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Influence of prostacyclin and indomethacin on castor oil-induced gastrointestinal effects in rats

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The effects of castor oil, alone, as well as in combination with PGI_2 and indomethacin on gastrointestinal functions have been examined in rats. Oral administration of the oil to fasted rats induced severe diarrhoea, with increased intestinal motility and fluid volume. Pretreatment with PGI_2 (s.c.) inhibited the effect of the oil on intestinal fluid accumulation and decreased intestinal motility below control values, but only delayed the occurrence of mucoid diarrhoea. Indomethacin (i.p.) reduced the accumulation in intestinal fluid after castor oil administration to a much smaller extent (47%) than PGI_2 and depressed the increased intestinal motility to control values. In contrast to PGI_2, indomethacin inhibited the occurrence of diarrhoea after administration of castor oil. The present results do not definitely confirm the general opinion that the diarrhoeal action of laxative agents is due only to an altered intestinal electrolyte and water transport or an increase of intestinal motility.

It is generally believed that certain types of diarrhoea are related to a stimulation of intestinal motility and to an inhibition of water reabsorption.

Alteration of water and electrolyte transport in the small intestine has been reported for laxatives like deoxycholic acid (Wanitschke et al 1977), anthraquinones (Lemmens & Borja 1976) and ricinoleic acid (Ammon et al 1974). Other gastrointestinally active substances like cholera toxin (Pierce et al 1971), colchicine (Beubler et al 1978) and prostaglandins E_1 and E_2 (Robert 1976; Matuchansky & Bernier 1973) show similar effects.

These agents activate mucosal adenylate cyclase (Schäfer et al 1970; Field et al 1972; Kimberg et al 1974). It is also widely accepted that the above mentioned laxatives act by stimulation of PGE biosynthesis in the intestinal mucosa (Beubler & Juan 1978, 1979). Since stimulation of intestinal fluid secretion is usually followed by watery diarrhoea, it seemed possible that changes in intestinal water transport contribute predominantly to the occurrence of diarrhoea. Robert et al (1979), have also recently shown that diarrhoea induced by the action of a PGE_2 derivative was prevented by pretreatment with PGI_2. This antidiarrhoeal action of PGI_2 was associated with an inhibition of intestinal fluid accumulation.

We have investigated the influence of PGI_2 on castor oil-induced diarrhoea to evaluate the general validity of PGI_2 as an antidiarrhoeal agent and to confirm a possible linkage between intestinal water accumulation and occurrence of diarrhoea.

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MATERIALS AND METHODS

Animals

Male wistar rats, 190–220 g, were used. Standard food (Altromin) was withheld for 24 h before experiments; there was free access to drinking water.

Solutions and drugs

PGI_2: Prostacyclin sodium salt (Schering AG) was dissolved in a small amount (0·1 ml) of ethanol and then diluted (1 mg ml^{-1}) with 0·15 M Tris HCl-buffer (pH 8·6) and stored on ice. Indomethacin (Prodotti Gianni, Mailand, Italy) was suspended in a 0·9% NaCl solution containing 0·1% Tween 80. PGI_2 and indomethacin were administered in a volume of 0·5 ml for intestinal motility studies and intestinal fluid volume determination; for studies of diarrhoea the volume was 1 ml. Castor oil was of commercial grade and given orally as 1 ml per animal.

Intestinal fluid volume determination

For the estimation of intestinal fluid accumulation the enteropooling assay of Robert et al (1979) was used. PGI_2 (0·1, 0·25, 0·5, 0·75 or 1·0 mg kg^{-1} s.c.) or indomethacin (5, 10 or 20 mg kg^{-1} i.p.) were injected 10 or 60 min before oral castor oil administration, respectively. 30 min after gavage of castor oil the animals were decapitated, the small intestine removed and its contents collected in a test tube and its volume determined gravimetrically.

Intestinal motility studies

At the time of administration of castor oil, the rats also received 1·5 ml of freshly prepared graphit-agar suspension comprising 1·5% agar and 0·4% Indian ink. PGI_2 (0·25, 0·5 or 1·0 mg kg^{-1} s.c.) or indomethacin (5, 10, 20 or 40 mg kg^{-1} i.p.) were injected 10 min or 60 min, respectively, before administration of the castor oil and graphit-agar suspension.

The animals were killed by cervical dislocation 30 min after being given the castor oil and graphit-agar. The whole gastrointestinal tract was quickly removed and the entire small intestine from the pylorus to the caecum gently stretched and its length as well as the distance that the black marker had been transported were measured. Intestinal motility was expressed as percentage of the length traversed by the marker to the total length.

