Effect of sorbin on electrolyte transport in rat and human intestine

BRUNO ETO, MICHEL BOISSET, BERTRAND GRIESMAR, AND JEHAN-FRANÇOIS DESJEUX. Effect of sorbin on electrolyte transport in rat and human intestine. Am. J. Physiol. 276 (Gastrointest. Liver Physiol. 39): G107–G114, 1999.—Stimulating water absorption in the colon represents an important target to reduce stool output in secretory diarrhea. Recently, a 153-amino-acid peptide was isolated from porcine upper small intestine and purified, taking into account the increase of water absorption in guinea pig gallbladder. Accordingly, this peptide was named sorbin. The aim of the present study was to determine if the COOH-terminal heptapeptide of sorbin (C7-sorbin) participates in the regulation of electrolyte transport in the colon. Different regions (from duodenum to colon) of stripped intestinal mucosa from rats or humans were mounted in Ussing chambers to measure the changes in short-circuit current (Isc) and net Na and Cl fluxes (JNa and JC) after serum exposure of 10−7 to 10−3 M C7-sorbin. In fasted rat intestine, C7-sorbin (10−4 M) induced an immediate reduction in Isc in the distal ileum and proximal and distal colon but not in the duodenum and jejunum. In the colon, Isc reduction and JNa and JC stimulation were dose dependent (EC50 = 2 × 10−5 M). At 10−3 M, maximal effect was observed (∆Isc = −1.14 ± 0.05, ∆JNa = +4.97 ± 1.38, and ∆JC = +9.25 ± 1.44 µeq·h−1·cm−2). C7-sorbin (10−3 M) inhibited the increase in Isc induced by a series of 10 secretory agents such as secretin, vasoactive intestinal peptide, PGE2, and serotonin. In HT-29-C19A cells, C7-sorbin induced an increase in Isc with a maximal effect at 10−3 M (∆Isc = 0.29 ± 0.10 µeq·h−1·cm−2). In human intestine, a dose-dependent decrease in Isc was observed in right and sigmoid colons in basal and stimulated conditions (EC50 = 10−5 M; at 10−4 M, ∆Isc = −2.66 ± 0.17 µeq·h−1·cm−2) but not in the jejunum. The results indicate that C7-sorbin stimulated NaCl neutral absorption and inhibited electrogenic Cl− in rat and human intestinal epithelia. In addition, the antisecretory effect was essentially observed in the distal part of both rat and human intestine and the magnitude of the proabsorptive effect was directly related to the magnitude of the previously induced secretion.

The epithelial cells lining the gastrointestinal tract are the key players in the absorptive and secretory events. Electrolytes are actively transported from lumen to blood or in the opposite direction as a result of the activity of the Na+-K+-ATPase and asymmetric distribution of specific transporters or channels, between luminal and basolateral membranes of the enterocytes. Regulation of intestinal electrolyte absorption or secretion occurs via intracellular messengers that are modulated by various external stimuli, including neurotransmitters. Several of them, including peptide YY (PYY) (9, 20), somatostatin (5, 16, 28), ANG (7, 17), enkephalin (16, 24, 27), and norepinephrine (28), reduce intestinal secretion in physiological and pathological conditions. However, the role of these hormones in stimulating colonic absorption as a response to increased secretion is ill defined.

Sorbin is a peptide of 153 amino acids that was isolated and purified, taking into account the increase in water absorption, from porcine upper small intestine in guinea pig gallbladder (32, 37). In the rat, the COOH-terminal heptapeptide of the natural molecule was found to be the minimal biologically active fragment. The synthetic modified fragment [Pro-Val-Thr-Lys-Pro-Gln-(d-Ala)-NH2 (C7-sorbin)] was found to in-
crease water and electrolyte absorption in the rat duodenum and to decrease intestinal secretion induced by vasoactive intestinal peptide (VIP) perfusion (7, 23, 29). In addition, it reduced water secretion induced by cholera toxin in the rat (J. Fioramonti and L. Bueno, personal communication). Because sorbin has been selected and identified based on its effect on water absorption in the gallbladder, its main activity might be the stimulation of an NaCl neutral absorptive process (18, 21). In addition, if sorbin is mainly active on NaCl absorption, it may be predicted that its effect would be observed in the ileum and the colon where the neutral NaCl transport is primarily located (6).

