PGE-Mediated Laxative Effect of Diphenolic Laxatives

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Summary. 1. In the tied off colon of the anaesthetized rat in situ, the effect of diphenolic laxatives (bisacodyl and phenolphthalein) and of osmotic laxatives (mannitol, sodium sulfate and lactulose) on water net flux was studied. All the laxatives reduced water net flux from lumen to blood or reversed it into water net flux from blood to lumen. Pretreatment with indomethacin reduced or abolished the effect of the diphenolic laxatives on water net flux but did not change the effect of the osmotic laxatives.

2. In the perfused colon of the rat in situ, the effect of the diphenolic and the osmotic laxatives on water net flux and on PGE-release into the colonic lumen was investigated. All laxatives reduced water net flux from lumen to blood in this preparation, too. PGE-release into the lumen during perfusion with osmotic laxatives was not different from the control. The diphenolic laxatives increased PGE-release dose-dependently about 2-fold.

3. Intraluminal perfusion of the jejunal loop of the rat in situ with bisacodyl increased intestinal blood flow by about 50%. Addition of indomethacin to the perfusion fluid abolished this increase.

4. It is concluded that diphenolic laxatives exert their laxative action at least partially via stimulation of intestinal PGE-biosynthesis.

Key words: Rat colon — Bisacodyl — Phenolphthalein — PGE-release — Indomethacin — Water net flux.

Introduction

Diphenolic laxatives like bisacodyl and phenolphthalein inhibit absorption of water and electrolytes in the colon and in the jejunum. They increase the transfer of water and electrolytes from blood to lumen which results in an increased intestinal fluid volume. Bisacodyl and phenolphthalein inhibit further the absorption of glucose in the jejunum (Forth et al., 1966; Adamic and Bihler, 1967; Nell et al., 1971). The basic mechanism of action of the diphenolic laxatives, as well as that of other “contact (stimulant) cathartics”, like castor oil and anthraquinones is still unknown (Fingl, 1975).

The prostaglandins (PGs) E1 and E2 inhibit absorption of water and electrolytes in the jejunum of man and animal, cause an increase of sodium and chloride flux from blood to lumen and, therefore, increase intestinal fluid volume (Pierce et al., 1971; Matuchansky and Bernier, 1973; Robert, 1976; Beubler and Juan, 1977; Beubler et al., 1978). PGs inhibit glucose absorption in the rat jejunum (Coupar and McColl, 1975) and are assumed to play a physiological role in the regulation of intestinal blood flow and transmucosal water movement (Beubler and Juan, 1977).

The present study was initiated to determine whether bisacodyl and phenolphthalein exert their action via stimulation of PGE-release in the gut (preliminary results: Beubler and Juan, 1978a). The diphenolic laxatives were compared with the osmotic laxatives sodium sulfate, mannitol and lactulose.

Methods

Animals. Female Sprague Dawley rats (Chemie Linz, 200 ± 10 g) were used after being deprived of food for 20h prior to the experiment.

Preparations

a) Tied Off Colon. In urethane anaesthesia (1.25 g/kg), the entire colon was rinsed with 20 ml warm saline solution in situ. After an interval of 30 min, the colon was filled with 2 ml Tyrode’s solution and tied off (Forth et al., 1966). The Tyrode’s solution contained the drug to be tested and the unabsorbable marker 14C-polyethylene...
glycol 4000 (PEG) (1.5 mg = 0.25 μCi/ml) to measure water net flux. After 60 min, the colon was removed, the intraluminal fluid was collected and 14C-activity was measured.

The experiments were performed in untreated rats and in rats pretreated with indomethacin, 4 mg/kg and day, s. c., starting 2 days prior to the experiments.

Water net flux was calculated from the concentration of PEG in the intraluminal fluid:

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\text{water net flux (ml/h)} = \frac{2 \times (\text{PEG})_a}{(\text{PEG})_b} - 2
\]

(PEG) \(a\) = 14C-PEG concentration in the Tyrode's solution; (PEG) \(b\) = 14C-PEG concentration in the intraluminal fluid after 60 min.

A negative value denotes net absorption and a positive value net secretion. Differences from control and differences between the untreated rats and the indomethacin pretreated rats were assessed by analysis of variance and the multiple range test of Duncan (1955). \(P < 0.05\) was considered to be significant; "n" always refers to the number of animals.

b) Perfused Colon. The rats were prepared as described above but the colon was continuously perfused (Perfusor, Braun-Melsungen 3.8 ml/h) with Tyrode's solution. The perfusate was collected in two 1-h periods and weighed. In the first (= control) period, the colon was perfused with Tyrode's solution, in the second period the Tyrode's solution contained the drug to be tested. In the perfusate, PGE was measured by radioimmunoassay.

Water net flux was calculated from the perfusion-rate and the weight of the perfusate. Water net flux (ml/h) = weight of the perfusate minus perfusion-rate. Negative values again denote net absorption and positive values net secretion.

Differences in water net flux and PGE-release between control-periods and test-periods were assessed with paired t-test; "n" always refers to the number of animals.

