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Stimulation of PGE_2 synthesis and water and electrolyte secretion by senna anthraquinones is inhibited by indomethacin

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The effect of dried senna pod extract, containing 10% sennoside B, on colonic electrolyte and fluid transport was examined in the anaesthetized rat in-situ. Oral administration of senna pod extract dose-dependently (17·5–30 mg kg^{-1}, calculated as sennoside B) reversed net absorption of water, sodium and chloride to net secretion and increased potassium secretion. Senna pod extract stimulated the output of prostaglandin E_2 into the colonic lumen. Inhibition of prostaglandin biosynthesis by pretreatment of the rats with indomethacin (10 mg kg^{-1}) significantly inhibited the effects of senna pod extract (17·5–30 mg kg^{-1}) both on net fluid transport and on prostaglandin E_2 synthesis. The inhibitory effect of indomethacin on net fluid transport induced by senna pod extract (30 mg kg^{-1}) was dose-dependent. It is concluded that anthraquinones exert their laxative action at least partially via stimulation of colonic fluid and electrolyte secretion, and that this secretion is mediated by stimulation of endogenous prostaglandin E_2 formation.

Laxatives containing anthraquinone derivatives are classified as 'contact cathartics' together with the diphenylmethane derivatives bisacodyl and phenolphthalein, castor oil, the docusates and the bile acids (Fingl 1980). They are thought to act mainly in the large intestine by altering peristalsis (for ref. see Garcia-Villar et al 1980) as well as water and electrolyte transport (Straub & Triendl 1934; Lemmens & Borja 1976; Beubler & Juan 1979; Leng-Peschlow 1980). Several mechanisms are supposed to be involved in the effect of anthraquinones on water and electrolyte transport (for ref. see Beubler 1983). Inhibition of $Na^+ - K^+$-activated adenosinetriphosphate (Na-K-ATPase) indicates an inhibition of the active sodium absorption (Chignell 1968; Phillips et al 1965). An alteration of mucosal permeability by anthraquinones has been suggested to be involved (Nataf et al 1979) but has not been investigated systematically. As for other laxatives, the stimulation of active secretion has been thought to be responsible for the diarrheogenic effect of anthraquinones (Beubler & Juan 1979). Bisacodyl, phenolphthalein and ricinoleic acid have been shown to stimulate PGE_2 synthesis and the secretory effect of these laxatives was shown to be reduced by indomethacin, indicating that their laxative effect is, at least partially, mediated by PGE_2 formation (Beubler & Juan 1978a, b, 1979; Rachmilewitz et al 1980; Luderer et al 1980; Autore et al 1984).

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Anthraquinones also stimulate PGE synthesis (Beubler & Juan 1979; Cohen 1982; Capasso et al 1983), however, the participation of PG formation in the mediation of active secretion caused by anthraquinones has not yet been proved.

The aim of the present study was to investigate whether anthraquinone induced colonic fluid and electrolyte secretion is accompanied by an increase in PGE_2 synthesis and whether inhibition of endogenous PG formation by indomethacin changes the secretory effect of these laxatives.

METHODS

Animals

Female Sprague Dawley rats (Himberg, strain OFA, S.D., SPF, 150–240 g) were used. They were deprived of food for 15 h before the experiment and had free access to water.

Treatment and preparation

The rats were fed with 0·9% NaCl (saline) or with the aqueous suspension of the dried senna extract via a gastric cannula (0·1 ml kg^{-1}). Four hours later, the rats were anaesthetized with urethane (1·2 g kg^{-1} intraperitoneally). The abdomen was opened, an incision was made within 0·5 cm distal to the caecum, and the entire colon was rinsed gently with a syringe using 20–40 ml warm saline solution (37 °C, 0·15 M NaCl) in-situ. After an interval of 90 min, that is 5·5 h after oral administration of senna pod extract, the entire colon was filled with 2 ml Tyrode solution,

ligated and replaced in the abdominal cavity. In some experiments, indomethacin (0·64–10 mg kg^{-1}, final doses) was injected subcutaneously in two cumulative doses, one at the same time as the senna extract and one at the time of anaesthesia to assure effective blood levels throughout the experiment.

