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Phenolphthalein stimulates the formation of histamine, 5-hydroxytryptamine and prostaglandin-like material by rat jejunum, ileum and colon

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1 The effects of phenolphthalein on the formation of histamine, 5-hydroxytryptamine (5-HT) and prostaglandin-like material by rat intestine were examined *in vivo*.

2 Phenolphthalein, in a dose that causes laxation increased the formation of histamine, 5-HT and prostaglandin-like material, and indomethacin reduced these increases.

3 The data support the idea that the laxative effect of phenolphthalein is due to increased intestinal production of prostaglandin, histamine and 5-HT.

Introduction

Rat intestine can form substantial amounts of prostaglandin-like material (Bennett & Charlier, 1977; Cohen, 1982), and can release them in response to various stimuli (Bennett, Friedmann & Vane, 1967; Herman & Vane, 1976). Increased prostaglandin formation may augment intestinal peristalsis and cause diarrhoea (Karim, 1974; Bennett, 1976). These considerations have stimulated investigations into the possibility that laxative drugs such as phenolphthalein act by stimulating prostaglandin biosynthesis (Beubler & Juan, 1979). However, high amounts of other substances such as 5-hydroxytryptamine (5-HT) and histamine are present throughout the gastrointestinal tract (Thompson, 1966; Bertaccini, 1982). Since diarrhoea may be caused by over-production and release of prostaglandins or 5-HT (Karim, 1974), it is important to examine any interrelation between these substances, and to determine whether the laxative effect of phenolphthalein involves the release of mediators other than prostaglandins (Beubler & Juna, 1979).

Methods

Male Wistar rats (120–130 g) were deprived of food overnight but allowed water *ad libitum*. Phenolphthalein 16 mg kg^{-1} was administered by gastric gavage and 4 h later the rats were killed by exposure to ether and bled. Jejunum, ileum and colon were then removed, rinsed in saline and immediately weighed. For extraction of 5-HT, 1 M HCl was added to the tissue (w/v 1:2), cut finely with scissors and then homogenized. The homogenates were boiled for 1–2 min and centrifuged at a low speed (5000 *g* for 10 min). The supernatants were collected, neutralized with 1 M NaOH and assayed on rat isolated stomach strips. The bathing solution was 5 ml Krebs-Henseleit solution bubbled with 5% CO_2 plus 95% O_2 at 37°C, containing atropine 0.1 μg ml^{-1}, propranolol 0.2 μg ml^{-1}, mepyramine 0.1 μg ml^{-1} and phenoxybenzamine 0.5 μg ml^{-1}. The specificity of the action was checked by using methysergide (0.1 μg ml^{-1}). For extraction of histamine the same procedure was employed except that the pH of the boiled solution was 2. The extract was then assayed on the ileum of guinea-pigs (200 g). The bathing fluid was 5 ml Tyrode solution bubbled with 5% CO_2 plus 95% O_2 at 37°C, containing atropine, methysergide, propranolol and phenoxybenzamine in the concentrations used above. The specificity of the action was checked with mepyramine (0.1 μg ml^{-1}). For extraction of prostaglandin-like material ethanol was added to the tissue, removed and carefully evaporated to dryness under nitrogen. The tissue was homogenized in Krebs solution buffered with Sorensen's citrate/HCl 0.1 M solution (1:1) final pH 3.0:ethyl acetate (2.5:5). The dry extract was dissolved in 1 ml Krebs solution and bioassayed on rat stomach strips in the presence of atropine, mepyramine, methysergide, propranolol and phenoxybenzamine at the same concentrations used above. Some experiments were performed on rats pretreated with indomethacin (4 mg kg^{-1}) injected 48, 24 and 12 h before starting the experiment.

