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Inhibition of intestinal water and electrolyte absorption by senna derivatives in rats

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Three intestinal segments were simultaneously perfused with Tyrode solution in the anaesthetized rat using a perfusion rate of 12 ml h^{-1}. Dried purified senna extract containing 60% Ca-sennosides A + B (I) administered with the perfusion fluid had no or only minimal effects on intestinal absorption. Large doses (4 g $litre^{-1}$) slightly reduced net Na, Cl and H_2O absorption predominantly in the colon and the ileum; the jejunum was unaffected. Dried purified senna extract containing 18% oxidized Ca-sennosides (II) reduced net Na, Cl and H_2O absorption in all three intestinal segments, the effects increasing from the jejunum to the colon. Large doses (4 g $litre^{-1}$) induced net Na and H_2O secretion. II being administered with the colon perfusion fluid affected H_2O and electrolyte absorption in the colon and to a lesser degree, in the ileum. Pure rhein (III, 0·28 g $litre^{-1}$) had similar effects to II. Pure sennosides A + B (IV, 2 g $litre^{-1}$) had no effect on intestinal absorption. These results confirm that changes in the absorptive behaviour of the gut mucosa are, at least partly, responsible for the laxative action of anthraquinone drugs and that this effect is mainly dependent on the presence of oxidized derivatives. There is evidence that the drug's effect is not locally restricted to the colon as is seen from the ileal response after intracolonic administration. Side-effects like griping which are sometimes observed after senna treatment seem to originate from motility changes rather than from changes in absorption.

Senna glycosides stimulate colonic peristalsis after having been broken into aglycones and converted into pharmacologically active derivatives by the microorganisms of the large intestine (Hardcastle & Wilkins 1970; Okada 1940). They also inhibit colonic water and sodium absorption in cats and rats (Straub & Triendl 1934; Lemmens 1976; Lemmens & Borja 1976). It has been shown that senna extracts containing Ca-sennosides or oxidized Ca-sennosides, given by mouth, rapidly affect not only the motility pattern of the colon, but also of the upper gastrointestinal tract of dogs and rats (Garcia-Villar et al 1980). I have now examined whether comparable influences exist on small intestinal absorption.

To facilitate comparison of the different intestinal parts, jejunal, ileal and colonic segments were simultaneously perfused in the same rat. Test substances were added to the perfusion fluid of all segments or to the colonic perfusion solution only, to establish possible effects deriving from the colon on the absorption of water and solutes in the small intestine. Rhein was chosen as reference anthraquinone substance because of its ability to inhibit colonic water absorption (Lemmens & Borja 1976). In addition, the effect of pure sennosides A + B in non-salt form was examined.

METHODS

Animals

Female Wistar rats, 200–220 g, were used for the experiments. Food (Ssniff, Versuchstierdiäten-GmbH, Soest) was withheld 24 h before the experiments, but water was freely available.

Method

Rats were anaesthetized with urethane (25%; 0·5 ml per 100 g body wt. i.p.) and tracheotomized. The abdomen was opened with a midline incision and 3 intestinal segments in each animal were prepared for perfusion by inserting 2 silicone tubes for inflow in the proximal end of the segment (internal diameter 1·5 mm) and for outflow in the distal end (internal diameter 2·0 mm). The jejunal segments were isolated distal to the ligament of Treitz in a length of 20–25 cm, the ileal segments consisted of the last 20–25 cm of the small intestine, and the colon and rectum were taken as one of approximate length 15 cm. The outflow from the colonic segment was through a tube inserted in the anus.

Bowel contents were washed out with 0·9% NaCl (saline), prewarmed to 38 °C. Perfusion tubes left the abdomen through lateral openings of the skin. The intestine was returned to abdominal cavity and the

midline incision was covered with gauze kept moist with saline. Throughout the experiments, the animals were on a heated table.

