 3.** Glucose-dependent increment in net intestinal water and electrolyte movement in control and serotonin treated animals. Glucose values were obtained with an isotonic Ringer-HCO₃⁻ perfusate containing 15 mM glucose. Results represent increase in net absorption caused by glucose. Results are expressed as mean ± SE. NS, comparisons of controls and serotonin-treated animals.

not reach the level of net water and Na absorption present in control animals perfused with glucose-free perfusate. Thus, serotonin treatment for 14 days affected neither glucose absorption nor glucose-dependent water and Na absorption, although it markedly affected the level of water and electrolyte transport in the absence of luminal glucose.

**Effect of 5-hydroxyindoleacetic acid treatment on intestinal water transport.** To determine whether the effect of serotonin treatment on intestinal transport was related to the serotonin per se, rather than to a metabolic breakdown product of serotonin, 5-hydroxyindoleacetic acid (5-HIAA), the principal urinary metabolite of serotonin, was injected for 14 days in a comparable dose (52 µmol/kg per day). 5-HIAA treatment did not significantly affect net water movement in either the midjejunum (36.9 ± 7.4 (6) µl/20 min per cm vs. 38.4 ± 5.0 (7) in controls) or ileum (22.5 ± 6.4 (6) µl/20 min per cm vs. 27.9 ± 9.1 (9) in controls).

**Effect of methysergide treatment on serotonin-induced alterations in intestinal water transport.** If serotonin per se were responsible for the alterations in intestinal transport, reversal of these effects by a peripheral antagonist of serotonin would be predicted. Methysergide bimaleate (Sansert) is a derivative of LSD and a competitive inhibitor of the peripheral actions of serotonin (13). Methysergide, 0.008 µmol/kg, was injected subcutaneously every 12 h for the 48 h prior to the perfusion studies in rabbits treated with serotonin for 14 days. Net water absorption was observed in both the midjejunum (29.1 ± 1.5 (5) µl/20 min per cm) and ileum (28.6 ± 3.7 (5) µl/20 min per cm) of these serotonin-plus methysergide-treated animals. The level of water absorption in these animals was similar to the level in untreated control animals, indicating that methysergide completely reversed the serotonin-induced alterations in small intestinal water absorption.
Effect of duration of serotonin treatment on intestinal water transport. To determine whether the duration of serotonin treatment altered the serotonin effect on intestinal transport, animals were studied after 1, 4, and 14 days of serotonin treatment. Measurements made 24 h after a single serotonin injection revealed no change in intestinal water transport in midjejunum or ileum as compared to control animals. Serotonin treatment for 4 days decreased jejunal water absorption to a similar extent as the full 14 days of treatment. In contrast, serotonin treatment for 4 days reduced ileal water absorption to a level intermediate between the control level (8.0 ± 4.0 (8) μl/20 min per cm vs. 27.9 ± 9.1 (9) in controls, P < 0.05) and the level of net secretion found after 14 days of serotonin treatment (8.0 ± 4.0 (8) μl/20 min per cm vs. −19.5 ± 8.1 (8), in 14 day-treated animals, P < 0.05).

Effect of serotonin treatment on intestinal histology. The possibility that anatomic alterations in serotonin-treated animals contributed to the alterations in intestinal transport was examined by comparing histologic sections of jejunum, ileum, and colon from serotonin-treated animals and control tissues. No alterations in intestinal histology were apparent by light microscopy.

Effect of serotonin treatment on intestinal mucosal enzyme activities (Figs. 5, 6). Immediately after the intestinal transport studies, the specific activities of adenylate cyclase, cyclic nucleotide phosphodiesterase, Na-K-ATPase, and Mg-ATPase were determined (Fig. 5). Increases in the activities of adenylate cyclase and Na-K-ATPase have been linked to alteration in the intestinal transport of water and electrolytes (7, 8, 15, 27). As in our previous studies (8), adenylate cyclase activity was slightly higher in the ileum than in the jejunum in control rabbits. Cyclic nucleotide phosphodiesterase, Na-K-ATPase, and Mg-ATPase were similar in the jejunum and ileum of control animals. Serotonin treatment for 14 days did not affect the specific activities of any of the four mucosal enzymes measured.

Since the wide range of values in control and serotonin-treated animals might have obscured a significant change in the intestinal mucosal adenylate cyclase activity, we measured this enzyme in individual animals both before and after each animal was treated with serotonin. Six untreated animals were anesthetized, and a surgical mucosal biopsy was obtained from the distal ileum. The biopsied tissue was immediately assayed for adenylate cyclase activity. The animals were allowed to recover from surgery for 1 wk and then all were treated with serotonin for 14 days. The adenylate cyclase activity in the scraped ileal mucosa in these treated animals was compared to the pretreatment values. As shown in Fig. 6, when each animal served as its control, 14 days of serotonin treatment did not affect ileal adenylate cyclase activity.

