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J. Pharm. Pharmacol. 1986, 38: 627–629
Communicated January 14, 1986

Laxatives and the production of autacoids by rat colon

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The effects of some laxatives were examined on the formation of histamine, 5-hydroxytryptamine (5-HT) and prostaglandin-like material (PG-LM) by rat intestine in-vitro. Castor oil, senna, sulphosuccinate and bisacodyl, but not mannitol or lactulose, in doses that cause laxation, increased the formation of histamine, 5-HT and PG-LM. Indomethacin or hydrocortisone reduced the increase of PG-LM formation. The data support the idea that the laxative effects of these intestinal secretagogues are due to increased intestinal production of PG-LM, histamine and 5-HT.

Prostaglandins (PGs) inhibit absorption of water and electrolytes in the gut and consequently increase intestinal fluid volume (Pierce et al 1971). Such effects are similar in many respects to those caused by intestinal secretagogues (Beubler & Juan 1979). Histamine and 5-hydroxytryptamine (5-HT) also stimulate intestinal fluid and ion secretion (Koskowski 1926; Lee & Silverberg 1976; Donowitz et al 1977), and we have recently shown that phenolphthalein increases the formation of histamine, 5-HT and prostaglandin-like material (PG-LM) by rat intestine (Autore et al 1984). Our findings support the hypothesis that these three substances contribute to the laxative effect of phenolphthalein. In this paper we present evidence that other luminal secretagogues stimulate the output of histamine, 5-HT and PG-LM by rat colon.

Materials and methods

Male, Wistar Nossan rats (Correzzana, Italy), 150–160 g, were deprived of food overnight but allowed free access to water. Bisacodyl (5 mg kg^{-1}), castor oil (2 ml/rat), lactulose (5 g kg^{-1}), mannitol (10 g kg^{-1}), senna (50 mg kg^{-1}), sulphosuccinate (20 mg kg^{-1}) and water (control, 1 ml/rat) were administered by gavage. When diarrhoea was evident, the rats were killed by exposure to ether and bled. Specimens of colon were removed, rinsed in 150 mM NaCl and immediately weighed. For extraction of 5-HT, 1 M HCl was added (w/v 1:2), the tissue cut finely with scissors, homogenized, boiled for 1–2 min and centrifuged at 5000*g* for 10 min. Supernatants were neutralized with 1 M NaOH and assayed on rat gastric fundus strips in 5 ml Krebs-Henseleit solution bubbled with 5% CO_2 in O_2 at 37 °C. The bathing fluid contained (μg ml^{-1}) atropine 0·1, propranolol 0·2, mepyramine 0·1 and phenoxybenzamine 0·5. The specificity of the assay for 5-HT was checked with methysergide 0·1 μg ml^{-1}. Histamine was extracted by the same procedure except that the pH of the boiled solution was 2. The extract was bioassayed on the guinea-pig ileum in 5 ml Tyrode solution bubbled with 5% CO_2 in O_2 at 37 °C. The bathing fluid contained atropine, methysergide, propranolol and phenoxybenzamine in the concentrations used above, and the specificity of the histamine assay was checked with mepyramine 0·1 μg ml^{-1}. For extraction of PG-LM, ethanol was added to the finely cut tissue, decanted and evaporated to dryness under N_2. The tissue was then homogenized in ethyl acetate: Krebs solution buffered with Sörensen's citrate/HCl 0·1 M solution (1:1), final pH 3·0 (5:2·5). The dry extract was dissolved in 1 ml Krebs solution and bioassayed on rat gastric fundus strips in the presence of atropine, mepyramine, methysergide, propranolol, phenoxybenzamine (in the concentrations used above) and indomethacin (0·2 μg ml^{-1}). Some experiments were performed on rats pretreated with indomethacin (4 mg kg^{-1}) or hydrocortisone (20 mg kg^{-1}) injected, 48, 24 and 12 h before starting the experiment.

The following drugs were used: bisacodyl, dioctyl sulphosuccinate, mannitol, lactulose, hydrocortisone, histamine, 5-HT (all from Sigma); indomethacin (Gianni); PGE_2 (Upjohn); castor oil (Carlo Erba); senna (Senade: Andard mount). All other chemicals

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were analytical grade preparations obtained from usual commercial sources.