Diarrhoea

Rats, from which food had been withheld, kept in individual cages were divided into three groups. The first group received only castor oil (1 ml orally), the second group was pretreated subcutaneously with PGI_2 (0·25, 0·5 or 1·0 mg kg^{-1} s.c.) 10 min before the castor oil and the third group received saline only and served as control.

A similar experimental protocol was used for the indomethacin study. Indomethacin at doses of 0·5, 5, 10 or 40 mg kg^{-1} i.p. was injected 60 min before administration of castor oil.

After the castor oil challenge, rats were observed over 4 h for the assessment of characteristic diarrhoea.

Statistics

Results are expressed as arithmetric mean and standard error of the mean (s.e.m.) although non-parametric statistics were used. Significant differences compared with the control groups were evaluated by the non-parametric Kruskal-Wallis test (Kruskal 1952).

RESULTS

Effect on intestinal fluid volume

In a preliminary experiment the time course of enteropooling activity of castor oil after pretreatment with PGI_2 was studied (Fig. 1). Castor oil caused a significant increase in intestinal fluid volume with a maximal effect at 30 min, followed by a decline to normal level after about 60 min, then the fluid content fell significantly below controls.

PGI_2 (1 mg kg^{-1} i.p.) given 10 min before castor oil abolished its rapid enteropooling effect. 90 min after castor oil the intestinal fluid volume was slightly increased but was normal again at 120 min.

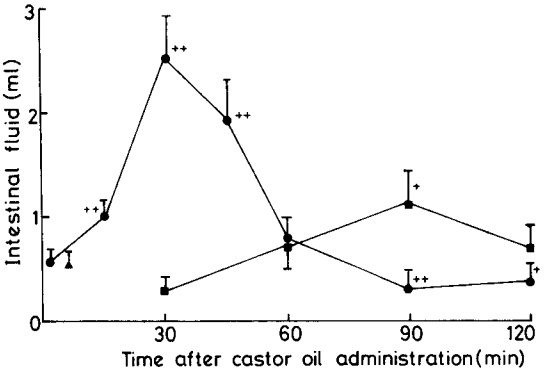

FIG. 1. Time course of the castor oil induced increase of intestinal fluid volume and its alteration by PGI_2. Changes in intestinal fluid volume were induced by oral administration of 1 ml castor oil (●) per animal and the estimation of intestinal fluid volume occurred at the time points indicated. PGI_2 (■); 1·0 mg kg^{-1} s.c. given 10 min before castor oil administration. [The points and the vertical bars represent the mean values and s.e.m. from 10 rats; + ($P < 0·05$) and ++ ($P < 0·001$) significantly different from control (▲)]

In the following experiments the influence of PGI_2 and indomethacin was assessed at the time of maximal enteropooling activity of castor oil (30 min). Pretreatment with PGI_2 dose-dependently reduced castor oil-induced intestinal fluid accumulation, reaching 99% inhibition at a dose of 250 μg kg^{-1}. Higher doses of PGI_2 (500 and 750 μg kg^{-1}) decreased intestinal fluid volume below control values (Fig. 2).

Indomethacin reduced the enteropooling activity of castor oil induced enteropooling less than PGI_2 (Fig. 2). The highest dose of indomethacin (20 mg kg^{-1}) reduced the increased fluid volume by 47%.

Intestinal motility

Castor oil increased the movement of the graphit-agar suspension through the small intestine (Fig. 3). 30 and 60 min after administration significant differences between controls and treated animals ($P < 0·01$) were found. The influence of PGI_2 and indomethacin on intestinal propulsion is summarized in Table 1. PGI_2 0·25, 0·5 or 1·0 mg kg^{-1} s.c. alone dose-dependently reduced rat intestinal propulsion. In combination with castor oil the same inhibitory result was achieved by PGI_2 at a dose of 1 mg kg^{-1} s.c. This reduction of intestinal motility induced by PGI_2 lasted for 60 to 90 min and thereafter control values were reached (data not

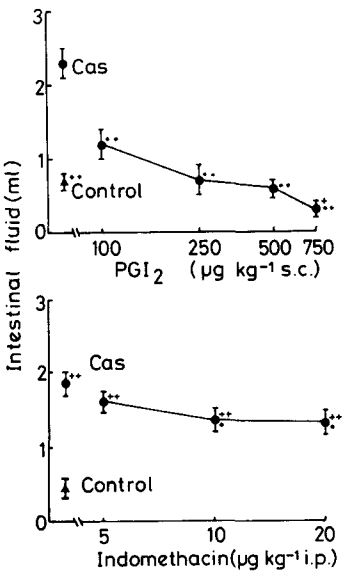

FIG. 2. Influence of prostacyclin and indomethacin on castor oil-induced increase of intestinal fluid volume. 10 min or 60 min before castor oil (Cas) treatment (1 ml per animal) animals received different doses of PGI_2 s.c. or indomethacin i.p., respectively. Changes in small intestinal fluid volume were registered 30 min after castor oil administration. Control animals received saline solution. [The points and the vertical bars represent the mean values and s.e.m. from 6 rats; abscissa: log scale; * ($P < 0.05$) and ** ($P < 0.001$) significantly different from the group treated with castor oil alone; + ($P < 0.05$) and ++ ($P < 0.001$) significantly different from control].

