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Senna still causes laxation in rats maintained on a diet deficient in essential fatty acids

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Abstract—The laxative effect of senna has been investigated in normal and essential fatty acid deficient (EFAD) rats. Oral administration of senna pod extract (7·5–90 mg kg^{-1}) produced a dose-dependent increase in the number of soft faeces excreted by normal rats. Senna 30 mg kg^{-1} also reversed net absorption of water and increased the prostaglandin (PG) production in the colonic lumen of normal rats by about four times. Oral administration of senna pod extract to rats, maintained on a fat-free diet for 30–90 days, produced diarrhoea and reversed net absorption of water as in normal rats. However, a fat-free diet reduced the PG production drastically in the colonic lumen both in senna-free rats and in senna-treated rats. In EFAD rats carrageenan oedema, but not dextran oedema, was also drastically reduced. Since PG mediation is not present in EFAD rats we conclude that the PG are not essential for laxation induced by senna and that water secretion and PG production in the rat intestinal lumen are unrelated.

Senna increases the secretion of water and electrolytes by rat intestine, as does prostaglandin (PG) E_2 (Beubler & Juan 1979). Senna and sennosides A and B, and their metabolite rhein anthrone, stimulate PG output in the intestine of rats (Beubler & Juan 1979; Cohen 1982; Capasso et al 1986) and mice (Yagi et al 1988) and their secretory effect was shown to be reduced by indomethacin, indicating that senna and anthraquinone derivatives act partly by stimulating the biosynthesis of PG (Beubler & Kollar 1985; Capasso et al 1986; Yagi et al 1988). However, doses of indomethacin sufficient to prevent PG production did not alter the effect of senna on rat colonic transport under conditions that inhibited the colonic water secretion caused by other laxatives (Beubler & Juan 1979). Furthermore, Donowitz et al (1984) found that quinacrine, a phospholipase A_2 inhibitor, did not alter the senna-induced changes in colonic electrolytes transport, and Capasso et al (1986) found that laxation caused by senna could be greatly reduced, but not prevented, by anti-inflammatory drugs. Thus the role of PG in mediating the action of senna remains uncertain. In the present investigation we have further studied the possible participation of PG in the mechanism of action of senna in rats maintained on a diet deficient in essential fatty acids and have found that the senna effect is not significantly modified in rats almost unable to produce PG. Some of the results reported were presented at the First International Symposium on Senna (Mascolo et al 1988).

Materials and methods

Animals. Male albino rats were used and unless stated otherwise, they were of an inbred Wistar strain (Lewis-Nossan, 150–280 g). Rats were maintained under standardized environmental conditions (room temperature 25 ± 1°C, relative humidity 58 ± 2%, light/dark cycle 12/12 h) and fed a standard rat cake diet with free access to water.

Essential fatty acid-deficient (EFAD) rats were obtained by putting 4 weeks old animals on a fat-free diet for 1–3 months. The EFAD diet (Italiana Mangimi) contained wheat starch, casein, dextrose and vitamins A, D_3, B_1, B_2, B_6, B_{12}, K, E, H, and PP, but not vitamin F, represented by the group of linolenic and linoleic acids. The animals receiving the EFAD diet exhibited a growth retardation, reaching 20–40% at the time of the experiments. Growth retardation was not due to an unpalatable diet because the weights of food consumed in 24 h by EFAD or normal rats were identical. As animals were selected according to body weight, the EFAD rats were older. The effect of this age difference, however, was ruled out since the senna effect was identical in rats on a normal diet at 4 and 16 week of age. Besides retarded growth, several rats showed hair loss, scaly paws and tails, poorly formed coats, which are recognized symptoms of EFAD condition (Ziboh & Hsia 1972).

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Laxative effect. The procedure was that described elsewhere (Mascolo et al 1988). Senna, 7·5–90 mg kg^{-1}, was administered into the stomach of rats by means of a polyethylene tube. Each animal was placed in a separate cage with a wire-meshed floor through which faeces fell onto blotting paper; faecal output was measured by counting the number of normal (dry) and soft (unformed) faeces at 2, 4, 6, 8, 10 and 24 h.

Net water flux and PG production. 5 h after senna treatment (30 mg kg^{-1}) rats were anaesthetized (urethane 1·30 g kg^{-1} subcutaneously) and the entire colon rinsed with warm saline solution (20 mL of 0·15 M NaCl). Thirty min later the colon was filled with 2 mL of Tyrode solution and ligated. After 60 min the colon was removed and net water transport estimated as described by Beubler & Juan (1979). PG production was determined in the intestinal fluid by radioimmunoassay as described previously (Autore et al 1987).

Rat paw oedema. Oedema of the right hind-paw of male Wistar rats was produced by an intraplantar injection of carrageenan 1% (0·1 mL/rat) or dextran 6% (0·1 mL/rat). The paw volume was determined immediately and then at hourly intervals for 5 h using a differential volume measuring instrument (Capasso 1981).