One hour after filling the colon, a sample of intestinal fluid was withdrawn for determination of PGE_2. To avoid PGE_2 formation due to mechanical stimulation (Beubler & Juan 1978c), the gut wall was punctured with a hypodermic needle and a small sample of fluid (0·2–0·5 ml) was withdrawn carefully into a 1 ml syringe. At the end of the experiment, the colon was removed and weighed. The remaining intestinal fluid was drained and analysed for sodium and potassium (flame photometer, Lange) and chloride (Chlor-o-counter, Mark II Labo International). Ion transport rates were expressed as μmol g^{-1} wet weight h^{-1}. Net fluid transport rates were determined gravimetrically (Beubler & Juan 1979). Net water flux was expressed as ml g^{-1} wet weight h^{-1}. Net absorption is indicated by a negative value and net secretion by a positive value. The samples for PGE_2 measurement were stored at −20 °C and analysed within three weeks by radioimmunoassay (Bukhave & Rask-Madsen 1981). PGE_2 synthesis was expressed as ng h^{-1} g^{-1} wet weight.

Preliminary experiments were performed to determine the onset of action of senna pod extract on net water transport in the colon after oral administration. If the experiment was started 3·5 h after oral administration by ligating the colon, no change in net water transport in the following 1 h was observed. When the colon was ligated 4·5 h after oral administration, a distinct net water secretion was measured in the colon. Since the effect was more pronounced after 5·5 h, the following studies were made as described above, 5·5 h being the minimum time required to establish a good effect.

Statistical significance of the differences of the means was evaluated by the two sample Student's *t*-test and all values given are mean ± s.e.m. In the Figs, the error bars are sometimes covered by the symbols. Values of $P < 0·05$ were considered significant.

Drugs

Dried senna pod extract (*Cassia angustifolia*) containing 10·3% sennoside B (roha-arzneimittel, Bremen, FRG) was used. The doses were calculated according to the sennoside B content and were 10·0, 17·5, 20·0, 23·0 and 30·0 mg sennoside B kg^{-1}. Indomethacin was purchased from Merck, Sharp and Dohme (Rahway, NJ). All other reagents were of analytical grade (Merck, Darmstadt, FRG).

RESULTS

Effects of senna pod extract on fluid transfer rates

Net fluid absorption was observed in all control rats (Fig. 1). Senna pod extract, containing 10·3% sennoside B dose-dependently from 17·5–30 mg kg^{-1} (calculated as sennoside B) reversed net fluid absorption to net fluid secretion (Fig. 1, $P < 0·01$).

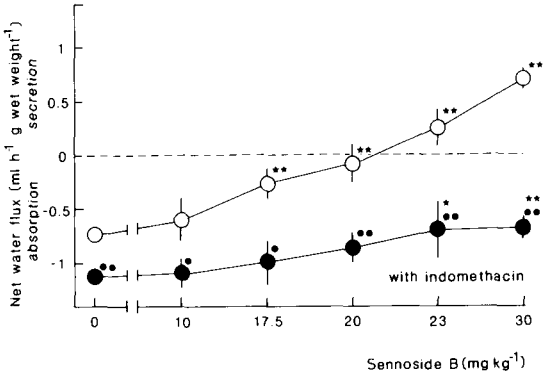

FIG. 1. The effect of senna pod extract (10–30 mg kg^{-1}, calculated as sennoside B) on net water flux in the tied off colon of the rat in-vivo, without (○, n = 22–42) and with (●, n = 17–28) pretreatment with indomethacin (10 mg kg^{-1}). Negative values denote net absorption, positive values net secretion. Each point represents the mean ± s.e.m. $*P < 0·05$, $**P < 0·01$ relative to control; $●P < 0·05$, $●●P < 0·01$ relative to results without indomethacin.

Effects of senna pod extract on ion transfer rates

In all control rats sodium and chloride was absorbed and potassium was secreted (Fig. 2). Senna pod extract (17·5–30 mg kg^{-1} calculated as sennoside B) dose-dependently reversed net sodium and chloride absorption to net secretion ($P < 0·05$ or 0·01, respectively). Net potassium secretion was slightly, but significantly increased by senna pod extract ($P <$ 0·05 or 0·01, respectively).

