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The antagonistic effect of morphine on rhein-stimulated fluid, electrolyte and glucose movements in guinea-pig perfused colon

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Rhein (1,8-dihydroxy-3-carboxyanthraquinone), in a concentration of 6×10^{-4} M, inhibits water absorption from the colon and causes a net transfer of fluid and electrolyte into the intestinal lumen. Morphine (4×10^{-4} M) counteracted the water and electrolyte secretion. Prior perfusion with morphine protected the large intestine from the laxative effect of a rhein perfusion. Differences in absorption rate of ^{99m}Tc-EDTA, a poorly absorbable marker, were found, as morphine caused nearly all radioactive compound to be retained in the colon, while rhein significantly facilitated the transfer of marker from colon through mucosal barrier to blood. The route followed by the ^{99m}Tc-EDTA complex was not the same as that followed by water, suggesting that ^{99m}Tc-EDTA travels by a paracellular route. Morphine counteracted the inhibition of Na^+ absorption caused by rhein and antagonized the massive loss of K^+ incurred by the presence of rhein in the colon. Cl^- absorption is reversed to secretion in the presence of rhein while normal values were restored by morphine. Neither the HCO_3^- content nor the pH were affected by either drug. Active absorption of glucose was completely blocked in the presence of rhein; the block could be antagonized by morphine.

Laxatives of the diphenolic type inhibit the absorption of sodium and water from the intestine and cause a net transfer of fluid and electrolytes into the intestinal lumen. This was shown for bisacodyl (Ewe & Holker 1974), for oxyphenisatin (Forth et al 1966) and for natural and synthetic anthraquinone laxatives (Lemmens 1974; Lemmens & Borja 1976).

Rhein was found to influence the water, electrolyte and carbohydrate transport in the human jejunum and colon (Ewe 1980). In contrast, Beubler & Lembeck (1979) reported on the inhibitory effect of opiate agonists on fluid secretion, stimulated by several substances. An in-vitro technique enabled measurement of a significant increase in chloride absorption in the distal ileum in the presence of 2×10^{-5} M morphine. Net Na^+ absorption was increased by morphine in fed, but not fasted, dogs (Mailman 1980). We investigated the effect of morphine on the accumulation of intestinal fluid induced by rhein, one of the anthraquinone laxatives, and one of the main active metabolites of sennoside (Lemmens 1979). The inhibitory effect of morphine on the activity of a variety of secretogogues, including cholera toxin, prostaglandin E_1, VIP, carbachol and bisacodyl has already been studied (Valiulius & Long 1973; Coupar 1978). As Verhaeren et al (1981a) found the antidiarrhoeal agent loperamide to reduce the permeability of the colonic mucosa, by using ^{99m}Tc-EDTA as a marker molecule the effect of morphine on the same mucosal transfer rate has been similarly evaluated.

As we used an in-vivo closed loop perfusion technique, the time required for stimulation and/or inhibition of secretion by rhein and morphine could be measured. A possible central effect of morphine on secretion could be minimized.

METHODS

Perfusion technique

The surgical technique of Schanker et al (1957) was used with minor modifications. Guinea-pigs of either sex, 350–450 g, had free access to pelleted food and water until they were fasted 12 h before the experiment. Anaesthesia was induced by peritoneal injection of ethylurethane ($0{\cdot}9$ g kg^{-1}) and body temperature was maintained at 37 °C by a thermostatically controlled heated table. A midventral abdominal incision was made and the large intestine exposed. The distal rectum was located and a small incision was made followed by a second incision at a distance of 7 branches of the superior mesenteric vein, this was to obtain a physiological length of tissue.

Two cannulae were introduced after removal of

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the faeces. The preparation was then covered with gauze sponges moistened with buffer at 37 °C which were changed throughout the experiment. The segment was gently flushed with 15 mL Krebs solution until the effluent was clear, drained by passing air and then replaced in the abdomen. Most of the cannulae and the whole closed loop system was made of glass to minimize adsorption. Solutions at 37 °C were perfused at 2·4 mL min^{-1}, to avoid pressure damage (Lewis & Fordtran 1975).