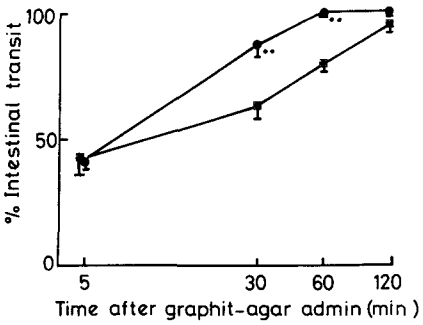

FIG. 3. Intestinal motility under the influence of castor oil. Animals received castor oil (●) [1 ml per animal] simultaneously with 1·5 ml of a graphit-agar suspension and at the time intervals indicated the percentage of small intestinal stained by the black marker referred to the total length of the small intestine was calculated. Control animals (■) received only saline solution. [The points and the vertical bars represent the mean values and s.e.m. of 6 animals. ** ($P < 0.001$) significantly different from control].

shown). In contrast, indomethacin alone had no significant effect on intestinal motility, but at 20 and 40 mg kg^{-1} reduced castor oil enhanced intestinal transport to control levels.

Diarrhoea

Administration of castor oil caused diarrhoea in over 90% of starved rats within 1 h of treatment. PGI_2 prevented diarrhoea during the first hour at all doses but during the following (second) hour, diarrhoea appeared at lower PGI_2 doses (250 and 500 μg kg^{-1}) in 100% of the animals and at the highest dose used (1 mg PGI_2 kg^{-1}) in 88% of the rats (Fig. 4).

In contrast, indomethacin dose-dependently inhibited the occurrence of diarrhoea during the 4 h observation period and moreover, the highest dose of indomethacin (40 mg kg^{-1}) prevented the development of diarrhoea in all rats (Fig. 4).

DISCUSSION

The induction of diarrhoea is a well-known action of castor oil and several investigations have sought to explain its cathartic property. Observations of the intestinal action of castor oil or its active component, ricinoleic acid, suggest a stimulation of endogenous prostaglandin biosynthesis. In-vivo studies in rats demonstrated a significant increase in the portal venous PGE_2 concentration following oral administration of castor oil (Luderer et al 1980). Ricinoleic acid markedly increased the PGE content in the gut lumen and also caused an increase of the net secretion of water and electrolytes into the small intestine (Beubler & Juan 1979). Inhibitors of

Table 1. Effect of PGI_2, indomethacin and castor oil on intestinal motility. Castor oil (1·0 ml) was administered orally simultaneously with the graphit-agar suspension. PGI_2 (s.c.) was given 10 min. indomethacin (i.p.) 60 min before the graphit-agar suspension. [Percentage (mean ± s.e.m.) of small intestine stained by the black marker in 30 min was determined (n = 6 rats); † ($P < 0.05$) and ‡ ($P < 0.001$) significantly different from control; * ($P < 0.001$) significantly different from castor oil alone].

	Dose (mg kg^{-1})	% Intestinal transit
Control	—	70·5 ± 2·5
PGI_2	0·25	25·0 ± 5·5‡
	0·50	5·9 ± 4·1‡
	1·00	3·5 ± 2·8‡
Castor oil alone	—	87·2 ± 4·4†
PGI_2 + castor oil	1·00	10·2 ± 1·0†
Control	—	65·2 ± 3·8
Indomethacin	5·00	58·3 ± 0·6
	10·00	58·5 ± 3·4
	20·00	61·7 ± 2·7
Control	—	77·4 ± 3·8
Castor oil alone	—	92·1 ± 5·9
Indomethacin and castor oil	20·00	59·7 ± 5·6*
	40·00	56·5 ± 1·7†*

