Is the effect of diphenolic laxatives mediated via release of prostaglandin E?

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Summary. The diphenolic laxatives, bisacodyl and phenolphthalein, and the osmotic laxative mannitol increase intestinal fluid volume in the rat colon in situ. The diphenolic laxatives stimulate the biosynthesis of prostaglandin E (PGE) whereas mannitol does not. Inhibition of PG-biosynthesis by pretreatment with indomethacin only reduces the effect of the diphenolic laxatives. It is suggested that diphenolic laxatives increase intestinal fluid volume via stimulation of PGE biosynthesis in the colon.

Diphenolic laxatives like bisacodyl and phenolphthalein inhibit absorption of water and electrolytes in the colon and in the jejunum. They increase the water net flux and the flux of electrolytes from blood to lumen which results in an increased intestinal fluid volume[1,2]. The basic mechanism of action of bisacodyl, phenolphthalein and other diphenolic laxatives is still unknown. The prostaglandins (PGs) E_1, E_2 and $F_{2\alpha}$ also inhibit absorption of water, electrolytes and glucose, cause an increase of sodium and chloride flux from blood to lumen and, therefore, increase intestinal fluid volume. It is supposed that PGs play a physiological role in the regulation of intestinal blood flow and transmucosal water movement[3-6]. We investigated whether bisacodyl and phenolphthalein exert their action via stimulation of PG-biosynthesis. The diphenolic laxatives were compared with the osmotic laxative mannitol.

Material and methods. The experiments were performed on female Sprague Dawley rats (200 ± 20 g). The rats were deprived of food for 20 h prior to the experiment and had free access to water. 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 (method according to Forth et al.[1]). The Tyrode's solution contained the unabsorbable marker ^{14}C-polyethyleneglycol (150 mg = 0.25 μCi) for determination of water net flux. After 60 min, the colon was removed and the intraluminal content was collected. Water net flux was calculated from the change in concentration of ^{14}C-polyethyleneglycol in the intraluminal fluid.

A second preparation was used to measure intraluminal PG-release in the colon, in order to avoid possible destruction of released PGs in the tied off 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 2 1-h periods and weighed. The 1st period served as control period and in the 2nd period the Tyrode's solution contained the appropriate drug. Water net flux was calculated from perfusion rate and the weight of the perfusate-sample. In the perfusate PGE was determined by radioimmunoassay[7,8]. The substances Indomethacin (Merk, Sharp & Dohme), bisacodyl (Bender), phenolphthalein (Mallinckrodt, ethanolic dilution) and mannitol (Merck) were all dissolved in Tyrode's solution. PGE_1 was kindly supplied by Dr J. Pike, Upjohn Co. Kalamazoo. The experimental data were evaluated by Student's t-test.

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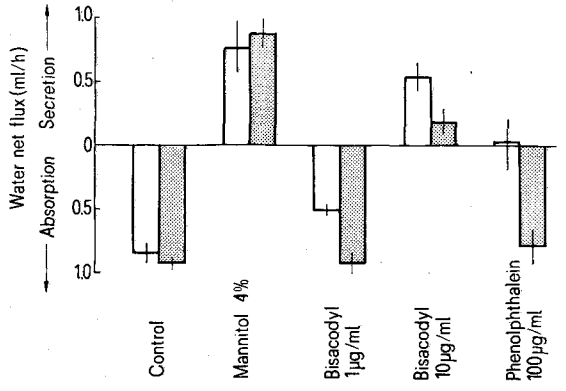

Fig. 1. Tied off colon: The effect of bisacodyl, mannitol and phenolphthalein on water net flux in tied off colon loops of the rat without pretreatment (open columns) and after pretreatment with indomethacin (crosshatched columns) ($x \pm s_x$, n = 6).

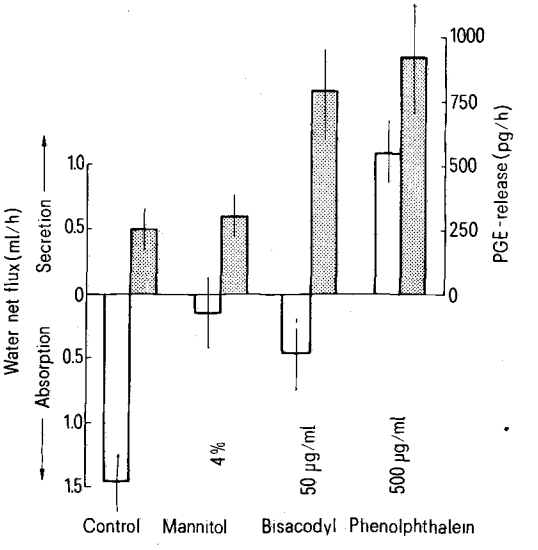

Fig. 2. Perfused colon: The effect of bisacodyl, phenolphthalein and mannitol on water net flux (open columns) and on PGE-release (black columns) in the perfused rat colon ($x \pm s_x$, water net flux: n = 6, PGE-release: n = 4).