A catheter filled with heparin (10–25 iu mL^{-1}) was inserted into the jugular vein to permit blood sampling (Waynforth 1980) and the free end was stopped with a Dieffenbach pincette; the exact amount of heparin, required to fill the tube was noted. Blood samples (50 μL) were taken every 5 min.

The composition of the perfusate was (mM): NaCl 130, KCl 4, glucose 2, $NaHCO_3$ 25. The pH was adjusted to 7·0 with CO_2. For the rhein perfusion a saturated solution was used (6×10^{-4} M) (Lemmens & Borja 1976).

Morphine hydrochloride was dissolved in the buffer at 4×10^{-4} M. The osmolarity of solutions, as determined with the Osmette precision osmometer, was between 211 and 284 mOsm kg^{-1}.

K^+ and Na^+ determination in the perfusate was measured by flame photometry with an internal lithium standard (Beckman Klina Flame).

HCO_3^- concentration was calculated from the gasometric determination of P_{CO_2} and pH by means of the Henderson-Hasselbach equation using a AVL 940 blood gas analyser.

Cl^- determinations were by coulometric titration on a Corning chloride meter 920.

A low glucose concentration was chosen because its luminal disappearance rate reflects active transport and mucosal metabolism and thus tissue viability. Glucose was analysed by the CHOD-PAP method (Boehringer Mannheim).

Calculations

Absorption rates of fluid and solute were calculated using ^{99m}Tc-EDTA as a poorly absorbed marker. Each point was the mean of at least 6 experiments. Every sample in each experiment was taken 3 times yielding also a mean value. The significance of the differences between mean values was assessed by the paired Student's *t*-test. All results are expressed as the mean ± 1 s.d.

^{99m}Tc-EDTA was prepared in-situ according to Verhaeren et al (1981a).

Operation procedure

Before an experiment, 35 mL perfusate was mixed with 1 mL ^{99m}Tc-EDTA, and the starting volume was taken as 36·0 mL and the activity as 0·15–0·13 mCi mL^{-1}.

Every 5 min three 20 μL aliquots of perfusate were taken and their radioactive content measured in a scintillation well counter (Berthold Gamma Sample Changer bf 5300) at 140 ± 10 keV. One sample of 200 μL was immediately frozen (−20 °C) and stocked for the determination of Na^+, K^+, glucose, Cl^- and pH. The mean and the s.d. of the radioactive counts were calculated and a time correction made, based on the $t_{\frac{1}{2}}$ of the ^{99m}Tc being 6·02 h. Also every 5 min 50 μL blood was sampled. As the absorption of ^{99m}Tc-EDTA was low in comparison with the circulating ^{99m}Tc-EDTA pool, this absorption could be omitted in the calculation of water movements.

As ^{99m}Tc-EDTA has essentially a very low penetration rate into the intestinal wall (Verhaeren et al 1981b), the mucosal tissue can be considered as nearly impermeable to it.

To investigate the effect of one compound on the effect of perfusion of a second, each drug was perfused for 1 h after which the colon was drained by syringing air through it over 5 min. Saline pH 7·2 was then perfused for 15 min to remove any trace of previously administered drug and after a further air draining procedure, the second compound was administered for 1 h. In this way each animal served as its own control.

Rhein was prepared from aloin (Bellaart 1952). All reagents used were of analytical grade.

RESULTS

As the solution containing NaCl, KCl, $NaHCO_3$, and glucose was perfused, the colon began to absorb water, after a stabilization period of 5–10 min.

The presence of 6×10^{-4} M rhein in the perfusing solution not only inhibited absorption but caused absorption to change to secretion (Fig. 1A). The net effect of rhein on water movements by perfusion was 5·4 ± 0·6 mL h^{-1}.

