J. Pharm. Pharmacol. 1993, 45: 951–954
Received January 19, 1993

Sennoside-induced Secretion is Not Caused by Changes in Mucosal Permeability or Na^+,K^+-ATPase Activity

ELKE LENG-PESCHLOW

Department of Pharmacology, Madaus AG, Ostmerheimer Str. 198, D-5000 Köln 91, Germany

Abstract—The effect of sennosides (50 mg kg^{-1}) on the rat colon in-situ was studied 6 h after oral treatment when the laxative effect was maximal. In a second experiment, rhein (4×10^{-3} M), an active sennoside metabolite, was administered into the lumen of the colon for 1 h. Both sennosides and rhein reduced net H_2O and Na^+ absorption or reversed it to net secretion. Paracellular permeability, as measured using erythritol as a small marker molecule, was increased 2- to 3-fold; permeability to a large molecule, PEG 1000, was unchanged. The activity of Na^+, K^+-ATPase in the colon mucosa was not affected. There was no damage of the epithelial cells as determined by lactic acid dehydrogenase release. These results indicate that neither inhibition of Na^+,K^+-ATPase nor damage of the colon epithelium are involved in the secretory effect of sennosides or rhein. The increased paracellular permeability of small molecules fits into the concept of stimulation of active chloride secretion by sennosides, which is electrochemically and osmotically balanced by an increase in Na^+ and H_2O flow via the paracellular pathway.

Sennosides are the active constituents of *Cassia angustifolia* Vah. and *Cassia acutifolia* Del. The leaves and pods of these species are used as laxatives. The laxative effect is due to changes in colon motility and secretion. It has been shown that the motility changes induced by sennosides occur earlier than the stimulation of fluid secretion (Leng-Peschlow 1986) and that the increase in fluid secretion alone is not sufficient to induce acceleration of transit and the laxative effect (Van Hoestenberghe et al 1991, 1992). Thus, the effect of sennosides on large bowel motor function is not a secondary process due to fluid secretion and accumulation as frequently interpreted, but an independent mechanism.

Nevertheless, an increase in fluid secretion contributes to the laxative effect of sennosides. The secretory effect of many laxatives such as bisacodyl, phenolphthalein, ricinoleic acid, docusate sodium and magnesium sulphate has been associated with damage or inflammation of the intestinal epithelium and an increase in mucosal permeability (Bright-Asare & Binder 1973; Cline et al 1976; Gullikson et al 1977; Wanitschke 1980a; Farack & Nell 1984). Another mechanism proposed is the inhibition of the mucosal Na^+,K^+-ATPase thereby blocking active Na^+ absorption (Rachmilewitz et al 1980; Schreiner et al 1980; Wanitschke 1980b). For sennosides, insufficient data are available concerning either of these possible mechanisms of action. Therefore, paracellular and cellular permeability of the colon epithelium and mucosal Na^+,K^+-ATPase activity were studied in rats after oral treatment with sennosides or local treatment with the active metabolite rhein.

Materials and Methods

Animals

Female Wistar rats, 200 g, were maintained under standard environmental conditions (room temperature 24 ± 1°C, relative air humidity 50 ± 2%, light/dark cycle 12/12 h). Commercial pelleted rat diet was freely available but was removed 20 h before the absorption studies. Water was available throughout. Ten to fifteen rats were used in each experimental group.

Absorption studies

Animals were anaesthetized with pentobarbitone sodium 50 mg kg^{-1} intraperitoneally. The colon was cannulated at the caecocolic junction and rinsed with 50 mL warm physiological saline. A second cannula was inserted proximal to the rectum. Two millilitres of an electrolyte solution was introduced into the colon. The electrolyte solution consisted of (g L^{-1}): NaCl 6·72, KCl 0·37, $NaHCO_3$ 2·1, PEG 4000 2·0 and [^{14}C]PEG 4000 (5 μCi L^{-1}) or [^{3}H]PEG 4000 (50 μCi L^{-1}) as a nonabsorbable fluid marker. pH was adjusted to 6·5 and the osmolality was 290 mOsmol kg^{-1}. After an incubation time of 1 h, the colon contents were removed, their volume was measured and the length of the colon segment determined. For analysis of the ATPases, the epithelial cells were isolated according to the methods of Stern (1966) and Weiser (1973).

For measurement of paracellular permeability (Farack & Nell 1984), both renal pedicules were ligated and [U-^{14}C]erythritol (mol. wt 125, 1 μCi per rat) together with non-labelled erythritol (2×10^{-3} M physiological saline, 0·5 mL per rat) was injected via the tail vein 10 min before the start of colon incubation. A blood sample was taken from the retro-orbital plexus immediately before the start of the incubation and a second blood sample after incubation. In a different experiment a larger molecule, 1,2-[^{3}H]PEG, mol. wt 1000 (6·25 μCi per rat), mixed with non-labelled PEG 1000 (2 g L^{-1} physiological saline) was used as a paracellular marker instead of erythritol.