Results

Data obtained from laxative-treated rats are shown in Table 1. All extracts contained histamine, 5-HT and PG-LM. The mean concentrations of these endogenous substances were higher in all the tissue extracts from laxative-treated rats than from controls. Castor oil, the most active secretagogue, approximately produced a four-fold increase in PG-LM ($P < 0.01$) and a three-fold increase in histamine and 5-HT ($P < 0.01$). The effect of senna was in the following rank order: PG-LM ($P < 0.01$) > 5-HT ($P < 0.05$) > histamine ($P > 0.05$). The production of PG-LM, histamine and 5-HT was also increased by sulphosuccinate and bisacodyl ($P < 0.05$), but mannitol or lactulose had little or no effect.

Table 1. Histamine, 5-hydroxytryptamine (5-HT) and prostaglandin-like material (PG-LM) recovered from rat colon after in-vivo treatment with one of several laxatives.

Treatment	Histamine $\mu g\,g^{-1}$ tissue	5-HT $\mu g\,g^{-1}$ tissue	PG-LM ng PGE_2 equivalent g^{-1} tissue
None	4·1 ± 1·5	4·8 ± 1·7	7·3 ± 1·8
Bisacodyl 5 mg kg^{-1}	8·1 ± 1·0*	9·0 ± 1·4*	14·5 ± 1·4*
Castor oil 2 ml/rat	12·0 ± 1·0**	13·9 ± 1·5**	29·4 ± 2·9**
Mannitol 10 g kg^{-1}	5·0 ± 1·7	5·1 ± 1·9	8·4 ± 2·3
Lactulose 5 g kg^{-1}	5·7 ± 2·0	5·9 ± 1·4	9·9 ± 1·6
Senna 50 mg kg^{-1}	11·6 ± 2·0*	12·0 ± 2·3**	23·3 ± 2·4**
Sulphosuccinate 20 mg kg^{-1}	8·3 ± 1·4*	10·3 ± 1·0**	17·9 ± 1·3**

Each result is the mean ± s.e. of 6–8 experiments.
* $P < 0.05$, ** $P < 0.01$ compared with control, Student's t-test.

Pretreatment of the animals with indomethacin or hydrocortisone reduced the production of castor oil-, senna-, sulphosuccinate- or bisacodyl-induced PG-LM (Table 2). Indomethacin or hydrocortisone given to control rats decreased PG-LM values by 35% ($P < 0.05$). All the drugs produced unformed faeces in 2–4 h, but pretreatment with indomethacin or hydrocortisone reduced the effect by 60 and 73%, respectively ($P < 0.01$), and no gastrointestinal side effects were observed.

Discussion

The association of diarrhoea with tumours of different origin with high plasma levels of PG has been taken as evidence that the diarrhoea is mediated by PG (Williams et al 1968; Rask-Madsen & Bukhave 1981). PGE inhibits intestinal absorption of water and electrolytes (Pierce et al 1971; Beubler & Juan 1979; Al-Awqati & Greenough 1972) and may be released from the gut after mechanical stimulation (Collier 1974). Several laxatives also inhibit absorption or induce secretion of electrolytes and water, and it has been proposed that such drugs may act by liberating PGs (Beubler & Juan 1978).

Table 2. PG-LM (ng PGE_2 equivalent tissue) recovered from the colon of rats given laxatives alone or after pretreatment with indomethacin (4 mg kg^{-1} s.c.) or hydrocortisone (20 mg kg^{-1} s.c.).

Laxatives	Indomethacin	Hydrocortisone
None	5·1 ± 0·7**	3·0 ± 1·9**
Castor oil 2 ml/rat	15·3 ± 1·7**	10·4 ± 2·9**
Senna 50 mg kg^{-1}	16·0 ± 2·4**	12·0 ± 2·0**
Sulphosuccinate 20 mg kg^{-1}	12·4 ± 1·4*	9·8 ± 1·9**
Bisacodyl 5 mg kg^{-1}	10·4 ± 1·0*	9·8 ± 2·0*

Each result is the mean ± s.e. of 6–8 experiments. * $P < 0.05$, ** $P < 0.01$ (Student's t-test) compared with group treated with the laxative alone.