To find if the secretory effect of rhein lasted only during the perfusion, guinea-pigs were perfused with rhein after 1 h. Then the colon was thoroughly rinsed and the animal perfused with a saline solution for 1 h. It was clear (Fig. 1B) that some laxative effect due to rhein remained.

The morphine concentration used was comparable with that used in-vitro by McKay et al (1981). About 30 mg morphine was perfused through the gut for 1 h. No blood concentrations of morphine were

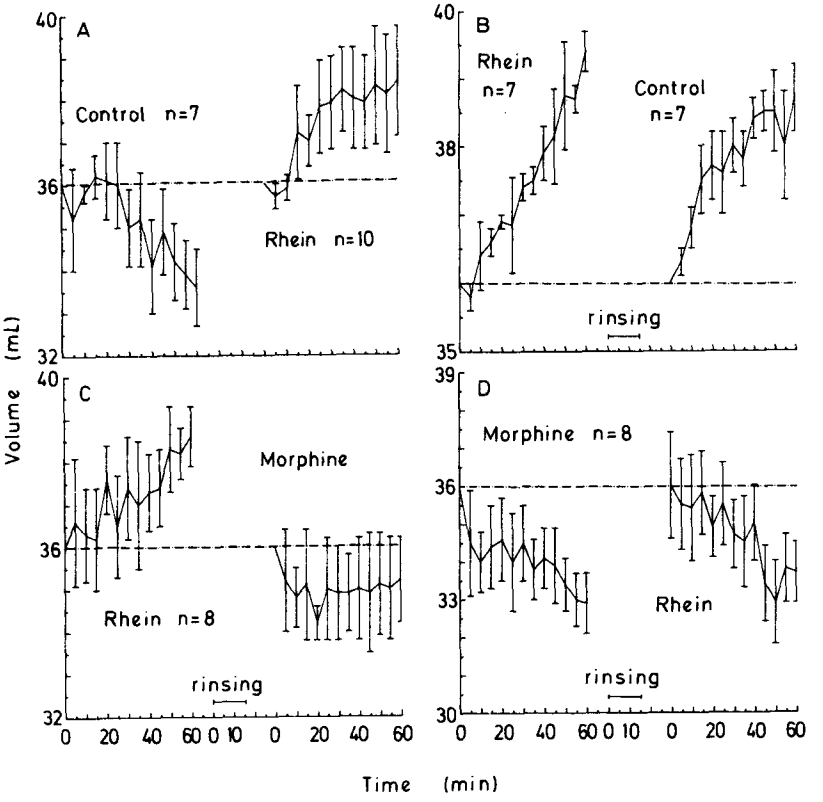

FIG. 1. A: Water absorption in control animals during perfusion. The presence of rhein reverses absorption into secretion. B: Secretion due to the presence of rhein is still present after a 15 min rinsing. C: Morphine (4×10^{-4} M) antagonized the rhein-stimulated fluid secretion. D: Prior perfusion in the presence of morphine inhibits the secretory effect of rhein.

measured. If morphine was perfused in a concentration of 4×10^{-4} M after rhein, the opiate drug counteracted the secretory effect of rhein (Fig 1C).

The differences are statistically significant for $P < 0{\cdot}05$ using the paired t-test. Prior perfusion with morphine even protects the large intestine from the secretory effect of rhein (Fig. 1D).

^{99m}Tc-EDTA did reach the bloodstream but there were differences in absorption rate as morphine retained nearly all the radioactive compound in the colon, while rhein facilitated significantly its transport from the colon through the mucosal barrier into the blood (Fig. 2). The results are expressed as the radioactivity of 50 µL blood sample versus the radioactivity of 50 µL perfusate in %. The absorption rate of ^{99m}Tc-EDTA was followed in double perfusion experiments, as previously described. It is obvious that morphine perfused after rhein did not significantly change the blood concentration of ^{99m}Tc-EDTA (Fig. 3A). On the other hand, rhein