Effect of serotonin treatment on cholera toxin-induced ileal secretion and activation of adenylate cyclase. Purified cholera toxin was used to provide a positive control for adenylate cyclase activation in rabbits treated with serotonin. Ileal loops in control animals and in animals treated with serotonin for 14 days were incubated with saline and choleragen (50 μg) for 5 h. After this incubation period, loop volume/length ratios and mucosal adenylate cyclase activities were determined (Table 3). In both untreated and serotonin-treated animals, choleragen incubation caused a significant increase in volume/length ratios compared to saline incubation. However, the increase in volume/length ratios caused by choleragen was significantly less in serotonin-treated as compared to untreated animals (0.27 ± 0.13 ml/cm (6)
EFFECT OF SEROTONIN TREATMENT ON INTESTINAL TRANSPORT

E91

vs. 1.14 ± 0.18 (6) respectively, P < 0.005). In both untreated and serotonin-treated animals, incubation of choleragen caused a significant increase in mucosal adenylate cyclase activity as compared to saline incubation. However, the increase in adenylate cyclase activity caused by choleragen was significantly less in serotonin-treated as compared to untreated animals (266 ± 58 (7) pmol cAMP/mg protein per 10 min vs. 560 ± 127 (6), P < 0.05).

Thus, choleragen did provide a positive control for the lack of a serotonin-induced effect on adenylate cyclase activity; however, we were surprised to find that serotonin decreased both choleragen-induced ileal secretion of water and activation of mucosal adenylate cyclase.

**Effect of serotonin treatment on intestinal permeability (Table 4).** An alteration in permeability has been suggested as a possible contributing factor in the pathogenesis of intestinal secretion (17). The effect of serotonin treatment on intestinal permeability was examined by comparing the streaming potential or ΔPD produced by the addition of 100 mM mannitol to the Ringer-HCO₃ perfusate in control and serotonin-treated animals. The method was initially tested in the rabbit ileum by adding 25 mM EDTA to the Ringer-HCO₃ and to the Ringer-HCO₃ plus 100 mM mannitol perfusate. EDTA has been shown to increase intestinal permeability and to prevent the hyperosmolar mannitol-induced increase in transmural PD in the monkey small intestine (29). As shown in Table 4, EDTA also prevented an increase in PD in the rabbit ileum perfused with hyperosmolar mannitol. Since in control and serotonin-treated animals, the hyperosmolar perfusion caused similar increases in ileal PD, we can conclude that serotonin treatment did not alter intestinal permeability at least as evaluated by this technique.

**DISCUSSION**

The hormone serotonin has been considered the likely cause of the diarrhea in the carcinoid syndrome since Lembeck (31) first described serotonin in a carcinoid tumor. To document and characterize the effect of serotonin on intestinal water and electrolyte transport, we developed a rabbit model in which a stable elevated blood level of serotonin was produced. Treatment with serotonin suspended in oil produced a stable blood serotonin level twice that of control animals for as long as 24 h after the last of 14 daily injections. The in vivo perfusion of intestinal loops and assay of intestinal mucosal enzyme activities during this 24-h period provided a comprehensive picture of net water, electrolyte, D-glucose, and L-amino acid transport, transmucosal permeability, and changes in ileal mucosal adenylate cyclase activity.

**TABLE 3. Effect of serotonin treatment on choleragen-induced ileal secretion and adenylate cyclase activation**

<table>
<thead>
<tr>
<th>Animal Group</th>
<th>Volume/Length Ratio</th>
<th>Adenylate Cyclase Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Choleragen&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>0 (6)</td>
<td>1.14 ± 0.18 (6)</td>
</tr>
<tr>
<td>Serotonin treated&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.52 ± 0.10 (6)</td>
<td>0.79 ± 0.08 (6)</td>
</tr>
</tbody>
</table>

P<sup>±E</sup> < 0.005

P<sup>±E</sup> < 0.05

Values are means ± SE. The numbers of animals studied are in parentheses. *Serotonin-treated animals were treated with 52 μmol/kg per day for 14 days. **One milliliter of saline or choleragen (50 μg) was inoculated in 10 cm ileal loops for 5 h. \<sup>±E</sup> Mean increase is the difference between choleragen- and saline-inoculated loops. *P values refer to comparison of saline and choleragen-inoculated loops (paired-t test). **P values refer to comparison of mean increase in control and serotonin-treated animals (unpaired-t test).