Radioimmunoassay of PGE. In the perfusate prostaglandin E \(_2\) was determined by radioimmunoassay. Antisera against PGE were obtained by immunization of rabbits according to Goodfriend et al. (1964). PGE was determined as described by Peskar and Hertting (1973). The antisera were used in a final dilution of 1:6000 and could bind 50--55% of the 3H-PGE1 or added 3H-PGE2, respectively. No differentiation between PGE1 and PGE2 in the perfusate of the colon was made. None of the substances in the concentrations used interfered nonspecifically with the radioimmunoassay.

c) Jejunal Loop. The influence of bisacodyl on intestinal blood flow was studied by using the method described by Beulher and Lemberg (1976): a jejunal loop was cannulated with polyethylene tubes on the oral and aboral end, the descending vein was punctured and the blood collected in 5-min periods. At the beginning of each 5-min period the loop was filled with 0.25 ml of control- or test-solution. Indomethacin (5 μg/ml) was added according to Beulher and Juan (1977) to the intraluminal fluid to inhibit PGE-biosynthesis.

Blood flow (ml/min and g wet weight) was calculated from the weight of the collected blood and the wet tissue weight of the loop. Differences were assessed with the paired t-test; "n" always refers to the number of animals.

Substances and Solutions. Carboximide hydrochloride (Story Chem. Corp., Muskegon), charcoal (E. Merck, Darmstadt), bovine serum albumin (Merek, Sharp and Dohme, Rahway, N.J.), \(^{3}H\)-prostaglandin \(E_1\) and \(E_2\) (The Radiochemical Centre, Amersham, U. K., spec. act. 59 Ci/mmol), prostaglandin \(E_1\) (Upjohn, Kalamazoo, Mich.), unisolve (Koch-Light Labs Ltd., Colnbrook U.K.), indomethacin (Merek, Sharp and Dohme, Rahway, N.J.), ethylene glycol (E. Merck, Darmstadt), \(^{14}C\)-polyethylene glycol, Radiochemical Centre, Amersham, U.K.).

Bisacodyl (Boehringer, Ingelheim), phenolphthalein (Mallinckrodt Chemical Works, N.Y.), mannitol and sodium sulfate (E. Merck, Darmstadt), and lactulose (Laevolac, Laevozan-Ges., Linz) were diluted in Tyrode’s solution. Phenolphthalein was stable in water containing 1% (v/v) ethanol. The concentration of 500 μg/ml phenolphthalein was stable in water after addition of 10% (v/v) ethylene glycol.

Results

A. Tied Off Colon

In the tied off colon of control rats, water net flux was directed from lumen to blood. Net water absorption was \(0.84 ± 0.08\) ml/h (Fig. 1). Intraluminal PGE\(_1\) (2μg/ml) reduced net water absorption (\(P < 0.01\)) (Fig. 1b).

Bisacodyl (1 μg/ml and 10 μg/ml) and phenolphthalein (10 μg/ml and 100 μg/ml) dose-dependently reduced net absorption of water or reversed it into net secretion (\(P < 0.01\)) (Fig. 1a). The osmotic laxatives, solutions of sodium sulfate (3.95% w/v), mannitol (4% w/v) and lactulose (2% w/v) caused net secretion of water (\(P < 0.01\)) (Fig. 1b).

Pretreatment of the rats with indomethacin did not influence net water absorption in the colon of control rats (Fig. 1); the effect of 1 μg/ml bisacodyl, however, was abolished (\(P < 0.01\)) and that of 10 μg/ml bisacodyl was reduced (\(P < 0.05\)). Pretreatment with indomethacin also abolished the effect of 10 μg/ml (\(P < 0.05\)) and 100 μg/ml phenolphthalein (\(P < 0.01\)) (Fig. 1a). The effects of PGE\(_1\) and of the osmotic laxatives, sodium sulfate, mannitol and lactulose were not changed by indomethacin (Fig. 1b).

B. Perfused Colon

a) Water Net Flux. During control perfusion of the colon with Tyrode’s solution, water net flux was directed from lumen to blood (Fig. 2). Perfusion with solutions of bisacodyl (10 μg/ml and 50 μg/ml) and phenolphthalein (100 μg/ml and 500 μg/ml) dose-dependently reduced water net absorption or reversed it into net secretion (\(P < 0.001\)) (Fig. 2). Reduction of water net absorption or water net secretion was also observed with solutions of the osmotic laxatives sodium sulfate (3.95%), mannitol (4%) and lactulose (5%) (\(P < 0.01\)) (Fig. 2).

b) PGE-Release Into the Lumen. Perfusion with bisacodyl (10 μg/ml and 50 μg/ml) dose-dependently increased PGE-release (\(P < 0.01\)). Perfusion with phenolphthalein (100 μg/ml and 500 μg/ml) also significantly increased PGE-release (\(P < 0.01\) and \(P < 0.02\), respectively) (Fig. 2).

Perfusion of the colon with solutions of the osmotic laxatives sodium sulfate, mannitol and lactulose did not change PGE-release in comparison with the control period.