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Dual effect of orally administered sennosides on large intestine transit and fluid absorption in the rat*

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Quantity and consistency of the faecal output, large intestine transit time, and colonic net fluid absorption were investigated in rats after oral administration of sennosides A + B (12·5–200 mg kg^{-1}). The release of normal faecal pellets was accelerated 3–4 h after drug administration; excretion of soft faeces was evident within 4–5 h and reached its maximum 5–7 h after administration. Large intestine transit time was dose- and time-dependently influenced by sennoside treatment. A highly significant reduction in transit time from more than 6 h in controls to 90 min for a 2 h pretreatment and a nearly maximal reduction to 30 min for a 4 h pretreatment was induced by a dose of 50 mg kg^{-1}. Inhibition of net fluid absorption in the colon was maximal with the same dose, but clearly more pronounced after a 6 h pretreatment period than after a 4 h period. Since the increase in fluid volume due to net fluid secretion is delayed compared with the acceleration of large intestine transit, the early motility effect seems to be largely independent of the changes in absorption mechanisms. Therefore, the laxative effect of the sennosides consists of changes in colon motility as well as in colonic fluid absorption, but motility may be an earlier and more sensitive parameter than net absorption.

Sennosides, the main laxative components of various senna extracts, chemically belong to the anthraquinones. Due to their dianthrone-β-glycoside structure they are pharmacologically inert in the upper gastrointestinal tract and have to be regarded as prodrugs. Bacterial degradation in the large intestine releases the laxative metabolites which are characterized by a monoanthrone-aglycone structure (Breimer & Baars 1976; Lemli & Lemmens 1980).

Anthraquinones induce fluid secretion exclusively in the colon when administered orally as senna glycosides (Donowitz et al 1984; Beubler & Kollar 1985). Local administration of the active metabolites, e.g. rhein, inhibits fluid absorption in the small intestine also (Ewe 1980; Lemmens & Borja 1976; Leng-Peschlow 1980). Senna laxatives also cause changes in motility of the large intestine (Okada 1940; Hardcastle & Wilkins 1970; Garcia-Villar et al 1980), but the relationship between the motility and absorption effects is unclear. Therefore, the laxative action of pure sennosides A + B in rats in relation to changes in large intestine transit and fluid absorption dependent on time after oral administration, was studied under comparable conditions.

METHODS

Animals

Female Wistar rats, about 200 g, were maintained under standardized 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 all the time except before absorption studies where food, but not water, was removed 20 h before the experiment.

Faecal output

During the experiment the rats were kept individually in cages with a wire-meshed floor through which faeces fell onto blotting paper. After administration of the sennosides by a stomach tube, the number of normal (hard) and soft faecal pellets were counted over 14 h in hourly intervals and afterwards in a 10 h period until next day.

Transit studies

Animals used for transit studies had a chronically implanted caecal catheter which ran subcutaneously up to the animal's neck where it was fixed to facilitate injection of the marker substance. A carmine red suspension, as transit marker, was injected intracaecally at 2, 4, 6 or 8 h after oral administration of the sennosides. The time from carmine red injection to the first visual appearance of coloured faeces was registered as the large intestinal transit time. The faecal output and the large intestine transit were studied simultaneously in the same animals.

Absorption studies

Animals were anaesthetized with pentobarbitone Na (50 mg kg^{-1}). The colon was ligated and cannulated

* Dedicated to Dr Rolf Madaus on the occasion of his 65th birthday.

distal to the caecocolic junction (PE-tube, i.d. 1 mm) and, after thorough rinsing with 50 ml physiological saline to remove all contents, a second cannula (silicone, i.d. 3 mm) was inserted proximal to the rectum for fluid outflow. 4 h and 6 h after oral administration of the sennosides an open perfusion with an electrolyte solution (NaCl 6·72, KCl 0·37, $NaHCO_3$ 2·1, PEG (mol. wt 4000) 2·0 g litre^{-1}, [^{14}C]PEG 5 μCi litre^{-1}; pH 6·5; osmolality 275 mosmol kg^{-1}) was started at a rate of 12 ml h^{-1} for 2 consecutive 1 h periods.

