J. Pharm. Pharmacol. 1979, 31: 681–685 Received March 1, 1979

Effect of ricinoleic acid and other laxatives on net water flux and prostaglandin E release by the rat colon†

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Ricinoleic acid, oleic acid, dioctyl sodium sulphosuccinate, deoxycholic acid, sennoside A + B and mannitol reduced or reversed water flux from lumen to blood in rat colon in situ. Stearinic acid was without any effect. Ricinoleic acid, oleic acid, dioctyl sodium sulphosuccinate, deoxycholic acid and sennoside A + B stimulated release of PGE-like material into the colonic lumen whereas the osmotic laxative mannitol and stearinic acid did not. Inhibition of PGE biosynthesis by pretreatment of the rats with indomethacin significantly reduced (but did not abolish) the effect of ricinoleic, oleic and deoxycholic acids on net water flux and PGE release. Indomethacin reduced the effect of dioctyl sodium sulphosuccinate and of sennoside A + B on PGE release but not their effect on the net water flux. The effect of mannitol was not influenced by indomethacin. The amount of PGE release in experiments with ricinoleic acid, oleic acid, stearinic acid and dioctyl sodium sulphosuccinate (with and without indomethacin) showed a good correlation (r = 0·99) with the change in net water flux. Deoxycholic acid, sennoside A + B and mannitol did not show this correlation. It is assumed that the action of non-osmotic laxatives is partially mediated by PGE, although other mechanisms also seem to be involved in their mode of action.

Straub & Triendl (1934) showed that the laxative effect of senna in cats is due not only to stimulation of intestinal motility but also to inhibition of water absorption. Forth et al (1963) showed that diphenolic laxatives inhibit intestinal absorption of water and electrolytes, causing increased net secretion.

Ricinoleic acid (Bright-Asare & Binder 1973; Ammon & Phillips 1974; Ammon et al 1974; Gaginella et al 1977), deoxycholic acid (Nell et al 1976; Wanitschke et al 1977), anionic and non-ionic surfactants (Donowitz & Binder 1975; Sund & Matheson 1978) and anthraquinones (Lemmens 1974; Lemmens & Borja 1976) also inhibit intestinal fluid absorption.

Prostaglandins (PGs) E_1 and E_2 inhibit absorption and cause secretion of water and electrolytes in the intestine of man and other species (Pierce et al 1971; Matuchansky & Bernier 1973; Robert 1976; Beubler & Juan 1977; Beubler et al 1978).

The diphenolic laxatives bisacodyl and phenolphthalein release PGE-like material in the rat colon, and the cathartic effect of these laxatives is reduced by the PG synthesis inhibitor indomethacin (Beubler & Juan 1978a,b). In contrast, osmotic laxatives do not release PGE and their effect is unaffected by indomethacin.

† This investigation was supported by the grant No. 3630 from the Fonds zur Förderung der wissenschaftlichen Forschung in Österreich and by the Jubiläumsfonds der Österreichischen Nationalbank No. 1239.

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The present studies investigate whether or not PGE is released by other non-osmotic laxatives, and mediates their cathartic effect.

MATERIALS AND METHODS

Female Sprague Dawley rats (Mus Rattus, Brunnthal, 200 ± 10 g) were deprived of food for 20 h before the experiment. The animals were anaesthetized with urethane (1·25 g kg^{-1}, intraperitoneally), and the entire colon rinsed cautiously with a syringe of 20 ml warm saline solution (0·15 M NaCl) in situ. Thirty min later the colon was filled with 2 ml Tyrode solution containing the drug to be tested and ligated (Forth et al 1966). After 20 or 60 min, the colon was removed, weighed, opened at one end, emptied and weighed again. In the experiments with sennoside A + B the method was modified: the rats were fed orally with the drug; 4 h later the rats were anaesthetized, the colon filled with 2 ml Tyrode solution and after 1 h the colon was removed. The experiments were performed in untreated rats and in rats pretreated with indomethacin (4 mg kg^{-1} per day, s.c., starting two days before the experiment). Net water transport was estimated from the weight of the filled colon minus weight of the empty colon minus 2 g initial instillate. A negative value denotes net absorption and a positive value net secretion. PGE-like material was determined in the intestinal fluid by radioimmunoassay as described previously (Beubler & Juan 1978c).