Thus the aim of the present study was to determine if C7-sorbin participates in the regulation of electrolyte transport in the colon in basal and stimulated secretion conditions. The results indicate that C7-sorbin stimulated NaCl neutral absorption and inhibited electrogenic Cl⁻ secretion in the rat colon. In addition, the antisecretory effect was essentially observed in the distal part of rat and human intestine. The magnitude of the proabsorptive effect was directly related to the magnitude of the previously induced secretion.

MATERIALS AND METHODS

Chemicals

Bumetanide, 5-hydroxytryptamine (serotonin, 5-HT), PGE₂, clonidine, amiloride, TTX, naxalone, and BSA (99% fatty acid free) were purchased from Sigma (St. Quentin-Fallavier, France). Gastrin, VIP, porcine motilin, peptide histidine isoleucine, secretin, helodermin, substance P, rat α-atrial natriuretic factor (1–28), neurotensin, ANG I, PYY, and somatostatin (somatotropin release-inhibiting factor, SRIF), at least 95% pure, were obtained from Neosystem (Strasbourg, France). H-sorbin (C7-sorbin) was a kind gift from Institut Henri Beaufour (Les Ulis, France). Scintillation fluid (Aqua-safe 300+) was purchased from BAI (Elancourt, France). \(^{36}\)Cl (0.2 M, specific activity 0.34 MBq/mg Cl\(^{-}\)) was from BAI (Elancourt, France). \(^{22}\)NaCl (specific activity 23.38 MBq/mg Na\(^{+}\)) was from DuPont NEN (Les Ulis, France). All other chemicals were analytic grade reagents.

Peptide Solutions

Stock solutions (10⁻² M) of C7-sorbin were made with 0.25% (wt/vol) BSA in ultrapure water, stored at −25°C, and thawed immediately before use. Working solutions were prepared extemporaneously at 4°C by serial dilution of stock solutions to achieve final concentrations of 10⁻⁷ to 10⁻³ M when added to serosal medium. All the other solutions of secretory agents were prepared as described above and used at 2 × 10⁻⁷ and 10⁻⁶ M.

Preparation of Tissues

Male mature Sprague-Dawley rats weighing 180–250 g were obtained from Iffa Credo (St. Germain s’Arbresle, France) and housed in individual cages and fed standard laboratory chow (UAR, Villemoisson s’Orge, France) until used in these studies. Food was withdrawn 18 h before the experiments, but animals had free access to drinking water.

For electrophysiology studies, animals were killed by cervical dislocation, and segments of intestine from fasted animals were removed and rinsed free of intestinal content by flushing with ice-cold Ringer solution. The animals’ stomachs were found to be empty. Tissues were stripped off the muscular layer, opened along the mesenteric border, and mounted as flat sheets between the two halves of acrylic Ussing chambers, as previously described (20, 35).

Human tissues were obtained at surgery from digestive cancer patients. Healthy pieces were stored in ice-cold Ringer medium until used (close to 1 h after the tissue was removed). Tissues were prepared as described above and mounted in Ussing chambers.

Short-Circuit Current Studies in Ussing Chambers

The isotonic Ringer solution used throughout the experiments contained (in mM) 115 NaCl, 25 NaHCO₃, 1.2 MgCl₂, 1.2 CaCl₂, 2.4 K₂HPO₄, and 0.4 KH₂PO₄. The pH was 7.40 at 37°C when bubbled with the 95% O₂–5% CO₂ mixture used to circulate the chamber fluid. The Cl⁻-free solution was made by replacing NaCl with sodium isethionate and CaCl₂ and MgCl₂ with the sulfate salts. The standard Na⁺-free solution was prepared by replacing NaCl with choline chloride. The osmolarity of the solution was checked by an osmometer and adjusted close to 300 mosM by the addition of mannitol.