[^{14}C]PEG activity was measured by liquid scintillation counting with Insta-Gel as scintillator in a Tri-Carb (Packard, Frankfurt). Na^+ and K^+ were analysed by flame photometry, Cl^- by coulometric titration, osmolality by freezing point depression and mucus as protein-bound total hexoses by the orcinol-sulphuric acid method (Winzler 1955). After perfusion, the lengths of the colon segments were measured. Net H_2O, Na^+, K^+ and Cl^- transport was calculated as previously described (Leng-Peschlow 1980) and expressed as ml or μmol h^{-1} and per 10 cm colon length. Positive values indicate net absorption, negative values net secretion.

Mean values with standard deviation were calculated for each period. Statistical significance was assessed by Student's *t*-test for dependent (comparison between the 1st and 2nd hour for each animal) and independent values (comparison between the different groups).

Treatment

Sennosides were obtained as an approximately 50/50 mixture of the stereoisomers A and B at a purity of at least 96% (Chemical Department, Dr Madaus GmbH & Co., Köln). They were suspended in 0·5% tragacanth (5 ml kg^{-1}) and given to the rats through a stomach tube 2–8 h before the experiment. The doses ranged from 12·5–200 mg kg^{-1} in the transit studies and from 25–100 mg kg^{-1} in the absorption studies. Each dose-group included 12–16 animals. The same animals were used several times for the transit studies with an interval of 1 week between each experiment. Each week the rats were divided into dose-groups randomly.

RESULTS

Faecal output

Sennosides exhibit a dose-dependent effect on excretion of soft faeces which is nearly maximal at 50 mg kg^{-1}. With this dose, about 70% of the faeces recovered within 24 h were not of normal consistency (Fig. 1). The loss of normal faecal pellets was

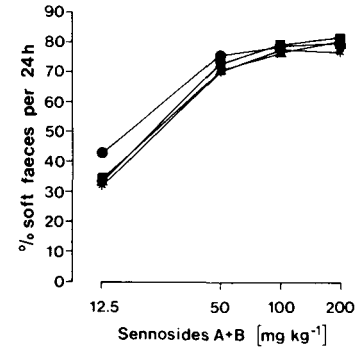

FIG. 1. Excretion of soft faeces as percent of the total faecal output during 24 h following oral sennoside A + B administration (4 identical experiments marked by different symbols, mean values of 12–16 rats per dose-group).

accelerated 3–4 h after drug administration and the excretion of soft faeces began 1 h later. Diarrhoea reached its maximum between 5 h and 7 h. Very high doses caused a slightly prolonged effect (Fig. 2).

Large intestine transit time

In controls, an intracaecally administered colour marker did not appear before at least 6 h in the faecal pellets. In sennoside-treated animals, large intestine transit was accelerated dose- and time-dependently (Fig. 3). With low doses (12·5 mg kg^{-1}), passage time was not reduced to a value below 6 h in all animals, but higher doses (50–200 mg kg^{-1}) induced transit times highly significantly different from controls for all pretreatment times ($P \leq 0{\cdot}001$). In 2 h pretreated animals, marker excretion was observed within 89 ± 30 min (50 mg kg^{-1}) and 85 ± 38 min (100 mg kg^{-1}). Acceleration was significantly more pronounced after 4 h pretreatment, mean transit times amounted to 33 ± 30 min (50 mg kg^{-1}) and 18 ± 13 min (100 mg kg^{-1}). A prolongation to 6 h only moderately increased further the effect resulting in marker excretion after 19 ± 16 min (50 mg kg^{-1}), 9 ± 10 min (100 mg kg^{-1}) and 7 ± 5 min (200 mg kg^{-1}). The dose of 50 mg kg^{-1} seems to be approaching that dose giving the maximal effect.

Absorption and secretion in the colon

Oral pretreatment with the sennosides 4 h before perfusion reduced H_2O, Na^+ and Cl^- net absorption in both perfusion periods compared with the controls. Maximal reduction was obtained with a dose of 50 mg kg^{-1} and amounted to about 50–60% of the control values (Table 1). Mucus and K^+ secretion did not differ from the untreated animals.