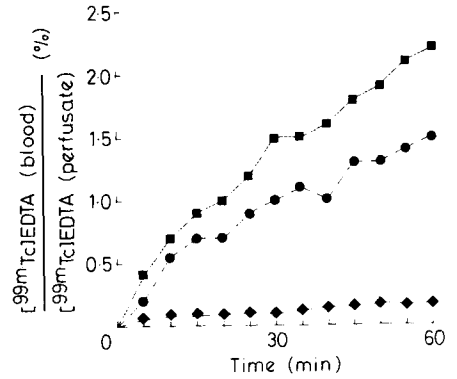

FIG. 2. Blood levels of ^{99m}Tc-EDTA in the presence of (◆) morphine, (●) control, (■) rhein.

was able to re-establish the absorption rate of the ^{99m}Tc-EDTA (Fig. 3B).

Morphine counteracted the inhibition of sodium absorption caused by rhein (Fig. 4A). With the

exception of the starting point, all differences are statistically significant (paired t-test for $P < 0{\cdot}05$).

Even more relevant was the significant rise in K^+ concentration under the influence of rhein. This effect was completely antagonized by morphine (Fig. 4B).

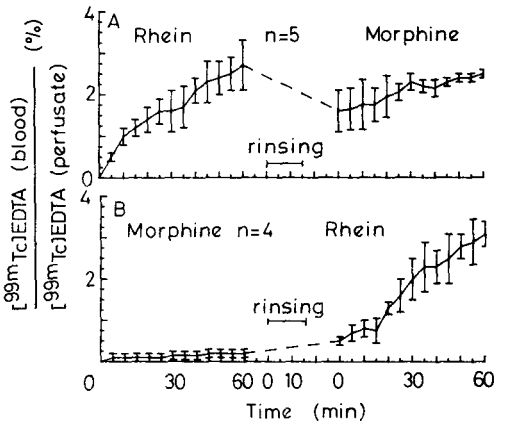

FIG. 3. A: The absorption rate of ^{99m}Tc-EDTA in double perfusion experiments. One perfusion in the presence of rhein is followed by a morphine perfusion. B: After a morphine perfusion, rhein is able to refacilitate the absorption rate of ^{99m}Tc-EDTA.

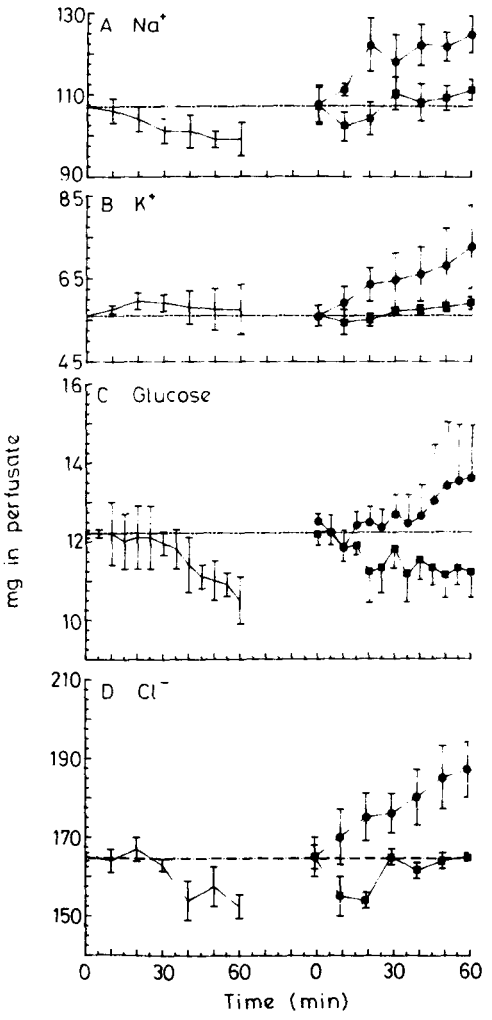

FIG. 4. A: The colon is actively absorbing Na^+ ions. The presence of rhein is responsible for a Na^+ secretion in the perfusing fluid (●). Administration of morphine counteracted the sodium secretion (■). B: A small K^+ secretion in control animals turned into a considerable K-loss in the presence of rhein (●). Morphine antagonized this effect (■). C: Controls actively absorbing glucose out of the perfusion fluid. This active absorption is inhibited by rhein (●) and antagonized by morphine (■). D: Absorption of Cl^- reversed to secretion in the presence of rhein (●). Morphine is able to inhibit Cl^- secretion (■).