Immediately before the perfusion tubes were connected to the infusion pump, each segment of intestine was rinsed with Tyrode solution containing [1,2-^{14}C] polyethylene glycol (PEG, mol. wt 4000; New England Nuclear, Dreieichenhain, Frankfurt). Perfusion was with the same solution heated to 38 °C at a rate of 12 ml h^{-1}. The outflow was collected at 15 min intervals. Control perfusion lasted 150 min. Before perfusion was continued with the test solution for another 150 min, the intestine was rinsed with Tyrode solution containing the test substance. Previous experiments have shown that absorption from Tyrode solution using the same experimental conditions did not change for at least 5 h.

After the experiments the animals were killed with urethane, and the lengths of the intestinal segments measured by stretching them gently on a glass rod. They were then dried to constant weight at 80 °C.

Solutions and drugs

Control solution was Tyrode solution containing (g litre^{-1}): NaCl 8·0, KCl 0·2, $CaCl_2 \times 2H_2O$ 0·134, NaH_2PO_4 0·05, $NaHCO_3$ 1·0, D-Glucose $1H_2O$ 2·7; ^{14}C-PEG 5 μCi litre^{-1} as volume marker, inactive PEG 2 g litre^{-1} as carrier. Osmolality was 305 mmol kg^{-1} and pH 7·69.

Test solutions consisted of a control solution to which the following drugs as described by Garcia-Villar et al (1980) had been added:

(1) Dried purified senna extract containing 60% Ca-sennosides A + B at doses of 0·2, 2 or 4 g litre^{-1}) soluble in perfusion fluid.

(2) Dried purified senna extract containing 18% oxidized Ca-sennosides A + B at doses of 0·2, 2 or 4 g litre^{-1} administered as a suspension. (A filtered solution resulted in similar absorption rates.)

(3) Sennosides A + B at 2 g litre^{-1}, readily soluble in Tyrode solution.

(4) Rhein (Roth, Karlsruhe, G.F.R.), mol. wt 284·23. Dose: 1 mmol litre^{-1} (≡ 284 mg litre^{-1}). This substance was partly soluble, and as it was impossible to get a well-distributed suspension, a filtered solution was used.

Analyses

The samples were centrifuged for 2 min and analysed for Na and K by flame photometry, Cl by coulometric titration, glucose by a hexokinase method (Boehringer, Mannheim) and osmolality by freezing point depression. The ^{14}C-PEG activity was measured by liquid scintillation counting with Insta-Gel as scintillator (Tri-Carb; Packard, Frankfurt).

FIG 1

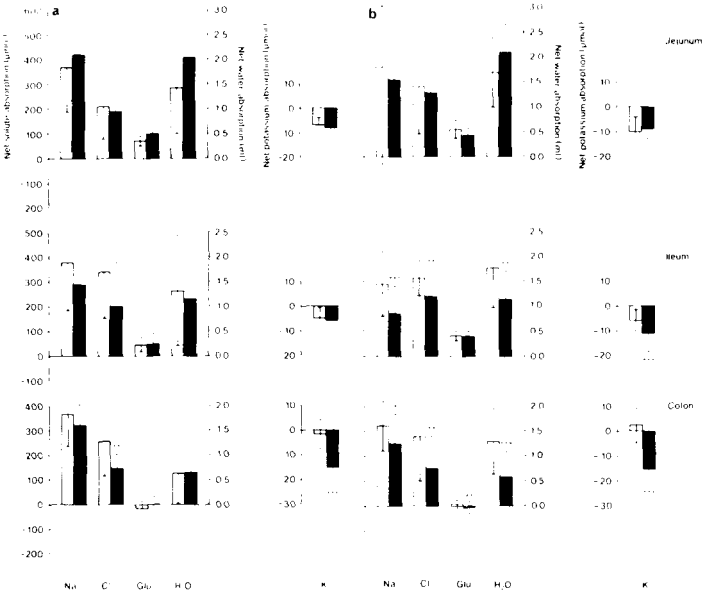


FIG. 1. Effect of a dried, purified senna extract containing 60% Ca-sennosides on jejunal, ileal and colonic net water and solute absorption (μmol per 1 g dried intestine; mean with s.d.). Concentration of senna extract in the perfusion fluid was 0·4 (g litre^{-1}) (1a), and 4 (1b). Open columns, control periods; solid columns, test periods.