A 6 h pretreatment before perfusion revealed

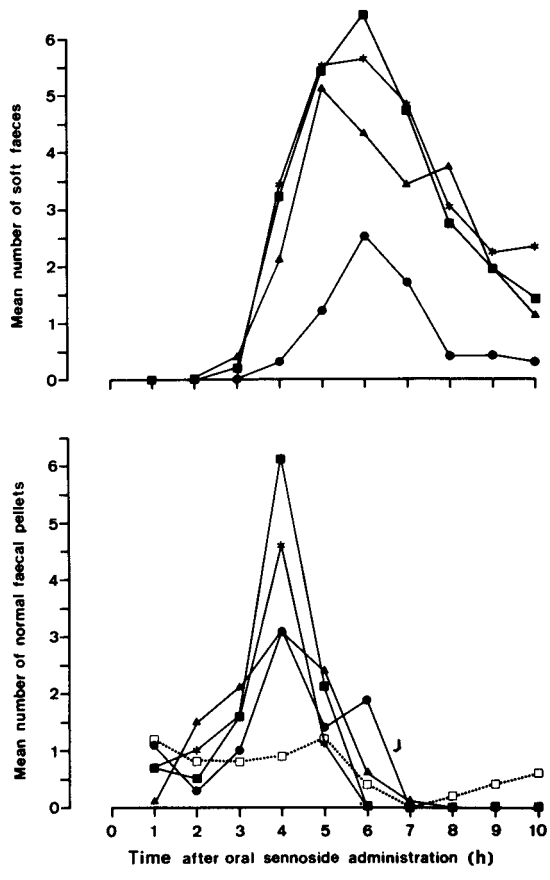

FIG. 2. Time course of the excretion of soft and normal faecal pellets following oral administration of sennosides A + B (mean values of 12–16 rats per dose-group), in the following doses: □, 0; ●, 12·5; ▲, 50; ■, 100; ★, 200 mg kg^{-1}.

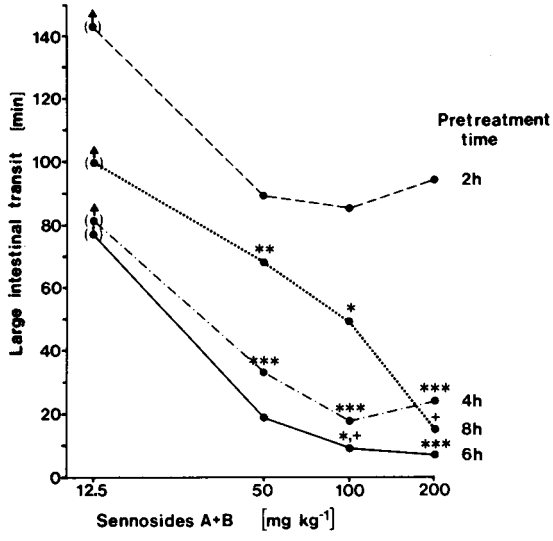

FIG. 3. Large intestine transit time as measured by faecal excretion of an intracaecally administered colour marker in rats pretreated orally with different doses of sennosides A + B (2, 4, 6 or 8 h before marker injection, mean values of 12–16 rats per group). In all controls and in 25–45% of the animals treated with 12·5 mg kg^{-1}, transit time is >6 h. *$P \leq 0{\cdot}05$, **$P \leq 0{\cdot}01$, *** $P \leq 0{\cdot}001$ as compared to the same dose of the preceding pretreatment (4 vs 2, 6 vs 4, 8 vs 6 h). †$P \leq 0{\cdot}05$ as compared to the preceding dose of the same pretreatment period.

significantly more pronounced changes in net absorption (Table 2). Net water and net Na^+ absorption was reversed to net secretion at a dose of 50 mg kg^{-1}, and to the same extent at the dose of 100 mg kg^{-1}. Cl^- net absorption was nearly abolished. Again, K^+ net secretion was unchanged and there was no statistically significant difference in mucus

Table 1. Effect of an oral pretreatment with sennosides A + B 4 h before perfusion on net absorption (+) and net secretion (−) during 2 consecutive 1 h periods in the rat perfused colon. Values are related to 1 h and 10 cm colon length and are given as mean ± s.d. (n = 16).