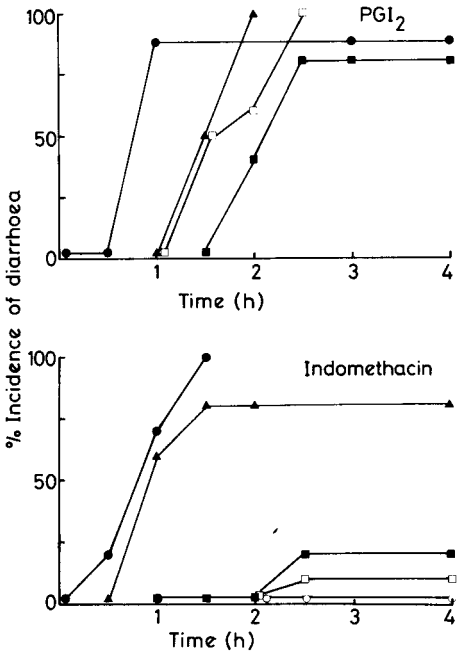

FIG. 4. Effect of PGI_2 and indomethacin on castor oil-induced diarrhoea. Animals received different doses of PGI_2 s.c. or indomethacin i.p. 10 min or 60 min, respectively, before castor oil administration (1 ml per animal). Occurrence of diarrhoea was observed during a time period of 4 h after castor oil challenge. Control animals received saline solution. [(●) castor oil alone; pretreatment with PGI_2: (▲) 250, (□) 500 and (■) 1000 μg kg^{-1} s.c.; pretreatment with indomethacin: (▲) 0·5, (■) 5, (□) 10 and (○) 40 mg kg^{-1} i.p.; n = 10 rats].

prostaglandin biosynthesis delayed castor oil-induced diarrhoea (Awouters et al 1978). These findings, together with the knowledge of the action of PGE_2 on intestinal water and ion transport, indicate that diarrhoea caused either by prostaglandins or through the induction of prostaglandin biosynthesis is the result of an altered net secretion into the intestine possibly connected with an increase in intestinal motility (Bennett et al 1968; Pierce et al 1971; Taub et al 1978).

This assumption was further supported by the observation that PGI_2, in contrast to PGE_2 and PGE_1, did not stimulate intestinal fluid and electrolyte transport. Moreover PGI_2 inhibited both diarrhoea induced by 16,16-dimethyl PGE_2 and prevented an accumulation of intestinal fluid (Robert et al 1979; Hardcastle et al 1980).

To clarify the interrelation of these effects found by others, the influence of indomethacin, an inhibitor of PG-synthesis (Vane 1971; Ferreira et al 1971; Smith & Willis 1971), and of PGI_2 on the gastrointestinal effects of castor oil was investigated. The observations presented here underline the strong carthartic activity of castor oil, which is accompanied by a pronounced increase in intestinal fluid volume and in small intestinal motility. Indomethacin, an inhibitor of the cyclo-oxygenase, had only a minor influence on enteropooling activity of the oil. Given alone, this drug did not cause any alteration of intestinal motility but in the presence of castor oil it reduced the increased intestinal motility to control values.

Despite the persistence of the enteropooling effect and a concomitant normal intestinal motility after indomethacin pretreatment, no castor oil-induced diarrhoea occurred. This finding shows that a stimulation of prostaglandin synthesis is not exclusively responsible for the increase of intestinal fluid volume after castor oil, and confirms also that an enteropooling effect and an increase in intestinal motility are probably necessary for the induction of diarrhoea (Robert et al 1979).

In contrast to the results obtained with indomethacin, PGI_2 reduced the enteropooling activity of castor oil and also when administered alone as well as in the presence of castor oil-depressed intestinal motility. In addition, PGI_2 delayed the appearance of diarrhoea after single oral administration of castor oil by 30 min but could not prevent the effect after that time. This discrepancy between antienteropooling effect, depressed intestinal motility and occurrence of diarrhoea might be explained by a rapid loss of PGI_2 activity 40 to 60 min after s.c. administration, so that thereafter the effect of castor oil on intestinal fluid volume and intestinal motility could emerge. From the experimental data presented, this explanation seems to be rather improbable, because the increase of intestinal fluid volume after castor oil administration is a transient effect which lasts for 20 to 30 min and, moreover, the slight increase of intestinal fluid content with a concomitant reduced or normal intestinal motility about 1·5 h after PGI_2 administration cannot account for the appearance of diarrhoea at the same time.

The differences between our experiments and the results reported by Robert et al (1979) could be explained by different mechanisms of action for castor oil and for 16,16-dimethyl PGE_2 and also by the different experimental protocol where repeated s.c. injections of PGI_2 were administered.

In conclusion, all the data presented do not confirm the idea that the diarrhoeal action of castor oil is due only to an altered intestinal electrolyte and water movement and/or an increase in intestinal

motility. The persistent increase of intestinal volume did not interfere with the antidiarrhoeal action of indomethacin. Also the strong antienteropooling effect of PGI_2 did not abolish the cathartic action of castor oil. It is conceivable that its cathartic action may be due to other still unknown gastrointestinal dysfunctions which are affected by indomethacin but not by PGI_2.

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