Calculations

Net water flux was calculated from the ^{14}C-PEG activity (counts min^{-1}) in the initial perfusion fluid (i) and the intestinal outflow (o), respectively:

Net water flux (ml) $= V - V\,(PEG_i/PEG_o)$

Net solute flux (μmol) $= S_iV - S_oV\,(PEG_i/PEG_o)$

where V indicates the perfusion rate (ml/15 min) and S represents solute concentrations (μmol ml^{-1}) before perfusion (*i*) and in the intestinal outflow (o). Positive values represent absorption, negative values secretion. Steady state conditions of absorption or secretion were normally present after 2–3 control perfusion periods and after 2–6 test periods depending on the drug and the dose used. All values before reaching steady state were discarded. Net absorption/secretion values were related to 1 g dried bowel substance. Means with s.d. of control periods and test periods were first calculated separately for each animal, then all animals of each experimental group were combined. Animals which did not show normal control values, were discarded. 3–5 animals were used for each substance and each dose. Statistical significance between control and test periods was assessed using the Student's *t*-test. The mean dry weight and length of the intestinal segments used were 242 s.d. 74 mg (22·7 s.d. 2·8 cm) for jejunum, 242 s.d. 95 mg (22·9 s.d. 3·3 cm) for ileum and 239 s.d. 51 mg (15·5 s.d. 2·5 cm) for colon (n = 36).

RESULTS

Compound 1 (containing 60% Ca-sennosides) in the doses of 0·4 and 2·0 g litre^{-1} did not have striking and uniform effects on the absorption in all three intestinal segments (Fig. 1a). The same was valid for jejunal absorption after 4 g litre^{-1}. Ileal and colonic water, sodium and chloride net absorption, however, were significantly reduced and net potassium secretion was increased (Fig. 1b).

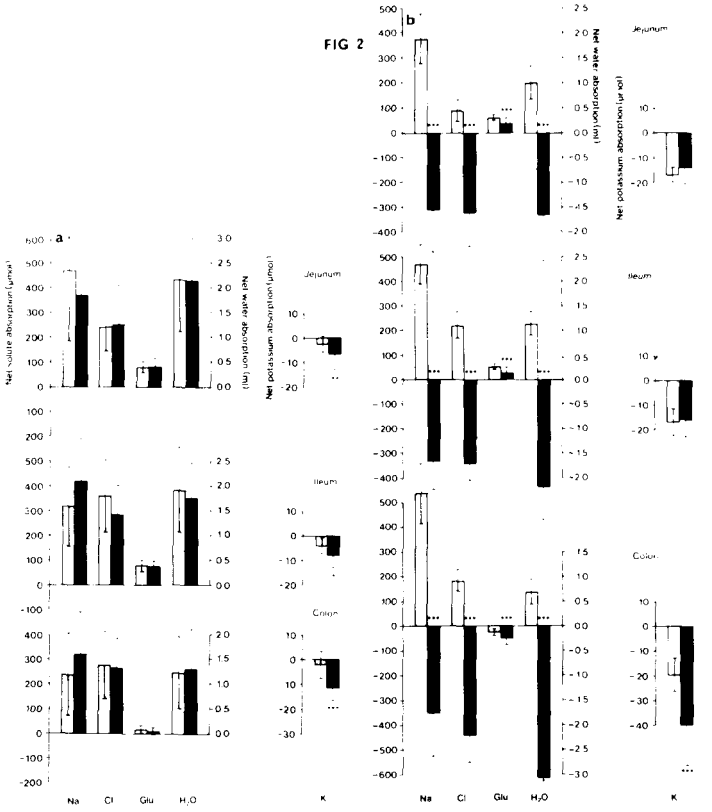


FIG. 2. Effect of a dried, purified senna extract containing 18% completely oxidized Ca-sennosides on jejunal, ileal and colonic net water and solute absorption (μmol per 1 g dried intestine; mean with s.d.). Concentration of senna extract in the perfusion fluid (g litre^{-1}) was 0·4 (2a), and 4 (2b). Open columns, control periods; solid columns, test periods.