The spontaneous transeptal electrical potential difference, reflecting the asymmetry of electrical charges between the luminal and serosal membranes, was measured via 3 M KCl solution in 4% (wt/vol) agar bridges. These bridges were placed on both sides of the tissue and adapted to calomel half-cells, linked to a high-impedance voltmeter. Potential difference was short-circuited throughout the experiment by a short-circuit current (Isc) via 3 M KCl in agar bridges placed in each reservoir, adapted to Ag-AgCl electrodes in relation with an automatic voltage-clamp system (DVC 1000, World Precision Instruments, Sarasota, FL). Delivered Isc, corrected for fluid resistance, was recorded continuously on a computer. Isc represents the sum of the net ion fluxes transported across the epithelium in the absence of an electrochemical gradient (mainly Na⁺, Cl⁻, and HCO₃⁻). Every 30 s, the tissue was automatically clamped at +1 mV for 3 s to calculate transepithelial electrical conductance, according to Ohm’s law. Peptides were added to the serosal medium after stabilization of Isc.

Effect of C7-Sorbin on Isc

C7-Sorbin was used at both single and cumulative doses. In the single dose experiments, one piece of tissue per chamber was exposed to only one given concentration that ranged from 10⁻⁷ to 10⁻³ M of C7-sorbin, until a new steady state for Isc was obtained. In cumulative dose experiments, one piece of tissue per chamber was successively exposed to increasing doses of C7-sorbin up to the maximal effect of peptide within a 20-min time period (i.e., from 10⁻⁷ to 10⁻³ M). After the maximal decrease in Isc was obtained, 5 × 10⁻⁵ M bumetanide was added to serosal fluid to measure residual electrogenic Cl⁻ secretion (20).

Effect of C7-Sorbin on Na⁺ and Cl⁻ Fluxes

At steady state of the electrical parameters, tissues were paired according to their conductance value (±20%). C7-sorbin was then introduced at 10⁻³ M into the serosal medium. Next, 74 kBq (2 µCi) of \(^{36}\)Cl⁻ or 37 kBq (1 µCi) of \(^{22}\)Na⁺ were introduced into the mucosal or the serosal bath of paired tissues. A 1-ml sample of the serosal or mucosal fluid was withdrawn at 15-min intervals for 60 min and replaced by 1 ml of Ringer solution at 37°C. Scintillation fluid (4 ml) was then added to the samples, which were counted for 10 min. The effects of C7-sorbin on unidirectional mucosal-to-serosal and serosal-to-mucosal Cl⁻ and Na⁺ fluxes were
determined during the steady state of transport (30–60 min). The net Cl$^{-}$ and Na$^{+}$ fluxes were the differences between the opposite unidirectional fluxes obtained on paired tissues. (For comparison, $I_{SC}$ was expressed both in µA/cm$^2$ and in µeq·h$^{-1}$·cm$^{-2}$ and the fluxes of Na$^{+}$ and Cl$^{-}$ were expressed in µeq·h$^{-1}$·cm$^{-2}$.)

Effect of C7-Sorbin on Secretion Induced by Intestinal Peptides

In this experiment, one piece of tissue per chamber was exposed to $10^{-3}$ M of each secretory agent until a new steady state for $I_{SC}$ was obtained. Then, $10^{-3}$ M C7-sorbin was added to the serosal medium.

Statistics

Results are reported as means ± SE. All determinations were performed in pieces of tissue (n) from at least seven rats or three humans. ANOVA was performed according to the general linear model procedure, and comparison of means was by the least-square difference test of the SAS package (SAS Institute, Cary, NC).

RESULTS

Effect of C7-Sorbin in Rat Intestine

Effect of C7-Sorbin on $I_{SC}$. Typical recordings of the effect of C7-sorbin on $I_{SC}$ in rat colon are presented in Fig. 1. In basal condition, addition of $10^{-3}$ M C7-sorbin in the serosal compartment was followed by an immediate and steady decrease in $I_{SC}$ (Fig. 1A). A similar effect was observed (Fig. 1B) after stimulation of $I_{SC}$ by $10^{-4}$ M 5-HT. Further addition of $5 \times 10^{-5}$ M bumetanide in the serosal solution did not decrease the current, strongly suggesting that C7-sorbin entirely inhibited electrogenic Cl$^{-}$ secretion. Previous treatment with naloxone ($10^{-6}$ M) or TTX ($2 \times 10^{-6}$ M) did not alter the response of colon to C7-sorbin.