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.

The drugs were used in concentrations known to inhibit absorption or cause intestinal section (for ref. see introduction): deoxycholic acid (5 mM), dioctyl sodium sulphosuccinate (2 and 4 mM), oleic acid (5 mM), ricinoleic acid (5 mM), sennoside A + B (75 mg kg^{-1}); for comparison we also investigated stearinic acid (5 mM) and mannitol (22 mM); the acids were used as their sodium salts.

RESULTS

Net water flux and PGE release in controls. Net water flux in control rats was directed from lumen to blood (Table 1). Pretreatment of the rats with indomethacin in controls did not significantly influence net water flux or release of PGE-like material (Table 1).

Ricinoleic acid. Ricinoleic acid (5 mM), the active principle of castor oil, reversed net water absorption into net secretion within 20 min. The effect was weaker after 60 min. Ricinoleic acid markedly increased the PGE content in the gut lumen. Both net water secretion and PGE output after 20 and 60 min were significantly less in indomethacin-pretreated rats (Table 1).

Oleic acid. Oleic acid (5 mM) was less potent than ricinoleic acid as a stimulus of water secretion and PGE release. Oleic acid reduced net water absorption but did not cause net secretion. Indomethacin reduced the effect of oleic acid on net water flux and PGE release (Table 1).

Deoxycholic acid. Deoxycholic acid (5 mM) reversed net water absorption into net secretion and significantly increased the PGE content in the lumen. Both net water secretion and PGE output were significantly less in indomethacin pretreated rats (Table 1).

Stearinic acid. Stearinic acid (5 mM) influenced neither net water flux nor PGE release, and indomethacin caused no significant changes (Table 1).

Dioctyl sodium sulphosuccinate. Dioctyl sodium sulphosuccinate (2 and 4 mM) reduced net water absorption and stimulated PGE release. Indomethacin reduced PGE release, but the tendency to reduce net water flux was not statistically significant (Table 1).

Sennoside A + B. Sennoside A + B (75 mg kg^{-1}, orally, 4 h before measuring net water flux) reversed net water absorption into net secretion and stimulated PGE release. Indomethacin pretreatment did not significantly reduce water secretion but the release of PGE (Table 1).

Mannitol. The osmotic laxative mannitol (22 mM) reversed net water absorption to net secretion but

Table 1. Effect of various substances on net water flux and PGE release in the tied off colon of the rat in vivo, with and without pretreatment with indomethacin; a negative value denotes net absorption, a positive value net secretion. The results are mean ± s.e.m. of 6 experiments each. The experimental data were evaluated by an unpaired *t*-test.

Drug	Dose mM	Time min	Net water flux (ml) No indomethacin	Net water flux (ml) With indomethacin	PGE-release (ng) No indomethacin	PGE-release (ng) With indomethacin
Control	—	20	−0·52 ± 0·08	−0·50 ± 0·04	0·71 ± 0·09	0·86 ± 0·12
Control	—	60	−1·18 ± 0·05	−1·27 ± 0·05	0·60 ± 0·08	0·79 ± 0·17
Ricinoleic acid	5	20	+0·55 ± 0·07*	+0·27 ± 0·03**	8·14 ± 1·13*	5·81 ± 0·36**
Ricinoleic acid	5	60	+0·23 ± 0·08*	−0·01 ± 0·06***	29·51 ± 2·10*	10·17 ± 0·75**
Oleic acid	5	60	−0·38 ± 0·10*	−0·98 ± 0·05***	5·33 ± 1·27*	1·46 ± 0·10**
Stearinic acid	5	60	−1·39 ± 0·11	−1·49 ± 0·19	0·37 ± 0·23	0·44 ± 0·22
Dioctyl sodium sulphosuccinate	2	60	−0·40 ± 0·11*	−0·56 ± 0·08	8·82 ± 1·22*	3·58 ± 0·63**
Dioctyl sodium sulphosuccinate	4	60	±0·00 ± 0·13*	−0·21 ± 0·10	17·80 ± 4·59*	7·25 ± 2·64***
Deoxycholic acid	5	20	+0·55 ± 0·03*	+0·46 ± 0·06	1·71 ± 0·14*	0·95 ± 0·19**
Deoxycholic acid	5	60	+0·28 ± 0·09*	−0·11 ± 0·16***	1·69 ± 0·19*	0·97 ± 0·17***
Sennoside A + B	75•	60	+0·16 ± 0·11*	+0·11 ± 0·26	3·73 ± 1·10*	0·97 ± 0·46***
Mannitol	22	60	+0·47 ± 0·16*	+0·27 ± 0·07	0·85 ± 0·11	0·80 ± 0·11