Compound 2 (containing 18% oxidized Ca-sennosides) decreased dose-dependently water and electrolyte net absorption in all three intestinal segments. The ileum and colon seemed to be more sensitive to the drug's action than the jejunum. The highest dose changed net absorption of water, sodium and chloride into net secretion (Fig. 2a, b). In most animals, potassium secretion was stimulated. A 50% inhibition of glucose was found in the jejunum and the ileum during perfusion of 4 g litre^{-1} of compound 2. Net glucose absorption in the colon approximated to zero in all control periods and was not uniformly influenced by any of the test compounds.

Compound 3 (pure sennosides) did not reduce salt and water absorption in any of the intestinal segments (Fig. 3).

Compound 4 (pure rhein) significantly decreased water, electrolyte and glucose net absorption in all three intestinal segments. As with compound 2, the ileum and colon were more affected than the jejunum (Fig. 4).

Administration of compound 2 (4 g litre^{-1}) with colonic perfusion only resulted in a considerable secretion of water and electrolytes into the colonic lumen similar to that found above. Ileal net absorption was also reduced, but to a lesser extent, whereas jejunal absorption was not significantly impaired (Fig. 5). The effect of rhein (0·248 g litre^{-1}) administered in the same way was restricted to the colon only (Fig. 6).

DISCUSSION

The present investigation confirms that anthraquinone derivatives are able to inhibit water net absorption in the colon (Straub & Triendl 1934; Schmid 1952). In addition, it can be concluded from these results that the oxidized derivatives are much more effective than the sennosides themselves. In a detailed study of the effects of 18 different dihydroxy-