Effect of C7-sorbin in different segments of rat intestine. A small antisecretory effect of C7-sorbin was found in both duodenum ($\Delta I_{SC} = -2.67 ± 0.67$ µA/cm$^2$) and jejunum ($\Delta I_{SC} = -5.27 ± 0.94$ µA/cm$^2$), as shown in Fig. 2. At $10^{-4}$ M, the effect of C7-sorbin was more marked in the ileum ($\Delta I_{SC} = -8.16 ± 3.37$ µA/cm$^2$, P < 0.05) and even more so in the colon ($\Delta I_{SC} = -17.69 ± 2.82$ µA/cm$^2$, P < 0.001).

Effect of C7-sorbin on $Cl^{-}$ and Na$^{+}$ fluxes in rat colon. The simplest explanation for the reduction in $I_{SC}$ is an inhibition of electrogenic Cl$^{-}$ secretion linked to a stimulation of neutral NaCl absorption that cannot be directly measured by $I_{SC}$. Therefore, fluxes were measured by use of $^{22}$Na$^{+}$ and $^{36}$Cl$^{-}$. Figure 4 shows that C7-sorbin reduced $I_{SC}$ and stimulated Na$^{+}$ and Cl$^{-}$ absorption in a dose-dependent manner. C7-sorbin also induced a dose-dependent decrease in tissue conductance together with an increase in unidirectional fluxes from serosa to mucosa (Table 1). In jejunum, however,
and Cl− fluxes were only noticed at 10−3 M (Table 2).

To confirm the results obtained by Na+ and Cl− fluxes in the colon, the effect of C7-sorbin was assessed on lsc using modified Ringer solution (see MATERIALS AND METHODS). When Na+ was replaced by choline, response to 10−3 M C7-sorbin was completely abolished (Δlsc = 0.67 ± 2.35 µA/cm2 vs. Δlsc = −19.70 ± 4.50 µA/cm2, P < 0.05). Replacement of Cl− by isethionate and sulfate in the medium also almost completely inhibited lsc reduction (Δlsc = −1.0 ± 0.50 µA/cm2 vs. Δlsc = −19.70 ± 4.50 µA/cm2, P < 0.05). These results suggest that the C7-sorbin-induced decrease in lsc could be interpreted as a reduction in electrogenic Cl− secretion together with a stimulation of neutral NaCl absorption.

Effect of C7-sorbin on secretion induced by secretory agents in rat colon. Several secretory agents present in the intestinal mucosa can stimulate Cl− secretion, including 5-HT, PGE2, and VIP. Thus a series of these compounds were first added to the serosal side of stripped colonic mucosa, followed by serosal addition of 10−3 M C7-sorbin. After a peak, lsc returned to a steady value greater than the basal value; further addition of C7-sorbin elicited a decrease in lsc until a new steady state was reached. For most tested compounds, subsequent addition of C7-sorbin was followed by a significant decrease in lsc. The magnitude of the lsc decrease after C7-sorbin was inversely related to the magnitude of lsc stimulated by secretory agents. The relationship between the steady-state lsc obtained after stimulation by secretory agents and the decrease in lsc elicited by further addition of C7-sorbin is presented in Fig. 5.

Comparison of different antisecretory agents in rat colon. The dose responses of four antisecretory agents on lsc were compared in rat colon; EC50 of SRIF was obtained at lower concentrations than that of other antisecretory agents such as PYY, C7-sorbin, and clonidine in the following sequence: SRIF > PYY > C7-sorbin > clonidine. When we compared the maximal effect of these antisecretory agents, the effect of C7-sorbin was similar to that obtained with SRIF, whereas the lowest maximal effect was obtained with clonidine (Fig. 6).

Effect of C7-Sorbin in HT-29 C51 Monolayers

The effect of C7-sorbin was assessed in vitro on human colonic cell line HT-29-C19A at concentrations ranging from 10−6 to 10−3 M (Fig. 7). C7-sorbin did not alter Jsc at concentrations up to 10−5 M. At 10−4 M and 10−3 M, C7-sorbin induced an increase in Jsc (ΔJsc = +0.29 ± 0.10 µeq·h−1·cm−2 at 10−3 M vs. 0.0 ± 0.01 µeq·h−1·cm−2 at 10−5 M; P < 0.05, n = 13) without modification of conductance. The increase in Jsc was not suppressed by pretreatment of cells with 10−4 M amiloride.