Effects of senna pod extract on PGE_2 synthesis

The output of PGE_2 into the colonic lumen was low in control rats (0·2 ± 0·05 ng h^{-1} g^{-1} wet weight). Senna pod extract (17·5–30 mg kg^{-1} calculated as sennoside B) dose dependently increased PGE_2 output into the colonic lumen (Fig. 3, $P < 0·01$).

Effects of indomethacin

Indomethacin (10 mg kg^{-1} s.c., final dose) slightly, but significantly reduced net fluid absorption in

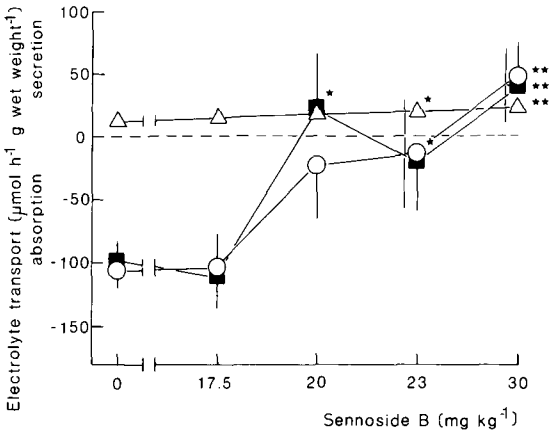

FIG. 2. The effect of senna pod extract (17·5–30 mg kg^{-1} calculated as sennoside B) on net transfer of sodium (○, n = 13–20), chloride (■, n = 9–14) and potassium (△, n = 11–18) in the tied off colon of the rat in-vivo. Negative values denote net absorption, positive values net secretion. Each point represents the mean ± s.e.m. In the case of potassium, the triangles cover the error bars. $^{*}P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$ relative to the control.

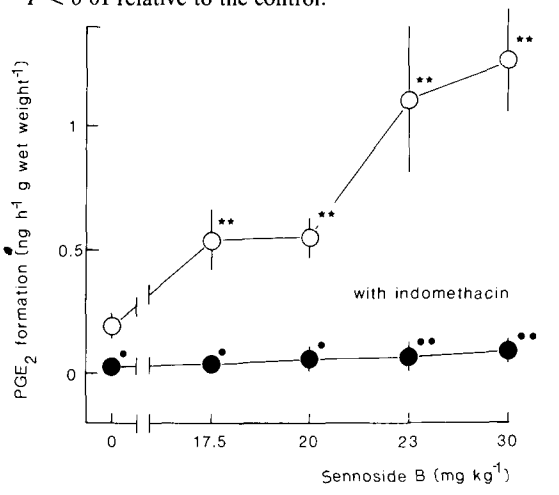

FIG. 3. The effect of senna pod extract (17·5–30 mg kg^{-1}, calculated as sennoside B) on PGE_2 output into the lumen of the rat colon in-vivo, without (○, n = 11–21) and with (●, n = 7–11) pretreatment with indomethacin (10 mg kg^{-1}). Each point represents the mean ± s.e.m., $^{**}P < 0{\cdot}01$ relative to the control; $^{\bullet}P < 0{\cdot}05$, $^{\bullet\bullet}P < 0{\cdot}01$ relative to results without indomethacin.

control rats. The effect of senna pod extract (10–30 mg kg^{-1} calculated as sennoside B) on net fluid transport rates was inhibited by indomethacin (Fig. 1, $P < 0{\cdot}05$ or 0·01, respectively). Increasing doses of indomethacin (1·6–10 mg kg^{-1}, final doses) dose-dependently reduced the effect of 30 mg kg^{-1} (calculated as sennoside B) senna pod extract (Fig. 4, $P < 0{\cdot}05$ or 0·01, respectively). Indomethacin (10 mg kg^{-1}, final dose) also reduced the output of