In-vitro studies

Colon epithelial cells were isolated from rats fasted for 20 h (Stern 1966; Weiser 1973). After a final centrifugation (380 g for 5 min at 4°C), cells from the whole colon were resuspended in 10 mL Tris-HCl buffer (pH 7·5) at 4°C and homogenized (Potter, Braun, Melsungen, Germany). The homogenate was incubated with rhein in Tris-HCl buffer

(final concentrations 0, 10^{-4} or 10^{-3} M) for 10, 30, 60 and 120 min at 37° C. An aliquot of the incubation solution was used for the assays of total ATPase, Mg^{2+}-ATPase and protein.

Analyses and calculations

Radioactivity in serum and incubation solution was measured by liquid scintillation counting with Insta-Gel as scintillator. Na^+ was analysed in the incubation solution by flame photometry, and LDH (lactic acid dehydrogenase, E.C.1.1.1.27) enzymatically (Roche, Basel, Switzerland). Net H_2O, Na^+ and LDH transport (mL, μmol or m units h^{-1}/10 cm colon) were calculated according to standard formulae (Leng-Peschlow 1980). The clearances (CL) of erythritol and PEG 1000 during the 1 h incubation period were calculated as follows (Farack & Nell 1984):

$$CL\ (\mu L) = \frac{C_{IE}}{(C_{SB}+C_{SA})/2} \times \frac{V_{IE}\ (\mu L)}{\text{Incubation time (h)}}$$

and related to a colon length of 10 cm. C_{IE} is the concentration (d min^{-1}) of radioactivity in the colon solution after incubation, C_{SB} and C_{SA} the concentrations of radioactivity in the serum before and after incubation, and V_{IE} the volume of the colon solution after incubation.

Total ATPase and Mg^{2+}-ATPase activities were measured in a homogenate of the isolated epithelial cells (Tris-HCl buffer, pH 7·5) according to Quigley & Gotterer (1969) and Schiffl & Loeschke (1977) and the Na^+,K^+-ATPase calculated as the difference between total and Mg^{2+}-ATPase. Protein was determined in the homogenate according to Lowry et al (1951).

Treatments

Rats were orally treated with sennosides (50 mg kg^{-1}), 6 h before the start of the colon incubation. It has previously been shown that 6 h after oral treatment, the laxative effect is at its maximum (Leng-Peschlow 1989). The very high dose of 50 mg kg^{-1} was chosen to induce a clear secretory effect and a pronounced diarrhoea.

In another set of experiments, an active metabolite of the sennosides, rhein, was used for local treatment. Rhein (as a sodium salt for better solubility) was administered with the incubation solution in a concentration of 4×10^{-3} M. Both drugs were obtained from the Chemical Department of Madaus AG, Köln, Germany.

Statistics

Mean values and standard deviations are given. Treatment groups were compared with control groups and statistical significance was assessed by Student's *t*-test. In the case of unequal variances, the Welch test was used.

Results

Oral treatment with sennosides

Sennosides (50 mg kg^{-1}) reversed net fluid and Na^+ absorption in the rat colon into net secretion 6 h after treatment (Fig. 1). These results were accompanied by a threefold increase in paracellular permeability for erythritol. Release of LDH, used as an intracellular marker, into the colon solution was, however, not influenced. The sennosides slightly decreased the activity of the total and Mg^{2+}-ATPase