Effect of C7-Sorbin on Human Colon

Effect of C7-sorbin on different segments of human intestine. The effect of C7-sorbin was tested on different segments of human intestine (Fig. 8). C7-sorbin dose dependently reduced basal lsc in the colon (right colon and sigmoid), as shown in Table 3. Addition of bumetanide did not further modify the lsc. In the jejunum, C7-sorbin had no effect on lsc.

Effect of C7-sorbin on human colon after stimulation of lsc by VIP. The effect of C7-sorbin was assessed in human colon after stimulation of tissue by VIP (Fig. 9). After lsc stimulation by 2 × 10−7 M VIP, C7-sorbin reduced secretion in human colon. The threshold for the C7-sorbin effect was 10−5 M, but significant reduction in lsc was observed at concentrations over 10−5 M.

DISCUSSION

Our study clearly suggested that C7-sorbin stimulated NaCl neutral absorption and inhibited electrogenic Cl− secretion in the rat colon. In addition, the antisecretory and proabsorptive effects were essentially observed in the ileum and colon, in rat and human intestine. The magnitude of the proabsorptive

### Table 1. Effect of C7-sorbin on unidirectional isotopic Na+ and Cl− fluxes in colonic epithelium in fasted rat

<table>
<thead>
<tr>
<th></th>
<th>JNa,m-1 cm2 s−1</th>
<th>JNa,m−1 cm2 s−1</th>
<th>JC,m-1 cm2 s−1</th>
<th>JC,m−1 cm2 s−1</th>
<th>G, mS/cm2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12.62 ± 0.81</td>
<td>12.83 ± 1.50</td>
<td>15.45 ± 0.91</td>
<td>18.41 ± 1.47</td>
<td>37.14 ± 4.77</td>
</tr>
<tr>
<td>C7-sorbin (10−5 M)</td>
<td>11.21 ± 0.75</td>
<td>9.19 ± 0.80</td>
<td>15.35 ± 0.93</td>
<td>16.44 ± 1.00‡</td>
<td>28.33 ± 2.74</td>
</tr>
<tr>
<td>C7-sorbin (10−4 M)</td>
<td>12.21 ± 1.15</td>
<td>9.13 ± 1.25‡</td>
<td>15.70 ± 1.27</td>
<td>13.02 ± 1.22‡</td>
<td>21.81 ± 2.81‡</td>
</tr>
<tr>
<td>C7-sorbin (10−3 M)</td>
<td>12.55 ± 1.57</td>
<td>6.64 ± 0.53‡</td>
<td>14.00 ± 1.73</td>
<td>9.30 ± 0.50‡</td>
<td>16.40 ± 1.88‡</td>
</tr>
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</table>

Values are means ± SE of 10 rats. All measurements were simultaneously performed on the same animals, i.e., 16 pieces of colonic epithelium per experiment. JNa,m−1 cm2 s−1 and JC,m−1 cm2 s−1, mucosal-to-serosal and serosal-to-mucosal Na+ flux; JC,m−1 cm2 s−1, mucosal-to-serosal and serosal-to-mucosal Cl− flux; G, conductance. *Different from control at P < 0.05; †different from control at P < 0.01; ‡different from control at P < 0.001 [general linear model (GLM) and least-square difference (LSD) test].
effect was directly related to the magnitude of the previously induced secretion.

The proabsorptive effect is constitutive of the peptide sorbin, which was selected from extracts from porcine mucosa on the basis of a capacity to stimulate absorption of water in guinea pig gallbladder (32, 37). The COOH-terminal heptapeptide has mainly been tested in intestinal loops in the rat (7, 23, 29). It was found that C7-sorbin induced absorption of water and electrolytes in duodenum and ileum. In addition, C7-sorbin decreased the VIP-stimulated secretion of water (37%), Na+, K+, and Cl- in duodenum and ileum. In our study using doses of neuropeptide Y, SRIF, and metenkephalin-amide in rat ileum ligated loops (29). In our study using isolated fragments of intestine mounted in Ussing chambers, we have confirmed the antisecretory and proabsorptive effects of C7-sorbin in the intestine of rats.