As glucose is readily absorbed under normal conditions, it is a measure of normal functioning of absorption and transport mechanisms. Addition of rhein in the perfusate inhibited the active absorption of glucose, which increased the circulating perfusate. Morphine has an opposite effect on glucose absorption (Fig. 4C).

Cl^- absorption was readily inhibited by rhein and was even changed to mild Cl^- secretion (Fig. 4D).

The absolute quantity of HCO_3^- present in the circulating perfusate showed no statistically significant variation. It was unaffected by either drug. Also the pH was continuously monitored. The pH of the perfusate, which was not buffered, increased rapidly to 8·69 ± 0·20 and was also unaffected by either drug.

DISCUSSION

Although most fluid absorption normally occurs in the proximal colon (Edmonds 1967; Hamilton & Roew 1977), the distal part also actively absorbs water and is easier to standardize so that a representative response is always obtained.

Rhein reversed absorption into secretion, as already reported in man by Ewe (1980). It is a fast process as the reversal of absorption into secretion began after 10–15 min. This time is probably required for the rhein molecule to penetrate the mucosal cells and to change biochemical cellular

equilibria, since anthraquinones also need a similar time to penetrate into an isolated mucosal layer. This results in an uncoupled mitochondrial oxidative phosphorylation as measured by an increased respiration rate (Verhaeren 1980). In agreement with Ewe (1980), the residual secretory effect of the rhein perfusion, had not been washed away after a 15 min rinsing. This slowly decreasing laxative effect was assumed to be due to the gradual fall of the laxative in the perfusate after changing to standard solution. However, this is not a satisfactory explanation for the finding that under identical experimental conditions the secretagogue effect of rhein was completely reversible in the jejunum within approximately 1 h, while it lasted longer in the colon. Although these experiments were performed with bisacodyl, the same retardation was found with anthraquinone. Morphine counteracted the remaining laxative action without any adaptation period. This result supports the earlier findings that PG-induced or -mediated secretion is blocked by opiates (Beubler & Kollar 1985). Moreover, prior perfusion with morphine protected the colon effectively from the laxative effect of a rhein perfusion, thus the effect of morphine exists even after a 15 min rinsing.

Rhein significantly facilitated the transfer of the labelled ^{99m}Tc-EDTA complex from colon through the mucosal barrier to blood, which is in good agreement with earlier results with 1,8-dihydroxyanthraquinone (Verhaeren et al 1981a).

On the other hand, morphine acted on the mucosa, to give a complete block of all ^{99m}Tc-EDTA transport. As 1,8-dihydroxyanthraquinone acts on mucosal permeability via tight junction permeability, an action of morphine on tight junction permeability as effected by TAP or loperamide cannot be excluded (Verhaeren et al 1981a, b).

To minimize biological variation, double perfusion experiments were carried out in which each animal functioned as its own control. Perfusion of morphine after rhein did not significantly change the concentration of ^{99m}Tc-EDTA in blood. This may be explained by a low elimination rate of the drug, by a retardation of the drug passage through the mucosal layer, or by its accumulation in cellular tissues, from which release would be the rate limiting step.

On the other hand, rhein was able to re-establish the mucosal transfer rate of ^{99m}Tc-EDTA, suggesting that the route followed by the ^{99m}Tc-EDTA complex was not identical with the route followed by water, since rhein exerted no laxative action even though ^{99m}Tc-EDTA blood content increases. Rhein perfusion, following morphine fails to effect secretion, although the ^{99m}Tc-EDTA concentration in blood increases.