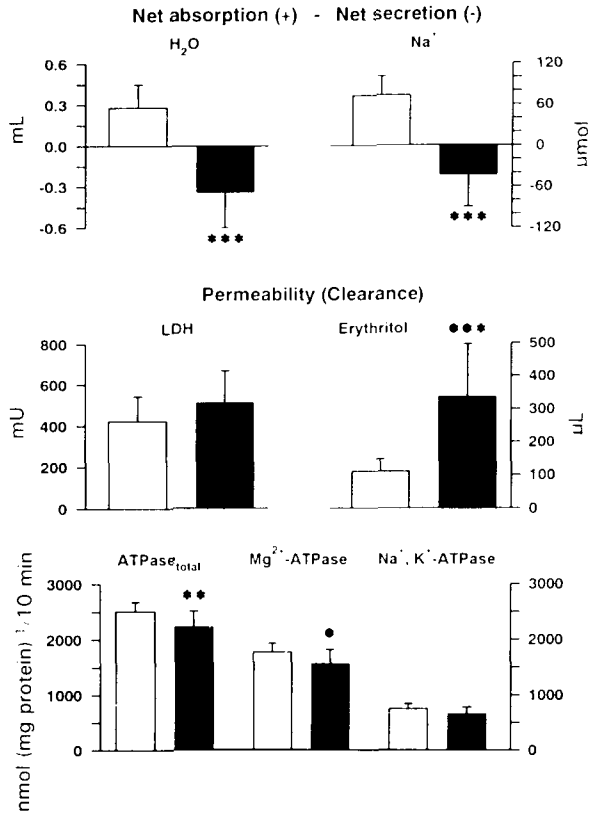

FIG. 1. Effect of oral treatment with sennosides (50 mg kg^{-1}, 6 h before colon incubation) on net transport of water and sodium, release of LDH as an intracellular marker, and clearance of [^{14}C]erythritol as a marker of paracellular permeability and on activities of total, Mg^{2+}- and Na^+,K^+-ATPase activities in rat colon in-situ (mean values ± s.d., 11–13 rats per group). ■ Sennosides, □ control. $*P \leq 0{\cdot}05$, $**P \leq 0{\cdot}01$, $***P \leq 0{\cdot}001$ compared with the control group.

in the colon epithelium, the Na^+,K^+-ATPase activity was not significantly affected.

Local treatment with rhein

Exposure of the colon epithelium to rhein led to a 50% decrease in net fluid and Na^+ absorption (Fig. 2). Permeation of erythritol, as a small marker molecule, from the blood into the colon lumen was twice that in untreated controls, whereas there was no difference with the larger molecule PEG 1000. LDH release from the epithelial cells was not significantly increased by rhein. The activity of the mucosal Mg^{2+}-ATPase and the activity of the Na^+,K^+-ATPase were also not influenced.

Effect of rhein on ATPase activities in-vitro

In isolated colon epithelial cells there was a time-dependent loss of ATPase when incubated at 37°C for up to 120 min (Table 1). Addition of rhein during incubation did not increase this loss for Mg^{2+}-ATPase and Na^+,K^+-ATPase, i.e. rhein in both concentrations has no inhibitory effect in-vitro on these ATPases.

Discussion

The present studies show that the secretory effect of the sennosides and their metabolite rhein in the colon mucosa is

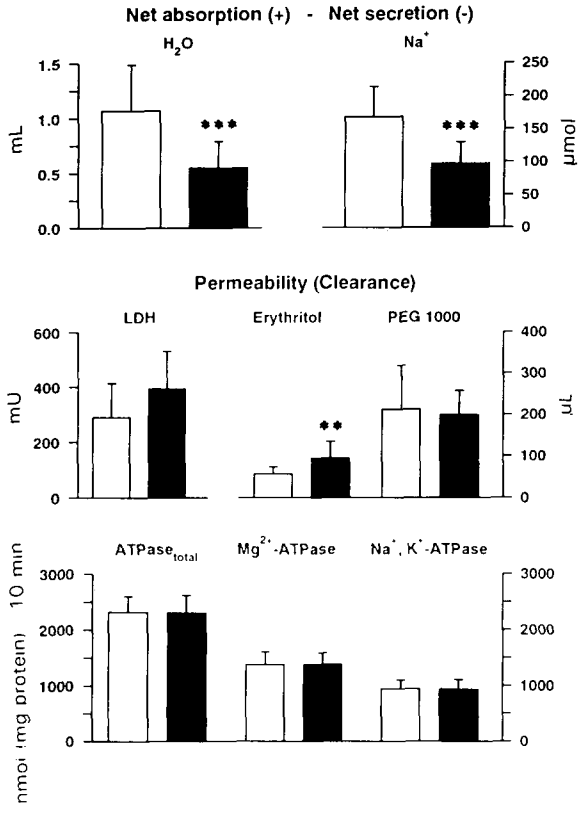

FIG. 2. Effect of rhein (4×10^{-3} M) administered locally into the rat colon for 1 h on net transport of water and sodium, release of LDH as an intracellular marker and clearances of [^{14}C]erythritol and PEG 1000 as markers of paracellular permeability and on total, Mg^{2+}- and Na^+, K^+-ATPase activities in incubated rat colon in-situ (mean values ± s.d., 10–13 rats per group). ■ Rhein, □ control. $*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$ compared with the control group.

accompanied by an increase in paracellular permeability for small molecules but not by an inhibition of the Na^+,K^+-ATPase. These findings are in contrast to results of other authors.

Table 1. Effect of rhein on ATPase activities (nmol (mg protein)$^{-1}$/10 min) in isolated colon epithelial cells of the rat (mean ± s.d., n = 5–7 in each group).