We also have extended the previous observations in terms of site and mechanism of action and relationship with the secretory status of the intestine. The present results clearly indicate that the C7-sorbin effect displays an aboral gradient: the decrease in $I_{sc}$ was hardly observed in the duodenum and jejunum, and in the ileum it was 46% lower than that observed in the colon. The present results might explain why large numbers of animals were necessary to demonstrate an effect of sorbin in the small intestine (7, 23, 29). The proabsorptive effect of sorbin in the colon is not unique, as several other agents have been found to display similar effects, including PYY (9, 20), somatostatin (5, 16, 28), ANF (7, 17), enkephalin (16, 24, 27), and norepinephrine (28). However, the present study clearly indicates that the main effect of sorbin is located in the distal part of the rat and human intestine.

The aboral gradient for the effect of sorbin on electrolyte transport may be due to several factors, including the distribution for the sorbin receptors and effectors. Very little is known about the receptors. In recent experiments, no specific binding for sorbin was found in basolateral membranes of intestinal epithelial cells (M. Laburthe and T. Voisin, personal communication). In terms of cellular effectors, the effect of sorbin on stimulating water absorption in the gallbladder pointed to the possibility of an effect on a neutral NaCl absorptive process (18, 21, 37). We thus measured on the same pieces of tissue the electrical parameters and the Na+ and Cl- isotopic transepithelial fluxes in absence of a transepithelial electrochemical gradient. Also, because the coefficients of variation are commonly much greater for the isotopic fluxes than for the electrical parameters, the determinations were made at four different concentrations of C7-sorbin, i.e., on 16 adjacent pieces

### Table 2. Effect of C7-sorbin on short-circuit current, conductance, and transepithelial Cl⁻ fluxes in the jejunum of fasted rats

<table>
<thead>
<tr>
<th></th>
<th>$I_{sc}$ μeq h⁻¹ cm⁻²</th>
<th>$J_{Cl}^{net}$ μeq h⁻¹ cm⁻²</th>
<th>$J_{Cl}$ μeq h⁻¹ cm⁻²</th>
<th>$J_{Cl}$ μeq h⁻¹ cm⁻²</th>
<th>G, mS/cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.55 ± 0.06</td>
<td>22.02 ± 2.85</td>
<td>28.21 ± 2.79</td>
<td>−6.19 ± 2.67</td>
<td>56.58 ± 2.44</td>
</tr>
<tr>
<td>C7-sorbin (10⁻⁵ M)</td>
<td>0.37 ± 0.04</td>
<td>15.43 ± 1.76</td>
<td>21.83 ± 2.86</td>
<td>−6.39 ± 1.70</td>
<td>50.60 ± 8.62</td>
</tr>
<tr>
<td>C7-sorbin (10⁻⁴ M)</td>
<td>0.59 ± 0.14</td>
<td>19.30 ± 2.94</td>
<td>27.78 ± 3.65</td>
<td>−8.48 ± 1.76</td>
<td>66.62 ± 9.62</td>
</tr>
<tr>
<td>C7-sorbin (10⁻³ M)</td>
<td>0.28 ± 0.09*</td>
<td>24.20 ± 2.76</td>
<td>26.06 ± 3.75</td>
<td>−2.58 ± 2.03*</td>
<td>62.82 ± 5.93</td>
</tr>
</tbody>
</table>

Values are means ± SE of 56 tissues from 7 rats (8 pieces per animal). $J_{Cl}^{net}$ = net Cl⁻ flux (l $J_{Cl}^{net}$ − l $J_{Cl}$). *Different from control at $P = 0.05$ (GLM and LSD test). Minus (−) indicates secretion from serosa to mucosa.