**TABLE 4. Effect of serotonin treatment on ileal permeability**

<table>
<thead>
<tr>
<th></th>
<th>ΔPD, mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (5)</td>
<td>1.7 ± 0.3</td>
</tr>
<tr>
<td>Serotonin treated* (5)</td>
<td>1.6 ± 0.9 NS</td>
</tr>
<tr>
<td>EDTA&lt;sup&gt;+&lt;/sup&gt; (4)</td>
<td>0.2 ± 0.3 &lt;0.02</td>
</tr>
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Permeability was estimated by measuring the streaming potential ( ΔPD) induced by the addition of 100 mM mannitol to an isosmotic Ringer-HCO₃ perfusate. Results are expressed as mean ± SE. The numbers of animals studied are in parentheses. P values refer to comparisons with control animals. *Serotonin treated animals were treated with 52 μmol/kg per day for 14 days. +ΔPD caused by the addition of 100 mM mannitol to a Ringer-HCO₃ perfusate containing 25 mM EDTA.
bility, and mucosal enzyme activities in normal and serotonin-treated rabbits.

Untreated normal rabbits exhibited a gradual decline in net water absorption from midjejunum to colon; however, the difference in net water absorption in the absence of glucose between midjejunum and ileum did not reach statistical significance. The lowest level of water absorption was found in the proximal jejunum adjacent to the ligament of Treitz. Qualitatively similar electrolyte transport was observed in all segments of the small intestine and colon and consisted of Na and Cl absorption and residual anion (bicarbonate) secretion. The only qualitative difference in electrolyte movement among the intestinal segments was that net K secretion occurred in the colon. A comparison of separate studies of absorption in normal rabbit midjejunum (21) and ileum (20) by Fromm and co-workers also showed no significant gradient in water and electrolyte absorption between midjejunum and ileum. That rabbit proximal jejunum acts differently than more distal jejunum was also shown by Fromm et al. (21) who demonstrated net water secretion in a somewhat more oral proximal jejunal segment than we studied.

Similar to the absence of a proximal-distal gradient for water absorption in the absence of glucose, we did not observe a difference between midjejunal and ileal p-glucose absorption, L-tryptophan absorption, or glucose-dependent water. Na, and Cl absorption. The ability of glucose to increase water and electrolyte absorption in the rabbit ileum has been reported previously [in vitro (44, 51)] and is similar in the effect of glucose in the rat ileum. However, the transport characteristics of normal rabbit small intestine distinguish it from normal human small intestine (18) by virtue of a) the presence of a less significant proximal-distal gradient for water, Na, and glucose absorption and b) the ability of glucose to increase ileal water and electrolyte absorption.

The chronic elevation of blood serotonin in our study was associated with net ileal secretion and decreased net jejunal absorption of water. That serotonin itself was the intestinal secretagogue was suggested by our findings that a) the ileal secretion produced by serotonin treatment was reversed in animals treated with serotonin followed by its peripheral antagonist methysergide and b) the administration of the major urinary metabolite of serotonin, 5-hydroxyindoleacetic acid, failed to affect intestinal transport. It is possible that serotonin may affect intestinal transport in another mediator. In fact, serotonin does modulate the release of other hormones. Serotonin has been reported to increase the release of growth hormone (2), prolactin (25), and cortisol (40) and decrease the release of LH (24), FSH (25), insulin (14), and glucagon (33). It is possible, therefore, that serotonin may affect the level of other hormones which themselves may mediate the intestinal transport alterations.

The effect of serotonin on the intestinal transport of water and electrolytes was of similar magnitude in the midjejunum and ileum. Although, in our model, net secretion was demonstrated only in the ileum, the difference in net water transport between control and serotonin-treated animals was not significantly different in the midjejunum and ileum. The absence of net secretion in the midjejunum appears to be due to the slightly higher control level of midjejunal water absorption. No effect of serotonin on proximal jejunum or colon was demonstrated in this study. Fromm and co-workers (19, 21) also found that the proximal jejunum immediately adjacent to the ligament of Treitz did not respond to intestinal secretagogues. These investigators studied cholera toxin and theophylline which cause net intestinal secretion of water in association with an increased intracellular cAMP content. The absence of an effect on this segment by serotonin, a secretagogue that does not appear to affect the adenylate cyclase-cAMP enzyme system, suggests that the proximal jejunum in the rabbit is refractory to intestinal secretagogues in a more general sense.

The apparent absence of an effect of serotonin on the colon, on the other hand, is somewhat surprising. Most small intestinal secretagogues that have been examined also exert marked effects on colonic absorption both in vivo and in vitro (15, 17). The normal colonic water and electrolyte absorption in the presence of elevated blood serotonin levels may explain the failure of serotonin-treated animals to develop gross diarrhea. In addition, the colonic conservation of water probably accounts for the equivalent hydration of serotonin-treated and control animals, as judged by their equivalent serum osmolalities, serum Na concentrations, and hematocrits. However, the effect of serotonin treatment on the colonic reserve capacity to absorb water was not directly measured in our studies. The fact that the percentage of stool water was increased in serotonin-treated animals may indicate that colonic compensation was incomplete.