• mg kg^{-1}, orally applicated.
* $P < 0·01$ relative to the controls.
** $P < 0·01$, *** $P < 0·05$ relative to results in rats without pretreatment of indomethacin.

did not affect PGE release. Indomethacin pretreatment did not significantly influence water secretion or PGE release (Table 1).

DISCUSSION

Diarrhoea is the most prominent side effect of PGs used to terminate pregnancy (Karim & Filshie 1970; Karim & Amy 1975) and PGs are thought to be involved in the pathogenesis of diarrhoea associated with certain tumours (Williams et al 1968; Sandler et al 1968). PGs reverse net absorption of water and electrolytes to a profuse net secretion (Pierce et al 1971; Matuchansky & Bernier 1973; Beubler et al 1978) and inhibit glucose absorption in the rat jejunum (Coupar & McColl 1975). PGs are released from the gut after weak mechanical stimulation in vitro (Collier 1974; Ferreira et al 1976) and in vivo (Beubler & Juan 1977).

Like PGs, diphenolic laxatives such as bisacodyl and phenolphthalein inhibit absorption and induce secretion of water and electrolytes in the colon and jejunum. These diphenolic laxatives release PGE-like material into the lumen of the rat colon. Their effect is reduced after inhibition of PGE biosynthesis by indomethacin. These findings suggest that diphenolic laxatives may act via PGE release (Beubler & Juan 1978a,b).

Diphenolic laxatives, like PGs inhibit the absorption or induce secretion of electrolytes and water. Ricinoleic acid, deoxycholic acid, dioctyl sodium sulphosuccinate and anthraquinones (for ref. see introduction) also inhibit absorption and induce secretion of electrolytes and water.

We therefore measured the effect of these laxatives on PGE release into the rat colon. With the exception of the osmotic laxative mannitol (Beubler & Juan 1978a–c) and the (inactive) stearinic acid, all substances tested stimulated PGE release into the colonic lumen. If the laxative action of a drug is mediated by PGE release, the effect on net water flux as well as on PGE release should be lower in indomethacin-pretreated rats, because indomethacin inhibits PGE biosynthesis in the gut (Ferreira et al 1976; Beubler & Juan 1978c).

Since indomethacin significantly reduced the effect of ricinoleic acid, oleic acid and deoxycholic acid on net water flux and PGE release, the laxative effect of these drugs may be partly caused by stimulation of PGE synthesis.

Several discrepances, however, make the interpretation of the results difficult: (i) the strongly stimulated PGE release induced by ricinoleic acid is not abolished by indomethacin. The remaining PGE release might be responsible for the remaining effect on water transport because in kinetic studies indomethacin is shown to be a competitive inhibitor of PG synthetase from sheep seminal vesicles. On the other hand, indomethacin is reported to inactivate PG synthetase in a non-reversible and time-dependent manner (for ref. see Gryglewski 1974).

(ii) In contrast, deoxycholic acid released only small amounts of PGE but influenced net water flux as strongly as ricinoleic acid. Although indomethacin abolished PGE release in this case, the effect on net water flux was not significantly changed at 20 min and was only slightly reduced at 60 min. No satisfactory explanation for these discrepancies is apparent.