![Fig. 5. Inhibition of $I_{sc}$ stimulation in response to secretory agents by $10^{-3}$ M C7-sorbin in rat proximal and distal colon. Circles and vertical bars represent means and SE of 4 tissues from 4 rats. The following concentrations were used: $2 \times 10^{-7}$ M vasoactive intestinal peptide (VIP), $5 \times 10^{-7}$ M ANG II (ANG), $10^{-6}$ M motilin (MOT), $10^{-6}$ M substance P (SP), $10^{-6}$ M gastrin (GAS), $10^{-7}$ M neurotensin (NT), $2 \times 10^{-7}$ M secretin (Sec), $10^{-6}$ M atrial natriuretic factor (1—28) (ANF), $2 \times 10^{-6}$ M PGE₂, $10^{-6}$ M 5-HT, $2 \times 10^{-7}$ M peptid histidine isoleucine (PHI), and $2 \times 10^{-7}$ M helodermin (Helod). Magnitude of $I_{sc}$ decrease after $10^{-3}$ M C7-sorbin (on the ordinate axis) is plotted against magnitude of secretory agent-induced $I_{sc}$ (on the abscissa axis). See MATERIALS AND METHODS for details.](image_url)

![Fig. 6. Comparison of dose-response profiles of various antisecretory agents on $I_{sc}$ in rat colon. Squares and circles represent mean percent decrease in $I_{sc}$ induced by bumetanide (7 tissues from 4 rats). Clo, clonidine; PYY, peptide YY; SRIF, somatostatin (somatotropin release-inhibiting factor). See MATERIALS AND METHODS for details.](image_url)
of colon or jejunum in the same experiment. The results confirmed that the colon is the main target for sorbin, whereas isotopic fluxes were not significantly modified in the jejunum. In the colon, a dose-dependent stimulation of the neutral NaCl absorption together with a smaller reduction in electrogenic Cl\(^{-}\) secretion was observed. The results obtained after substitution of Na\(^{+}\) or Cl\(^{-}\) indicate that Na\(^{+}\) and Cl\(^{-}\) are required for the effect of C\(_7\)-sorbin. In addition, comparison of the ionic unidirectional fluxes with the transepithelial conductance further strengthened the possibility that sorbin stimulates electrolyte absorption (transepithelial conductance expressed in mS/cm\(^2\) is similar to the sum of the diffusional ionic fluxes measured in the absence of an electrochemical gradient and expressed in µeq·h\(^{-1}·cm\(^{-2}\)) (13). Thus the change in conductance could be compared with the change in the unidirectional ionic fluxes. In the present experiments, conductance decreased with increasing sorbin concentrations; concurrently, the unidirectional ionic fluxes from serosa to mucosa decreased, suggesting a reduction in transepithelial ionic diffusion, whereas the flux in mucosa to serosa was not significantly altered. This may indicate a decrease in the diffusional flux compensated for by an increase in the nondiffusional flux from

<table>
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<tr>
<th>C(_7)-sorbin (M)</th>
<th>Right colon</th>
<th>Sigmoid colon</th>
<th>Midjejunum</th>
</tr>
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<tbody>
<tr>
<td>10(^{-6}) M</td>
<td>2.50±2.22(\mu)A/cm(^2)</td>
<td>2.10±2.22(\mu)A/cm(^2)</td>
<td>2±1.21(\mu)A/cm(^2)</td>
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<tr>
<td>10(^{-5}) M</td>
<td>14.6±7.03(\mu)A/cm(^2)</td>
<td>0.9±1.59(\mu)A/cm(^2)</td>
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</tr>
<tr>
<td>10(^{-4}) M</td>
<td>-45±7.43(\mu)A/cm(^2)</td>
<td>-36.83±6.17(\mu)A/cm(^2)</td>
<td>2.95±1.52(\mu)A/cm(^2)</td>
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<tr>
<td>10(^{-3}) M</td>
<td>21.05±0.71(\mu)A/cm(^2)</td>
<td>-8.30±7.03(\mu)A/cm(^2)</td>
<td>7.15±2.44(\mu)A/cm(^2)</td>
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</table>

Values are means ± SE of 4 (right colon and midjejunum) or 6 (sigmoid colon) tissues. *Different from control at P < 0.05; ‡ different from control at P < 0.001 (GLM and LSD test); § not different from 0 at P = 0.05. Cumulative amounts of C\(_7\)-sorbin were successively added to one tissue to reach concentrations indicated.
mucosa to serosa compatible with a stimulation of a neutral NaCl absorptive flux. Although it was beyond the scope of these experiments to identify the molecular structures supporting the NaCl absorption and the Cl− secretion, the literature suggests that Na+/H+ exchanger (NHE3) and cystic fibrosis transmembrane conductance regulator (2, 22) transporters are good candidates for membrane effectors that support the proabsorptive and antisercretory effects of sorbin.