Table 1 Histamine, 5-hydroxytryptamine (5-HT) and prostaglandin (PG)-like material recovered from intestine of rats treated with phenolphthalein and indomethacin alone and together

Treatment	*Intestinal region*	*Histamine* ($\mu g\,g^{-1}$ tissue)	*5-HT* ($\mu g\,g^{-1}$ tissue)	*PG-like material* (ng PGE_2 equivalents g^{-1} tissue)
None				
	Jejunum	13.0±3.0	4.8±0.6	6.5±0.8
	Ileum	3.9±0.6	4.4±0.7	7.6±1.0
	Colon	5.7±0.7	4.4±0.9	8.9±1.0
Phenolphthalein				
	Jejunum	11.1±2.7	7.4±0.9*	10.1±0.7*
	Ileum	12.7±1.5**	10.8±0.9**	12.3±1.0**
	Colon	11.8±1.5**	10.8±1.7**	16.1±1.3**
Phenolphthalein + indomethacin				
	Jejunum	1.7±2.0	5.6±0.7	6.0±0.7
	Ileum	5.0±6.7	4.9±0.9	7.9±0.5
	Colon	7.7±0.5	6.9±0.5	8.3±0.7
Indomethacin				
	Jejunum	11.9±1.7	5.0±0.4	4.3±0.9*
	Ileum	4.0±0.7	4.0±0.8	5.0±1.0*
	Colon	6.0±0.5	4.6±0.9	4.7±0.9*

Phenolphthalein ($16\,mg\,kg^{-1}$) was given orally 4 h before the animals were killed. Indomethacin ($4\,mg\,kg^{-1}$) was given s.c. 48, 24 and 12 h before starting the experiments. Each result is the mean ± s.e. of 5–7 experiments. *$P<0.05$; **$P<0.01$ compared with control, Student's t test.

Results

Data obtained are shown in Table 1. All extracts contained histamine, 5-HT and prostaglandin-like material. Mean levels of histamine, 5-HT and prostaglandin-like material were higher in all the tissue extracts from phenolphthalein-treated rats than from controls, except for histamine which did not increase in the jejunum. Pretreatment of the animals with indomethacin prevented the phenolphthalein-induced production of histamine, 5-HT and especially prostaglandin-like material in all intestinal regions. Indomethacin given alone to control rats decreased the levels of PGs but not those of histamine or 5-HT. Phenolphthalein $16\,mg\,kg^{-1}$ produced unformed faeces throughout the terminal ileum, caecum and colon. Pretreatment with indomethacin reduced this effect. No gastric ulceration was observed in any indomethacin-treated rat.

Discussion

These results demonstrate that the intestinal production of prostaglandin-like material increases after oral administration of phenolphthalein. Laxatives stimulate the output of prostaglandins into the colonic lumen of rats (Beubler & Juan, 1979, Capasso, Mascolo, Autore & Duraccio, 1983) and increase the net secretion of water and electrolytes into the small intestine (Beubler & Juan, 1979). Indomethacin, an inhibitor of prostaglandin biosynthesis, delayed or decreased castor-oil-induced diarrhoea (Awouters, Niemegeers, Lenaerts & Janssen, 1978; Vischer & Casals-Stenzel, 1982) and reduced the laxative effect of phenolphthalein (Capasso *et al.*, 1984). These findings, together with the knowledge of the action of prostaglandin E_2 (PGE_2) on intestinal water and ion transport, indicate that phenolphthalein and other non-osmotic laxatives act partially through prostaglandin release. However, Cohen (1982), using different laxatives, showed that only senna produced a statistically significant stimulation of prostaglandin-like material.

The present results also show that phenolphthalein increases the formation of histamine and 5-HT by the intestine except, for a reason that is not understood, in the jejunum. The failure of methysergide to affect prostaglandin-induced diarrhoea implies that 5-HT is not involved (Karim, 1974), and PGE_1 or PGE_2 do not alter the levels of 5-HT in the rat gastrointestinal tract (Thompson & Angulo, 1969).

Our results show that indomethacin, given alone, does not alter the normal intestinal production of histamine or 5-HT but reduces the increased formation of these substances induced by phenolphthalein. The stimulation of histamine and 5-HT output by phenolphthalein may be due to altered gut motility, to an over-production of prostaglandin-like material, or to a non-related direct effect of phenolphthalein. All 3 substances might contribute to the laxative effect obtained.

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