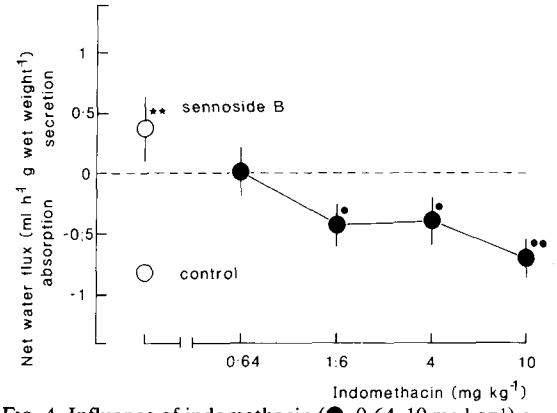

FIG. 4. Influence of indomethacin (●, 0·64–10 mg kg^{-1}) on senna pod extract (○, 30 mg kg^{-1}, calculated as sennoside B) induced net fluid secretion in the tied off colon of the rat in-vivo (n = 9–13). Negative values denote net absorption, positive values net secretion. Each point represents the mean ± s.e.m. $^{**}P < 0{\cdot}01$ relative to the control, $^{\bullet}P < 0{\cdot}05$, $^{\bullet\bullet}P < 0{\cdot}01$ relative to the effect of 30 mg kg^{-1} sennoside B alone.

PGE_2 into the colonic lumen in control rats ($P < 0{\cdot}05$) and the effect of senna pod extract (17·5–30 mg kg^{-1} calculated as sennoside B) on PGE_2 formation (Fig. 3, $P < 0{\cdot}05$ or 0·01, respectively).

DISCUSSION

As determined in preliminary experiments (see methods), the onset of action of senna pod extract on net water transport could not be seen earlier than about 4–5 h after oral administration. The reason for this latency is not the intestinal transit time, but the metabolism of the anthraquinones, which are present in the senna pod extract as inactive glycosides. Senna glycosides pass the stomach and the small intestine without being absorbed and are metabolized in the colon by microorganisms into a pharmacologically active form (Fairbairn & Moss 1970; van Os 1976). The prerequisite for the secretory effect of anthraquinones is the presence of free phenols, preferably in 1,8-position (Lemmens & Borja 1976). Accordingly, the sennosides must be transformed into the active metabolites rhein and rhein-9-anthrone (Lemli & Lemmens 1980). Since free phenolic groups have been shown to stimulate PG-formation (Pace-Asciak 1972), the free phenolic groups of rhein and rhein-9-anthrone might be responsible for the ability of these anthraquinones to increase PG synthesis in the colon. Diphenolic laxatives like bisacodyl and phenolphthalein have also been discussed as being able to stimulate PG synthesis due to their free phenolic groups (Beubler & Juan 1978a, b) and hence to stimulate colonic secretion.

To establish the role of PGs in anthraquinone mediated secretion, PGE_2, the major arachidonic acid metabolite in the intestinal mucosa (LeDuc & Needleman 1979), was measured in the colonic lumen, since this 'overflow' of PGs presently appears to provide the most reliable index of the balance between intestinal PG synthesis and degradation in-vivo (Rask-Madsen & Bukhave 1983).

In the present experiments, senna pod extract dose-dependently increased PGE_2 output into the colonic lumen. A high dose of sennosides (75 mg kg^{-1}, calculated as sennoside A + B) has been shown previously to stimulate PG synthesis (Beubler & Juan 1979), but indomethacin did not reduce the secretory effect in these experiments. The present study shows a very steep dose-response curve of the sennosides between 10 and 30 mg kg^{-1} (calculated as sennoside B). Within this dose-range, indomethacin (10 mg kg^{-1}) is able to inhibit stimulated PGE_2 formation (Fig. 3) and the secretory effect of the sennosides (Fig. 1) significantly, indicating that PGE_2 synthesis is causally involved in mediating this secretory effect. This notion is further supported by the fact that increasing doses of indomethacin dose-dependently reduced the effect of senna pod extract (Fig. 4).

Senna pod extract dose-dependently reversed sodium and chloride absorption to secretion as has been shown previously for anthraquinones (Phillips et al 1965; Lemmens & Borja 1976; Leng-Peschlow 1980). Colonic potassium secretion was slightly, but significantly, enhanced as it has been shown with other laxatives (Forth et al 1966; Schreiner et al 1980; Beubler 1985). It seems likely that anthraquinones possess a similar pattern of action as other contact carthartics, probably lead to the same severe side effects upon chronic administration, and should be prescribed with the same precautions (Wolff et al 1968; Cummings 1974; Beubler 1985).

In conclusion, the present results support the assumption that the laxative effect of anthraquinones is at least partly caused by colonic fluid and electrolyte secretion, and that this secretion is mediated by stimulation of endogenous PGE_2 formation.

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