Results and discussion. In the tied off colon of control rats, water net flux was directed from lumen to blood. Net absorption of water was 0.84 ± 0.08 ml/h. 1 µg/ml bisacodyl reduced net absorption of water ($p < 0.05$) and 10 µg/ml bisacodyl and 100 µg/ml phenolphthalein reversed net absorption into net secretion ($p < 0.01$). The latter was also found with mannitol solution (4%). PGE_1 (2 µg/ml) applied into the lumen of the tied off colon, totally abolished net absorption of water and caused a net secretion of 0.06 ± 0.12 ml/h (not shown in the figures). Pretreatment with indomethacin (4 mg/kg day, s.c., starting 2 days prior to the experiment) reduced the effect of 1 µg/ml and 10 µg/ml bisacodyl ($p < 0.01$ and $p < 0.05$) and of phenolphthalein ($p < 0.01$); it did not influence net absorption of water in control animals. The effects of mannitol, an osmotic laxative, was not changed by pretreatment with indomethacin. This indicates that diphenolic laxatives might exert their action via PG-release, since indomethacin inhibits PG-biosynthesis[9–11] (figure 1).

In the perfused colon of control rats, water net flux was also directed from lumen to blood. Net absorption was 1.46–0.20 ml/h, that means 1.74 times higher than in the tied off colon. Accordingly, the effects of all laxatives tested were weaker in the perfused colon. To get comparable effects, higher concentrations had to be used in the perfused colon than in the tied off colon. No explanation for this discrepancy can be offered at present. Bisacodyl (50 µg/ml) and mannitol both reduced net water absorption markedly ($p < 0.01$) and phenolphthalein (500 µg/ml) caused net water secretion ($p < 0.01$) (figure 2). The concentration of phenolphthalein was 10fold the concentration of bisacodyl, according to their therapeutic potency[12]. During control periods, the total amount of PGE, released into the perfusate, was 250 ± 97 pg/h. During perfusion with bisacodyl (50 µg/ml), PGE-release increased about 3fold ($p < 0.01$) and with phenolphthalein (500 µg/ml) about 4.5fold ($p < 0.01$). Mannitol (4%) did not increase PGE release (figure 2).

It is suggested that diphenolic laxatives exert their action via stimulation of PGE-biosynthesis in the colon.

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The effects of hydrocortisone and glycyrrhizine on the enzyme releases of arylsulfatase and hyaluronidase from lysosomes of liver

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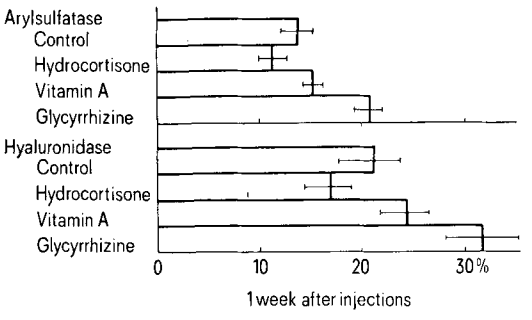
Summary. Hydrocortisone and glycyrrhizine act as both stabilizers and labilizers of the lysosomes of liver. The effect of both agents on the lysosomes is changeable according to the duration of their administration.

Lysosomes, which contain many hydrolytic enzymes, take part in the destruction and the restoration of tissue in inflammation. These lysosomal enzymes are released from the lysosome in the inflammation of the tissues. Some labilizing and stabilizing agents of lysosomes are well known[2–4].

Material and methods. Male albino rats of the Wistar strains, weighing approximately 200 g at the beginning of the experiement were used. Chronic hepatic damage was induced by i.m. injections, twice a week for 8 weeks, of 3ml of carbon tetrachloride per kg b. wt. Experimental animals were divided into 4 groups. Each group consisted of 7 rats. In the vitamin A group, vitamin A was administered by i.m. injection, of 10,000 IU/100 g b.wt, every other day for 2 weeks after the discontinuance of carbon tetrachloride. In the steroid hormone group, hydrocortisone was injected i.m. (dose 0.5 mg/100 g b.wt), every other day for 2 weeks after the discontinuance of carbon tetrachloride. A solution of 0.2% glycyrrhizine was administered by i.m. injection of 0.03 ml/100 b. wt every other day for 2 weeks after the discontinuance of carbon tetrachloride. The control animals received an appropriate volume of physiological saline after the discontinuance of carbon tetrachloride. The animals were killed by decapitation at 1 and 2 weeks after the administration of vitamin A, hydrocortisone and glycyrrhizine. The livers were taken out after being bled, and 8% liver homogenates were prepared. A supernatant of 20,000 × g in 8% homogenate was prepared. Each fraction of homogenate and supernatant was treated with ultrasound. Optimum liberation of particle-bound enzyme without loss of activity was obtained at 60 kW for 120 sec (Kubota 200 M). The enzyme assay of the total arylsulfatase activity was perfomed by the method of Worwood[4]. Hyaluronidase activity with hyaluronic acid as the substrate was measured by the method of Hutterer[5].

Results. The figure shows the pattern of the percentage of the release of the lysosomal enzymes at 1 week after dosing. The result of the administration of hydrocortisone



Percentage release of lysosomal enzymes at 1 week after dosing. Percentage release: Specific activity of supernatant specific activity of homogenate × 100.

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