Sennosides A + B (4 h before perfusion) (mg kg^{-1})	Perfusion period (h)	H_2O (ml)	Na^+ (μmol)	Cl^- (μmol)	K^+ (μmol)	Mucus (μg)
0	1	0·94 ± 0·40	208 ± 59	253 ± 53	− 7·5 ± 6·5	−96·8 ± 39·4
	2	0·80 ± 0·31†	196 ± 40	234 ± 40	−11·0 ± 6·6†	−64·8 ± 39·4†
25	1	0·65 ± 0·52	157 ± 72*	201 ± 64*	− 8·8 ± 7·7	−79·7 ± 39·2
	2	0·61 ± 0·27	158 ± 60*	194 ± 49*	−11·7 ± 7·6††	−85·7 ± 47·7
50	1	0·30 ± 0·77**	105 ± 109**	154 ± 108**	−10·7 ± 8·0	−97·4 ± 50·2
	2	0·39 ± 0·60*	128 ± 96*	165 ± 98*	−13·9 ± 6·6*	−91·9 ± 58·3
100	1	0·71 ± 0·34	159 ± 65*	191 ± 54**	− 6·2 ± 5·2	−86·0 ± 47·8
	2	0·71 ± 0 37	182 ± 60	207 ± 71	−11·3 ± 6·5**	−62·8 ± 25·1

†$P \leq 0{\cdot}05$, ††$P \leq 0{\cdot}01$: Compared with the 1st hour in the same animal.
*$P \leq 0{\cdot}05$, **$P \leq 0{\cdot}01$: Compared with the corresponding hour of the control group not pretreated with sennosides.

Table 2. Effect of an oral pretreatment with sennosides A + B 6 h before perfusion on net absorption (+) and net secretion (−) during 2 consecutive 1 h periods in the rat perfused colon. Values are related to 1 h and 10 cm colon length and are given as mean ± s.d. (n = 16).

Sennosides A + B (6 h before perfusion) (mg kg^{-1})	Perfusion period (h)	H_2O (ml)	Na^- (μmol)	Cl^- (μmol)	K^+ (μmol)	Mucus (μg)
0	1	0·68 ± 0·35	154 ± 57	193 ± 48	− 9·7 ± 8·2	− 96·5 ± 54·2
	2	0·76 ± 0·29	169 ± 48	217 ± 46†	−13·9 ± 8·5†	− 88·2 ± 53·2
25	1	0·14 ± 0·61**	66 ± 94**	111 ± 96***	−10·7 ± 8·3	−149·7 ± 93·2
	2	0·34 ± 0·46†,**	91 ± 97†,**	140 ± 89†,**	−11·2 ± 5·6	−108·4 ± 53·5
50	1	−0·37 ± 0·35***	−14 ± 61***	27 ± 57***	−12·8 ± 6·0	−121·2 ± 41·3
	2	−0·18 ± 0·29***	−8 ± 60***	50 ± 56†,***	−15·3 ± 6·1	−123·7 ± 91·3
100	1	−0·34 ± 0·42***	−16 ± 71***	27 ± 65***	−11·6 ± 5·9	−125·3 ± 79·6
	2	−0·15 ± 0·38***	−4 ± 64***	51 ± 62***	−15·3 ± 5·4†	−115·0 ± 65·4

†$P \leq 0{\cdot}05$, Compared with the 1st hour in the same animal.
**$P \leq 0{\cdot}01$ }
***$P \leq 0{\cdot}001$ } Compared with the corresponding hour of the control group not pretreated with sennosides.

release, although a tendency to higher values in sennoside-treated animals was found.

DISCUSSION

Sennosides A + B administered orally accelerate colonic transit and reduce fluid absorption in the colon of the rat. The most pronounced diarrhoea is obtained after 5–7 h and the most effective dose is 50 mg kg^{-1} for all 3 parameters. As far as investigated, other laxatives such as bisacodyl or castor oil also induce both motility changes resulting in augmented propulsion (Hardcastle & Mann 1968; Stewart et al 1975; Atchinson et al 1978; Mathias et al 1978; Rolemberg-Lessa et al 1981) and changes in fluid absorption (Bright-Asare & Binder 1973; Ammon & Phillips 1974; Ewe & Hölker 1974; Gaginella et al 1977; Saunders et al 1977; Beubler & Juan 1978), but the importance of each effect and the interaction between them are unclear. An accelerated passage shortens the contact time between intestinal contents and the absorptive surface thus reducing fluid absorption, but this can never be responsible for net fluid secretion. Abundant fluid secretion may influence motility by luminal distension but the importance of this in the action of a laxative is not certain.