The present results demonstrate that with the exception of the osmotic laxatives lactulose and mannitol, the laxatives tested stimulated colonic formation of PG-LM in rats, and this was reduced by indomethacin, an inhibitor of PG biosynthesis, and especially by hydrocortisone, a phospholipase A_2 inhibitor. Both drugs also reduced the laxative effect of castor oil, senna, sulphosuccinate and bisacodyl. These findings confirm previous results (Beubler & Juan 1979; Cohen 1982; Capasso et al 1983) and indicate that castor oil and the other non-osmotic laxatives studied may act through PG release.

Like PGs, histamine and 5-HT can stimulate fluid transport in the gut (Koskowski 1926; Donowitz et al 1977; Powell & Field 1980). High amounts of these substances are normally present throughout the gastrointestinal tract (Thompson 1966; Bertaccini 1982) and it is thought that some diarrhoea may also be caused by overproduction and release of 5-HT as well as of PGs (Bennett 1984). The present results show that the non-osmotic laxatives tested did increase the formation of 5-HT and histamine. It seems that 5-HT release is not brought about by PGs since methysergide does not affect PG-induced diarrhoea (Karim 1974) and PGE_1 or PGE_2 do not alter levels of 5-HT in the rat gastrointestinal tract (Thompson & Angulo 1968).

Ricinoleic acid (the active component of castor oil), bisacodyl and other laxatives are reported to cause mucosal injury (Beubler & Juan 1979), and this may release histamine and 5-HT, since disturbance of cellular membranes is known to release inflammatory mediators (Flower & Blackwell 1976). Histamine and 5-HT increase the availability of calcium to phospholipase A_2 and consequently increase PG production (Derksen & Cohen 1975; Rask-Madsen & Bukhave 1983). Some laxatives require the presence of calcium for their effects on electrolyte transport (Donowitz et al 1984) and stimulation of arachidonic acid metabolism (Capasso et al 1985), therefore, we must take into account the possibility that excess of 5-HT and histamine can activate membrane phospholipase and conse-

quently increase the yield of eicosanoids (Capasso et al 1984; Capasso unpublished observation), thereby increasing the laxative effect.

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J. Pharm. Pharmacol. 1986, 38: 629–630
Communicated February 20, 1986

Spectrofluorimetric analysis and buccal absorption of medifoxamine

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Medifoxamine is a new investigational antidepressant drug. Its buccal absorption at pH 5–9, which can be considered as an in-vivo model of passive drug transfer through a lipid membrane, was studied in six normal, healthy volunteers to predict its pharmacokinetic profile in man. Maximum absorption of medifoxamine occurred at pH 8, which is close to its pK_a (8·5).

A study of buccal absorption of drugs at various pH levels (particularly pH 5–9) can be considered as an in-vivo model of passive drug transfer through lipid membranes (Beckett & Triggs 1967) and may help to predict the extent of binding to plasma proteins (Henry et al 1981), the concentration in various body fluids and renal excretion at different urinary pH values (Meyer et al 1974; Ankier & Kaye 1976; Kaye & Long 1976).

Changes in pH relative to the pK_a of a drug alter the extent of its buccal absorption. For a basic drug, maximum absorption occurs at or above its pK_a, with the converse for an acidic drug. However, the controlling factor influencing the extent of absorption of a drug is its innate lipophilicity (Beckett & Triggs 1967).

Medifoxamine is a new investigational antidepressant drug (Bonnet et al 1984). Its buccal absorption at pH 5–9 was studied in normal, healthy volunteers using a method similar to that of Beckett & Triggs (1967).

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Materials and methods

Buccal absorption of medifoxamine. The study was carried out, with informed consent, in 6 normal, healthy, medically qualified volunteers (4 males, 2 females) aged between 25–35 years. They were requested not to eat or drink for at least 1 h before the study. After rinsing the mouth with 20 ml of buffer, 20 ml of medifoxamine solution (50 $\mu g\ ml^{-1}$, in the same buffer) was agitated in the mouth for 5 min, and expelled into a beaker. Immediately after that the mouth was rinsed with 20 ml of the buffer for 30 s and expelled into the same beaker. The volume of the fluid in the beaker was measured and the medifoxamine concentration estimated spectrofluorimetrically, indicating the amount