Interestingly, we observed a similar aboral distribution in the human intestine. This may indicate a similar distribution for the ionic transporters involved in the effect of sorbin in the rat and human intestine. Thus sorbin extracted from porcine intestine is also active on rat and human intestine. However, these results do not indicate the presence of sorbin or its receptors in the human intestine. When we measured the effect of C7-sorbin on HT-29-C19A, an intestinal epithelial cell line derived from a human colon cancer patient, it came as a surprise that the I_{sc} did not decrease but increased at a relatively high C7-sorbin concentration (10−4 M). This may indicate that sorbin does not act directly on the intestinal epithelial cells, but, like the many mediators located in the lamina propria, sorbin may interact with different cell types that control electrolyte transport by the epithelial cells (3, 8, 10, 12). However, neither naloxone nor TTX altered the C7-sorbin effect.

One of the most intriguing results is the effect of sorbin after secretion was stimulated: the intensity of the effect of sorbin appeared to be directly related to the intensity of the secretion previously induced by a variety of agents (Fig. 5). The physiological consequence of a regulatory link between absorption and secretion is obvious when considering the movement of water and electrolytes in the intestine after a meal (14, 34). During a meal, a large quantity of water enters the intestinal lumen as a result of the volume of the meal and the meal-induced secretion by the salivary glands, stomach, pancreas, biliary system, and intestine. Several hormones participate in the meal-induced secretion (30). Water and electrolytes thus secreted in the intestinal lumen are reabsorbed by the intestine, and the stimulation of intestinal absorption must be quantitatively adjusted to the amount secreted; the mechanism of meal-stimulated absorption involves the nutrients of the meal that stimulate electrolyte absorption by different mechanisms (4, 11, 14, 15, 25). In addition, several hormones display antisercretory and proabsorptive effects (5, 7, 9, 16, 17, 20, 24, 28). PYY acting in the upper part of the intestine is secreted in the blood from colonic cells at the beginning of the meal (19). However, it does not seem to display antisercretory effects until 1 h after the beginning of the meal (19). What is striking about sorbin is the link between the intensity of secretion and the intensity of the sorbin response. This suggests that if sorbin is a natural constituent of the human intestine it may play an important role in the economy of water and electrolyte handling. In addition, in diarrheal diseases, the symptoms may be reduced by stimulating colonic absorption in response to secretion. This has already been suggested for human cholera (36) and viral infection in piglets (1). In addition, sorbin has been found to display a beneficial effect in cholera toxin-induced diarrhea in the rat (J. Fioramonti and L. Bueno, personal communication).

At the cellular level, stimulated Cl− secretion has been found to be associated with a stimulated glucose and Na+ absorption (31). In cAMP-induced Cl− secretion, it has been suggested that the number of Na+/glucose transporter (SGLT1) molecules responsible for the glucose-Na+ cotransport is increased in the luminal membrane (26). In addition, it has been reported that methylated casein, an antidiarrheal drug, exhibits antisercretory activity only in cholera-treated rabbit ileum and has no effect in control conditions (33). However, we have no direct indication that a similar mechanism exists to explain the sorbin effect.

Finally, for the following reasons, the present results suggest that C7-sorbin is a good candidate for use in the reduction of water and electrolyte losses in diarrheal diseases: 1) it is mainly active in the distal part of the intestine; 2) its proabsorptive effect is apparent after intestinal secretion has been stimulated; 3) the intensity of its proabsorptive effect is grossly proportional to the intensity of previously induced secretion by a variety of mediators; 4) the size of the heptapeptide makes it feasible for synthesis in industrial environments; 5) there is no evidence of tachyphylaxis; the dose-response curves were similar when cumulative doses were given to the same tissue and when one dose was given to one tissue; and 6) the results of the dose-effect relationship may point to a limitation; apparently, in this in vitro system, the EC_{50} is one order of magnitude higher than other peptides, such as PYY and SRIF, that have antidiarrheal properties. However, the maximum effect appeared to be essentially the same as that of the most active peptides.

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