In the present study, the effect of the sennosides on fluid secretion in the colon is delayed compared with the effect on transit rate. Colonic transit is nearly maximally accelerated 4 h after oral administration, whereas net absorption of H_2O, Na^+ and Cl^- is only moderately diminished. Net secretion is observed only after a delay of 6 h. Since the perfusion rate was constant in these absorption experiments, an influence of individual motility and transit rate on fluid absorption can be excluded. On the other hand, colonic transit is reduced from more than 6 h in controls down to a few minutes by sennosides at a pretreatment time where absorption is only slightly affected and distension may not be an important factor being responsible for the observed motility changes. An augmented excretion of normal faecal pellets begins 3 h after oral sennosides and the first softer pellets appear 4–5 h after administration. This part of the laxative effect may be ascribed predominantly to motility changes, causing a faster passage, being able to accelerate the release of already present normal faecal pellets and to increase the moisture content of the stool corresponding to conditions present in the proximal colon or caecum. The appearance of liquid faeces is parallel to the change in net fluid transfer and dominates in the period 5–7 h after sennosides.

The cause for constipation is usually an abnormal motility pattern in the colon resulting in a delayed propulsion of intestinal contents. This includes, frequently, an augmentation in segmental contractions thus increasing resistance to aboral flow. Another reason is an atonic condition where peristaltic movements or the defecation reflex are too weak to cause efficient propagation. Sennosides pronouncedly reduce the total contraction frequency in the colon as investigated in an electromyographic study in rats and dogs (Garcia-Villar et al 1980; Bueno et al 1980). In man, oral treatment with senna diminishes intraluminal colonic pressure significantly (Waller 1975). Thus, it seems very likely that the powerful facilitation of colonic transit by sennosides, which has been shown in the present study, may be due to an inhibition of the predominantly segmenting motil-

ity in the colon. The fact that constipating agents, such as opioids, enhance segmenting contraction activity in the colon and delay transit also fits into this concept (Bass et al 1973; Bueno & Fioramonti 1982; Körner 1982; Stewart & Curd 1984). Recently, it has been shown in the cat colon that sennosides change the relation between long spike bursts and short spike bursts indicating that the propulsive movements dominate over segmenting contractions and that loperamide antagonizes these senna effects (Wienbeck et al 1985).

On the other hand, sennosides, or rather their active metabolites, influence water and electrolyte absorption in the colon by mechanisms independent from transit rate. These include inhibition of the $(Na^+ + K^+)$ATPase (Chignell 1968; Wanitschke 1980), stimulation of PGE_2-synthesis (Beubler & Kollar 1985), and a Ca^{2+}-dependent mechanism (Donowitz et al 1984). Net fluid secretion, which is evident 6 h after oral sennoside treatment in the washed and constantly perfused colon, can certainly be attributed to such mechanisms.

A striking phenomenon of the absorption studies presented here is that neither K^+ net secretion nor mucus release is stimulated by sennoside pretreatment. This is in contrast to experiments where an active metabolite of the sennosides, rhein, was added directly to the perfusion fluid in the colon (Leng-Peschlow 1980; Leng-Peschlow & Grimminger 1984), but confirms other findings with oral pretreatment with a senna extract (Donowitz et al 1984). Obviously, restitution to a normal condition in the absence of the active sennoside metabolites is faster for both of these parameters than for H_2O and Na^+ absorption.

In conclusion, the laxative effect of the sennosides includes two primarily independent components: acceleration of large intestine transit depending on motility changes and fluid accumulation depending on changes in absorption/secretion mechanisms. In an early stage or after low doses, motility changes seem to be the determining factor in increasing faecal output, whereas later both effects overlap and contribute to the laxative effect. Since it is not the aim of a laxative treatment to induce watery stools but to restore the disturbed colonic motility to normal, therapeutic doses should be as low as possible avoiding extensive net fluid secretion.

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