Also, morphine could be considered as an antagonist of ion movements induced by rhein. Anthraquinones dose-dependently reversed sodium and chloride absorption into secretion (Phillips et al 1965; Lemmens & Borja 1976; Leng-Peschlow 1980).

Morphine counteracted the inhibition of Na absorption caused by rhein but no statistically significant reversal of the mechanism could be seen. Anthraquinones enhance colonic potassium secretion, as has been shown with other laxatives (Forth et al 1966; Schreiner et al 1980). The loss of K^+ from the colon in the presence of rhein was antagonized by morphine.

The antagonistic effect of rhein and morphine on anion movements was also studied. Cl^- absorption was reversed to secretion by rhein, and reversed again to a nearly stationary state by morphine, probably because Cl^- acts as a counterion for Na^+ and to a minor degree for K^+.

Neither the HCO_3^- content nor the pH were affected during the perfusion by the two drugs.

As glucose is readily absorbed, it is a reflection of a normally functioning absorbing and transporting mechanism. Even an increase in the circulating perfusate was found. Glucose-free perfusate was tested for glucose but no trace was found, so the increase of glucose in the perfusate remains unexplained.

REFERENCES

Bellaart, A. G. (1952) Ph. D. Thesis, Groningen

Beubler, E., Kollar, G. (1985) J. Pharm. Pharmacol. 37: 248–251

Beubler, E., Lembeck, G. (1979) Naunyn-Schmiedeberg's Arch. Pharmacol. 306: 113–118

Coupar, I. M. (1978) Br. J. Pharmacol. 63: 57–63

Edmonds, C. J. (1967) J. Physiol. 193: 571–588

Ewe, K. (1980) Pharmacology 20 (suppl. 1): 27–35

Ewe, K., Holker, B. (1974) Klin. Wschr. 52: 827–833

Forth, W., Rummel, W., Baldauf, J. (1966) Naunyn-Schmiedeberg's Arch. Pharmacol. Exp. Pathol. 254: 18–32

Hamilton, D. L., Roew, E. (1977) Can. J. Comp. Med. 41: 241–250

Lemmens, L. (1974) IRCS 2: 1094–1097

Lemmens, L. (1979) Pharm. Weekbl. Sci. Ed. 1: 178–185

Lemmens, L., Borja, E. (1976) J. Pharm. Pharmacol. 28: 498–501

Leng-Peschlow, E. (1980) Ibid. 28: 498–501

Lewis, L. D., Fordtran, J. S. (1975) Gastroenterology 68: 1509–1521

Mailman, D. (1980) Br. J. Pharmacol. 68: 617–624

McKay, J. S., Linaker, B. D., Turnberg, L. A. (1981) Gastroenterolgy 80: 279–284

Phillips, R. A., Love, A. H. G., Mitchell, T. G., Neptune, E. M. (1965) Nature 80: 279–284

Schanker, L. S., Shore, P. A., Brodie, B. B., Hogben, C. A. (1957) J. Pharmacol. Exp. Ther. 120: 540–549

Schreiner, J., Nell, G., Loeschke, K. (1980) Naunyn-Schmiedeberg's Arch. Pharmacol. 313: 249–255

Valiulius, E., Long, J. F. (1973) Physiologist 16: 475–481

Verhaeren, E. H. C. (1980) Ph.D. Thesis, Leuven

Verhaeren, E. H. C., Dreessen, M. J., Lemli, J.A. (1981a) J. Pharm. Pharmacol. 33: 526–528

Verhaeren, E. H. C., Verbruggen, A. M., Lemli, J. A. (1981b) Pharm. Weekbl. Sci. Ed. 3: 111–115

Waynforth, H. B. (1980) in: 'Experimental and Surgical Technique in the rat', Academic Press, p. 50–52