FIG 3

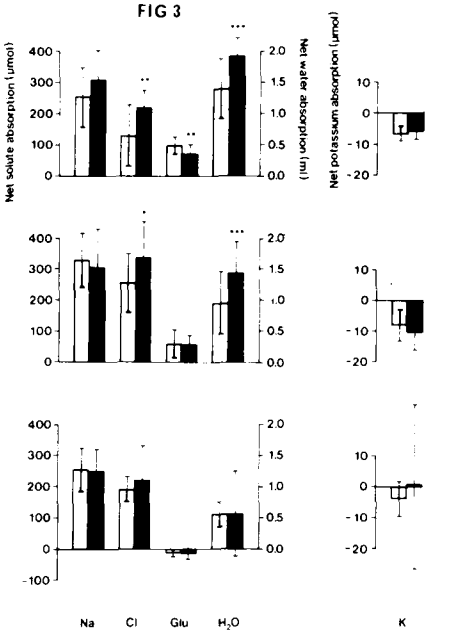


FIG 4

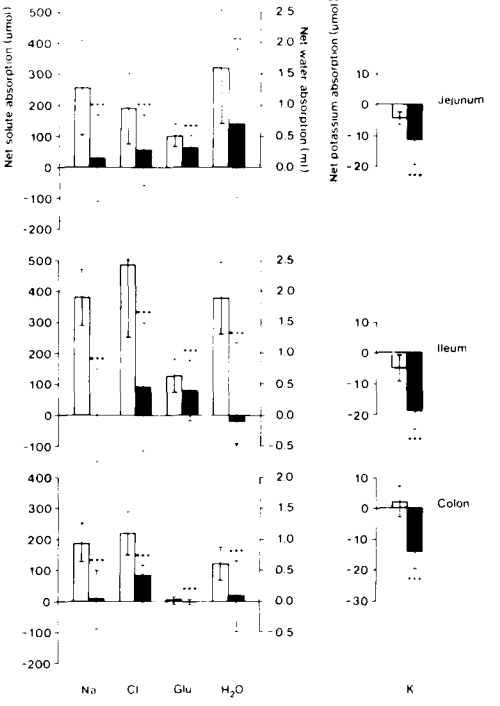


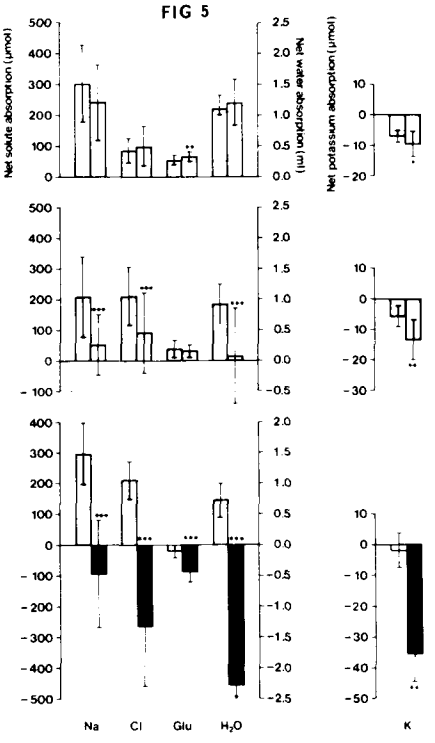
FIG. 3. Effect of sennosides A + B (2 g litre^{-1}) on jejunal, ileal and colonic net water and solute absorption (μmol per 1 g dried intestine; mean with s.d.). Open columns, control periods; solid columns, test periods.

FIG. 4. Effect of rhein (0·284 g litre^{-1}) on jejunal, ileal and colonic net water and solute absorption (μmol per 1 g dried intestine; mean with s.d.). Open columns, control periods; solid columns, test periods.

anthracene derivatives on water, sodium and potassium absorption in the rat colon, Lemmens & Borja (1976) proved rhein and rhein-anthrone to be the most active substances in inducing a net flow of water and sodium from the blood to the lumen. Pure sennosides did not show any effect on the colon (Lemmens 1976). The same result—extended to the ileum and jejunum—has been established by the present study, which also showed that the Ca-sennosides were able to reduce water and electrolyte net absorption at least in the colon and ileum. As the effect on absorption was weaker than on motility when compared with the completely oxidized drug, it is likely that passage through the stomach contributes at least partly to the pharmacological activity.

The present study has also shown that the effects of the anthraquinone drugs administered in the oxidized form are not specific to the colon only. In the presence of the oxidized drugs, jejunal and ileal net absorption rates of water and electrolytes were also much reduced. However, it was apparent that the inhibitory action of the drugs increased from jejunum to colon which may reflect an increasing sensibility of the gut mucosa to the drugs. Recently, a similar effect was established in perfusing the jejunum and colon of healthy volunteers: Rhein induced net secretion of water, sodium and chloride in both parts of the intestine which differed quantitively (Wanitschke et al 1978).

Glucose net absorption was not or only slightly affected by the oxidized senna derivatives including rhein. High concentrations caused a 50% inhibition in the jejunum and the ileum. As glucose normally is not absorbed in the rat colon, drug influences could not be established with certainty. Sennoside A, emodin (1,3,8-trihydroxy-6-methylanthraquinone) and danthron (1,8-dihydroxyanthraquinone) did not inhibit glucose absorption in the proximal rat small intestine, whereas diphenolic laxatives such as oxyphenisatin and bisacodyl were effective (Hart & McColl 1967). The diphenolic laxatives also reduce net water and sodium absorption from the human colon and tied jejunal and colonic loops in the rat in