Drugs used. Dried senna pod extract (*Cassia angustifolia*) containing 45% sennoside B (Indena, Settala, Italy), carrageenan and PGE_2 (Sigma), dextran (mol.wt 70.000, Pharmacia). All other reagents were of analytical grade. The doses of senna were calculated according to the sennoside B content and ranged from 7·5 to 90 mg kg^{-1}.

Statistics. The results are shown as means and s.e., analysed statistically by Student's *t*-test for paired data.

Results

Table 1 shows that not all the rats treated with 7·5 and 15 mg kg^{-1} of senna exhibited soft faeces while all the rats treated with 30–90 mg kg^{-1} provided satisfactory responses. The results also show that oral administration of 30–90 mg kg^{-1} of senna produced a dose-dependent increase in the number of soft faeces excreted by rats for the subsequent 24 h. Fig. 1 shows that 6–8 h after senna treatment faeces excretion reached a peak of soft faeces. In controls, only dry faeces were excreted during the 24 h. The fat-free diet caused no significant changes in the laxation induced by senna, 30 mg kg^{-1} over 90 days. The reduction of the

Table 1. Laxative effect of graded doses of senna.

Senna[a] mg kg^{-1}	Rats with soft faeces excreted %	Number of soft faeces excreted as % of total faeces produced in 24 h mean ± s.e.
7·5	37·5 (8)[b]	12·8 ± 3·7
15	50 (10)	15·7 ± 4·1
30	100 (10)	62·8 ± 4·7
60	100 (10)	82·6 ± 6·7
90	100 (10)	91·6 ± 7·0

Senna was given orally in a dose volume of 1 mL/rat. [a]Calculated as sennoside B; [b] Number of animals.

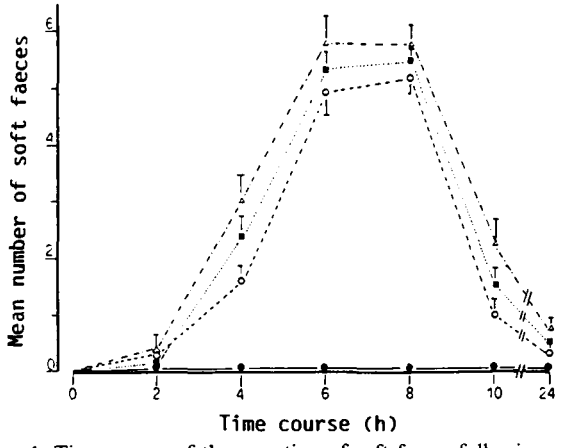

FIG. 1. Time course of the excretion of soft faeces following oral treatment with senna in the following doses: ●,0; ○,30; ■,60; △,90 mg kg^{-1}. Each point represents the mean ± s.e. of 10–12 rats.

number of soft faeces, produced within 24 h after senna, varied between 9 and 17%. Similarly, no significant changes were observed in rats kept on a fat-free diet (3 months) and treated with lower doses of laxative. In fact, senna 7·5 and 15 mg kg^{-1} had similar laxative effects (35 and 49%, respectively) and produced a similar number of soft faeces (11·7 ± 3·0 and 14·6 ± 4·1, respectively) as in controls. Table 2 shows that senna,

Table 2. Effect of senna 30 mg kg^{-1} on net water flux and PG production in the ligated colon of normal and EFAD rats in-vivo. The results are mean ± s.e. of 5 experiments each.

Treatment	Net water flux[(a)] (mL) Normal	EFAD rats	PG production (ng) Normal	EFAD rats
None	−1·08 ± 0·08	−1·16 ± 0·09	0·84 ± 0·11	0·05 ± 0·01
Senna	+0·21 ± 0·10	+0·20 ± 0·15	3·06 ± 0·21*	0·05 ± 0·01

[(a)] A negative value indicates net absorption, a positive value net secretion *$P < 0·001$

30 mg kg^{-1}, reversed net water absorption into net secretion both in normal and in EFAD rats. Table 2 also shows that the PG production in the colonic fluid of rats treated with senna increased four-fold. Fat-free diet reduced the PG production in the colonic fluid both in senna-free rats and in senna-treated rats. Fig. 2 shows that the carrageenan effect, which depends mainly on arachidonate metabolites, was drastically reduced in EFAD rats whereas dextran oedema, which depends mainly on histamine and 5-hydroxytryptamine, was not modified by a EFAD deficiency. Subplantar injection of arachidonic acid 1 h after carrageenan caused no further increase in the oedema in normal rats, but restored the inflammatory response of EFAD rats to the level of non-deficient control (data not shown).