Rhein (M)	Incubation time (min)	Total ATPase	Mg^{2+}-ATPase	Na^+/K^+ ATPase
0	0	2336 ± 361	1643 ± 350	693 ± 139
	10	2013 ± 165	1299 ± 247	714 ± 168
	30	1938 ± 81*	1243 ± 168	695 ± 129
	60	1799 ± 222*	1259 ± 195	540 ± 95
	120	1764 ± 145**	1129 ± 204	524 ± 135
10^{-4}	0	1994 ± 373	1323 ± 301	664 ± 183
	10	2006 ± 96	1294 ± 146	711 ± 115
	30	1990 ± 65	1314 ± 114	676 ± 117
	60	1883 ± 196	1348 ± 233	535 ± 63
	120	1802 ± 144	1272 ± 211	530 ± 97
10^{-3}	0	2071 ± 330	1474 ± 260	618 ± 104
	10	1880 ± 116	1186 ± 162	694 ± 163
	30	1872 ± 58	1180 ± 145	692 ± 98
	60	1690 ± 192	1111 ± 184	579 ± 107
	120	1491 ± 167**†	998 ± 167*	492 ± 113

$*P \leq 0.05$, $**P \leq 0.01$ compared with the 0 min value within the same treatment series. $\dagger P \leq 0.05$ compared with the corresponding incubation time of the control group (no rhein).

In rat colonic mucosa in-vitro, rhein (2×10^{-4} M) administered in the mucosal solution completely inhibited the Na^+, K^+-ATPase activity measured in mucosal scrapings after an incubation time of 2 h (Wanitschke & Karbach 1988). Despite a higher concentration of rhein in our studies, an inhibitory effect could not be confirmed in-vivo. The same negative results were obtained with mucosal scrapings instead of isolated cells (not shown). In addition, direct exposure to rhein of isolated colon epithelial cells from untreated rats also did not significantly inhibit Na^+, K^+-ATPase or Mg^{2+}-ATPase. As oral pretreatment with sennosides also did not influence the ATPases, inhibition of Na^+, K^+-ATPase leading to a decrease of active Na^+ absorption is obviously not involved in the mechanism by which sennosides and rhein exert their secretory action. Stimulation of active Cl^--secretion is the main mechanism responsible for rhein-induced secretion (Clauss et al 1988; Goerg et al 1988).

The primary event in stimulation of active Cl^--secretion seems to be a change in the conductive permeability of the luminal membrane, resulting in a selective opening of Cl^--channels (Field et al 1989). To compensate for the increase in negative charges at the luminal side due to Cl^- secretion, Na^+ (and also H_2O for osmotic equilibrium) follows passively via the paracellular pathway. The increased paracellular permeability for small molecules found in the present study during sennoside or rhein-induced secretion fits this well-acknowledged model. In contrast, in-vitro studies did not confirm an increase in paracellular permeability by rhein but indicated that rhein makes the mucosa even tighter, at least under conditions of higher hydrostatic pressure on the serosal side (Karbach & Wanitschke 1984; Wanitschke & Karbach 1988). These differences between in-vivo and in-vitro studies are not easily explained, but in this case may be a question of concentration. The increase in paracellular permeability by rhein in-vivo is restricted to small molecules, as larger molecules (as represented by PEG 1000) are not able to penetrate.

There has been much discussion about whether laxatives damage the intestinal mucosa and if those cytotoxic effects are involved in the secretory process and the laxative action. Cytotoxic effects have been found for bisacodyl, ricinoleic acid, and dioctyl sodium sulphosuccinate (Saunders et al 1975, 1977; Gaginella et al 1977; Meisel et al 1977). A recent study showed that castor-oil leads to an increased production of the platelet-activating factor (PAF) in the intestinal tissue and an increased release of acid phosphatase (AP) concentration in luminal contents indicating intestinal inflammation and damage (Pinto et al 1989). Long-term treatment of rats or mice with sennosides or senna extracts did not, however, show any histologically or electronmicroscopically detectable changes or cell damage of the intestinal mucosa (Dufour & Gendre 1984, 1988; Mengs 1988). Neither oral pretreatment with sennosides nor local administration of rhein, both drugs in a very high dose, lead to a significant leakage of lactate dehydrogenase (LDH), another sensitive biochemical marker of cell damage. Mascolo et al (1992) showed that a senna pod extract administered orally to rats once or daily for up to 90 days did not stimulate PAF production or enhance release of AP into the lumen. The same was found for rhein and rhein anthrone administered locally in doses comparable to ours. Studies in man confirm

that, in contrast to danthron and phenolphthalein, senna did not increase α_1-antitrypsin clearance indicating that there was no enhanced intestinal protein loss or leakage of macromolecules (Nataf et al 1981; Émeriau et al 1983).

In conclusion, in-vivo studies with oral sennosides in a very high dose or with rhein administered locally into the colon lumen gave no evidence that these drugs cause intestinal cell damage of any pathological significance. The increase in paracellular permeability for small molecules fits into the concept of electrochemical and osmotic balance of active Cl^- secretion into the lumen. An inhibition of the Na^+, K^+-ATPase as suggested from in-vitro studies seems not to be involved in the secretory action of sennosides.

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