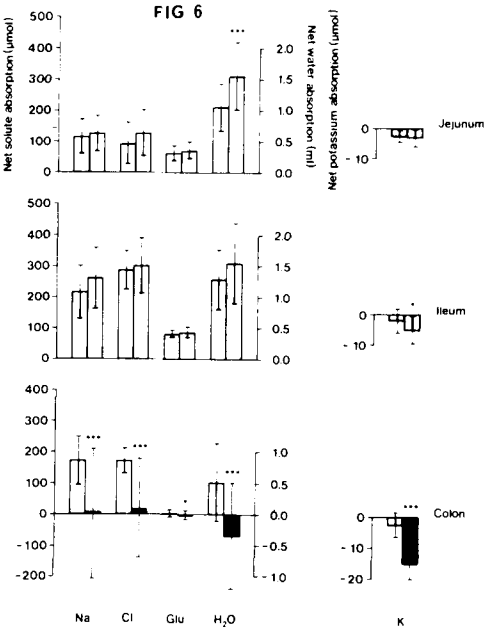


FIG. 5. Effect of a dried, purified senna extract containing 18% completely oxidized Ca-sennosides, administered with the colonic perfusion fluid only (4 g litre^{-1}) on jejunal, ileal and colonic net water and solute absorption (μmol per 1 g dried intestine; mean with s.d.). Open columns, control periods; solid columns, test periods.

FIG. 6. Effect of rhein, administered with the colonic perfusion fluid only (0·284 g litre^{-1}), on jejunal, ileal and colonic net water and solute absorption (μmol per 1 g dried intestine; mean with s.d.). Open columns, control periods; solid columns, test periods.

situ, whereby the colon proved to be more sensitive than the jejunum (Ewe & Hölker 1974; Forth et al 1966; Nell et al 1973). This corresponds to our findings with anthraquinone derivatives.

Administration of the extract containing oxidized Ca-sennosides with the colonic perfusion fluid only, resulted in decreased ileal net absorption rates, whereas rhein did not show a comparable effect. This difference may be because the extract may contain a mixture of different active oxidation products, rhein among them, the rest not having been identified. As it is unlikely that in vivo colonic micro-organisms produce only rhein as the active end-product of sennosides, similar actions on adjacent intestinal parts originating from the colon cannot be excluded in patients treated orally with senna glycosides.

The mechanism of action of anthraquinone laxatives on water and electrolyte absorption is poorly understood. It was suggested that inhibition of intestinal sodium transport and, as a consequence, retention of water in the lumen, results from inhibition of intestinal ($Na^+ + K^+$)-ATPase. Both bisacodyl and danthron inhibited the intestinal ($Na^+ + K^+$)-ATPase (Chignell 1968). Recently, the participation of prostaglandin E has been discussed, since the diphenolic laxatives stimulate the biosynthesis of PGE (Beubler & Juan 1978a, b).

Thus, the effects of the senna derivatives on intestinal absorption run parallel to motility changes (Garcia-Villar et al 1980) in that (i) the oxidized drugs are more effective than the non-oxidized compounds, (ii) the oxidized compounds are not specific to the colon, but also influence the upper gastrointestinal tract and (iii) there is a definite reaction of the ileum when the administration of the oxidized extract is restricted to the colon. Which effect—altered absorption or motility—is primary is unclear, but it appears likely that both actions contribute to the laxative effect of senna drugs. As senna glycosides normally are not present in their oxidized form in the drug in clinical use, their main action will be localized in the large intestine when they are transformed by colonic micro-organisms. The side-effects sometimes observed after senna-treatment (Glatzel 1972; Godding 1976; Richter 1966), and which seem to originate from the upper gastrointestinal tract, may be caused by the influence of traces of oxidized products in the drug on intestinal motility rather than on the influence on jejunal or ileal absorption, because small amounts of oxidized derivatives are not sufficient to induce changes in net absorption.

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