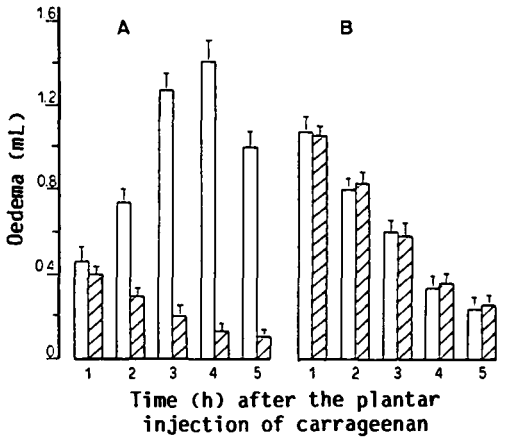

FIG. 2. Oedema of the rats paw produced by carrageenan 1% (A) and dextran 6% (B) in normal (open columns) and EFAD rats (hatched columns). Each bar represents the mean oedema ± s.e. induced in groups of 6–8 rats. In A the difference between normal and EFAD rats was significant ($P < 0·001$), except for the measurement at 1 h.

Discussion

Senna causes an increased excretion of normal faeces 4 h after drug administration and evident soft faeces 2–4 h later (Leng-Peschlow 1988) and this has been confirmed in the present study. However, we obtained a satisfactory and highly reproducible response with 30 mg kg^{-1} of senna whereas Leng-Peschlow used a dose of 50 mg kg^{-1}. We have used male rats of an inbred strain (Lewis-Nossan) and this could be a reason for explaining the differences observed. We have also confirmed earlier results (Mascolo et al 1988) showing that only 50% of the rats treated with senna, 15 mg kg^{-1}, exhibits laxation. A lower dose of senna, 7·5 mg kg^{-1}, produced laxation in 37·5% of the animals treated. Lack of arachidonic acid and subsequent shortage of PG precursor in peripheral tissues is a paramount biochemical property of EFAD rats (Bonta et al 1976) and our experiments carried out with carrageenan and dextran, strongly support this idea. However, increasing evidence suggests that tissues and/or their phospholipid constituents differ markedly in their ability to retain arachidonate during EFAD deprivation (Iritani & Narita 1984; Lefkowith et al 1985). Moreover, prostaglandin production in EFA-deficiency may be unaffected in response to receptor-mediated agonists, acting via phosphatidyl inositol turnover (Lefkowith et al 1985). The finding that colonic PG content is drastically reduced in EFAD rats (Mascolo et al 1988), led to the belief that there is a shortage of PG precursor in the gut of EFAD rats. This is now further supported by the experimental situation used in this study which shows clearly that PG production is markedly suppressed in the colonic lumen of EFAD rats.

PGs secreted in the colonic lumen are about 50 pg in EFAD rats eithers treated or not with senna and these small amounts are unlikely to increase water secretion and late laxation (Karim & Adaikan 1977). In the light of the accepted hypothesis that PGs may mediate the cathartic action of senna (Beubler & Juan 1979; Beubler & Kollar 1985; Capasso et al 1986) it was expected that rats which have a marked shortage of PG precursor would display a significant reduced response to senna. The present data show that laxation induced by senna in rats maintained on a fat-free diet for 30–90 days is only 9–17% less than in control rats. Since there is little change in the response of the EFAD rats to senna, the laxative seems able to act at least in part by a non-PG mechanism. Therefore, although senna can increase the colonic PG release (Beubler & Kollar 1985; Capasso et al 1986; and the

present results), PGs are not essential for the laxative activity. In the present investigation we have also observed that indomethacin (5 mg kg^{-1} s.e.) given 1 h before senna (30 mg kg^{-1}) to EFAD (90 days of deprivation) rats delayed the appearance of soft faeces by 2 h and reduced the total quantity by 26% ($67{\cdot}3 \pm 7{\cdot}3$). Because PG mediation is not present in EFAD rats it appears logical to consider the possibility that the inhibitory effect of indomethacin depends on non-prostaglandin (PG) mechanism(s). On the other hand, it has been shown that indomethacin has an intestinal antisecretory effect (Smith et al 1981) and inhibits carrageenan oedema (Bonta et al 1976), both via a PG-dependent and PG-independent mechanism.

A further observation made during the present investigation was that the senna was able to reverse net water absorption into net secretion both in normal and in EFAD rats. The finding that net secretion is similar in normal rats and in rats kept on a fat-free diet led to the proposal that water secretion and PG release in the intestine lumen are not necessarily related. Several other mechanisms are supposed to be involved in laxation induced by senna such as inhibition of the (Na^+-K^+)ATP-ase (Phillips et al 1965; Chignell 1968; Wanitschke et al 1977); a calcium-dependent mechanism (Donowitz et al 1984); stimulation of histamine and 5-hydroxytryptamine (Capasso et al 1986). Many other laxatives can release histamine and 5-HT (Autore et al 1984; Capasso et al 1986) and it is well known that 5-HT is a diarrhoegenic substance (Karim 1974) and histamine may also contribute to diarrhoea (Linaker & Turnberg 1983). Therefore both these autacoids might contribute by themselves to the laxative effect. However, senna and sennosides also affect intestinal motility (Waller 1975; Garcia-Villar et al 1980). Stimulation of intestinal motility seems to appear earlier and may influence faecal transit through the colon (Leng-Peschlow 1986, 1988).

Therefore fluid secretion, affected directly and/or via the release of endogenous substances, as well as the stimulation of intestinal motility must be taken into account when explaining the senna effect.

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