PGE release induced by dioctyl sodium sulphosuccinate is only lowered by indomethacin. The effect on net water flux is not significantly reduced. The stimulating influence of sennoside A + B on PGE release was prevented by indomethacin. Its effect on net water flux was not changed. Therefore, the present experiments cannot clarify whether the laxative effects of dioctyl sodium sulphosuccinate and of sennoside A + B are mediated by the released PGE.

Log-PGE release correlates well (r = 0·99) with net water flux in controls and in experiments with ricinoleic acid, oleic acid, stearinic acid and dioctyl sodium sulphosuccinate (with and without indomethacin) (Fig. 1).

Deoxycholic acid, sennoside A + B and mannitol

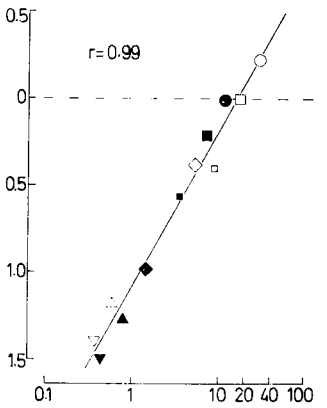

FIG. 1. Correlation between net water flux (ordinate: ml h^{-1}) and PGE-release (abscissa: log ng h^{-1}) in the ligated colon (60 min) of the rat in vivo, without (open symbols) or with pretreatment with indomethacin (full symbols). Controls (△▲); stearinic acid (▽▼); oleic acid (◇◆); ricinoleic acid (○●); dioctyl sodium sulfosuccinate 2 mM (□■) and 4 mM (□■). Each point is a mean of 6 determinations. The correlation coefficient r = 0·99.

do not fit in this correlation. Deoxycholic acid has the same effect as ricinoleic acid on net water flux, the PGE release, however, is 10 times lower for deoxycholic acid. No explanation for this discrepancy can be given. The failure of sennoside A + B to fit in the correlation may be caused by the different method used in this case (see methods).

The effect of mannitol is osmotic and not mediated by PGE release (Beubler & Juan 1978b).

To this date, no conclusive explanation for the mechanism of the laxative effect of the drugs tested has been given. Ricinoleic acid, dihydroxy bile salts, diphenolic laxatives and surfactants are reported to cause mucosal injury which might be responsible for increased permeability (for ref. see Gaginella & Bass 1978). Since disturbance of cellular membranes is known to evoke PGE release (for ref. see Flower & Blackwell 1976; Gilmore et al 1968), mucosal injury caused by laxatives may cause PGE release.

For several laxatives, a hypothesis based upon cAMP-mediated secretion has been discussed. Since PGE is shown to stimulate cAMP-formation in the gut in vitro (Kimberg et al 1974; Gaginella et al 1977) and in vivo (Beubler 1979), the cAMP-formation induced by ricinoleic acid (Binder 1974; Binder et al 1975; Conley et al 1976) and dioctyl sodium sulphosuccinate (Donowitz & Binder 1975) may be the consequence of the preceding release of PGE.

Another theory concerning the mechanism of action of diphenolic laxatives is based upon the observed inhibition of Na^+/K^+-ATPase in mucosal cells (for ref. see Gaginella & Bass 1978). Inhibition of the active sodium transfer, however, does not explain the net transfer of chloride and water from blood to lumen, an effect which is caused by PGE (Pierce et al 1971).

The hypothesis concerning the possible role of PGs as mediators of laxative actions of laxatives is further supported by the finding of Awouters et al (1978) who show a good correlation between the anti-inflammatory potency of non-steroidal anti-inflammatory compounds (inhibitors of PG biosynthesis) and their potency to delay castor oil-induced diarrhoea.

The above results lend support to the hypothesis that PGE may mediate the cathartic action of various laxatives. Whether other PGs such as prostacyclin (PGI_2) may be involved, cannot yet be decided. However, mucosal injury, inhibition of cellular energy production and the adenylate cyclase-cAMP-system may also be involved in the action of these agents.

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