The effect of serotonin on ileal electrolyte movement including net Na secretion, increased residual anion secretion, and decreased Cl and K absorption is similar to that of other intestinal secretagogues studied in vivo. The normal absorption of glucose and the amino acid L-tryptophan and the normal glucose-dependent Na absorption in serotonin-treated animals also are not unique for serotonin as compared to other secretagogues. What does appear to distinguish serotonin from other intestinal secretagogues is that it appears to alter water and electrolyte movement without affecting any of the usually identified mechanisms known to be associated with the production of net intestinal secretion. Altered intestinal mucosal enzyme activities, increased mucosal permeability, increased intraluminal osmolality, decreased absorption of nonelectrolytes, and mucosal histologic abnormalities have all been implicated or suggested as contributing to net intestinal water secretion (1, 15, 17, 39). In serotonin-treated rabbits perfused with an isosmotic solution, we found no intestinal histologic abnormalities by light microscopy, no alteration in absorption of the nonelectrolytes D-glucose and L-tryptophan, and no change in mucosal permeability as judged by a) the change in streaming potential generated by introduction of a hypertonic solution into the lumen and b) the clearance of [14C]mannitol from the blood into the intestinal lumen (unpublished observations).

Activation of the intestinal mucosal adenylate cy-
EFFECT OF SEROTONIN TREATMENT ON INTESTINAL TRANSPORT

clase-cAMP system characterizes such widely divergent groups of intestinal secretagogues as invasive bacteria, bacterial enterotoxins, and gastrointestinal hormones (23, 28). Therefore, the intestinal adenylate cyclase-cAMP system was evaluated in the jejunum and ileum of serotonin-treated animals. We did not observe mucosal adenylate cyclase activation or cyclic nucleotide phosphodiesterase inhibition in these serotonin-treated animals. In addition, to rule out the possibility that the wide range of mucosal adenylate cyclase activity in rabbits might have obscured a significant increase in the adenylate cyclase activity in serotonin-treated animals, we assayed the intestinal mucosal adenylate cyclase activity in individual rabbits both before and after serotonin treatment. No consistent increase in adenylate cyclase activity was noted in these rabbits after serotonin treatment. We conclude that activation of the adenylate cyclase-cAMP system plays no role in serotonin-induced intestinal secretion. This conclusion is similar to that of Schwartz et al. (45) who demonstrated that serotonin failed to increase adenylate cyclase in rabbit ileal mucosa in acute in vitro experiments.

As a positive control for adenylate cyclase activation, we incubated cholera enterotoxin in ileal loops of control and serotonin-treated rabbits. Cholera enterotoxin activated mucosal adenylate cyclase and caused net fluid secretion in both control and serotonin-treated animals. It was of interest that cholera enterotoxin produced a lower level of intestinal secretion, as previously reported (49, 50), and a smaller increase in adenylate cyclase activity in serotonin-treated than in control animals. This suggests that serotonin decreased cholera toxin-induced intestinal secretion at least in part by decreasing the ability of cholera toxin to increase adenylate cyclase activity. The origin and meaning of this effect remain unknown.

Mucosal Na-K-ATPase recently has been shown to be involved in intestinal water and electrolyte absorption (7, 8). In this regard, it is of interest that a gradient of Na-K-ATPase was not found along the rabbit small intestine in our study consistent with the absence of a gradient for water and Na absorption in these animals. In any case, a decrease in Na-K-ATPase activity might have accounted for the transport changes observed in our serotonin model. However, serotonin-treated animals exhibited unchanged, normal mucosal levels of Na-K-ATPase.

We have thus developed a rabbit model for serotonin-induced intestinal water and electrolyte secretion. Many characteristics of this model are similar to the findings in patients with the carcinoid syndrome and diarrhea (10), i.e., both exhibit: a) blood serotonin levels elevated to at least twice normal levels, b) net intestinal secretion or decreased net absorption of water and electrolytes, c) reversal of net intestinal secretion to net absorption by methysergide treatment, d) normal intestinal glucose absorption, and e) normal glucose-dependent intestinal water and electrolyte absorption with conversion by luminal glucose of net intestinal secretion of water into net absorption. We have not defined, however, the mechanism of the serotonin-induced changes in intestinal transport. It would be worthwhile investigating the possibility that alterations in intestinal blood flow, hydrostatic pressure, and/or motility play a pathogenetic role in serotonin-induced intestinal water secretion.

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In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences, National Research Council.

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