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Influence of Rhein on Rat Colonic Na^+, K^+-ATPase and Permeability in vitro

R. Wanitschke[a], *U. Karbach*[b]

[a] First Medical Clinic, University of Mainz, and [b] Medical Clinic, University of Munich, FRG

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Abstract. Rhein, an aglucone of a laxative-acting anthraquinone, which induces net secretion in the human jejunum and colon, does not induce net secretion under open circuit current in in vitro conditions but reduces net absorption of electrolytes and water. In the stripped rat descending colon at a concentration of $2 \cdot 10^{-4}$ mol/l, the Na^+, K^+-ATPase is almost completely inhibited resulting in a decreased net absorption of electrolytes and water. There are no changes of the diffusional resistance of the paracellular pathway. Results point to a cellular mechanism of rhein by which absorption is inhibited so that physiological cellular secretion dominates. This does not rule out an additional activation of cellular secretion but net secretion can be explained solely by inhibition of absorption without the assumption of any additional secretory mechanism.

Anthraquinone laxatives are widely used and have been popular for prompt purgative action for centuries. Nevertheless, the mode of action is still not well understood. Rhein, an aglucone of sennosides, induces net secretion in the jejunum as well as in the colon, which is in vivo functionally completely reversible [Ewe, 1980]. In order to differentiate whether active absorption is decreased, active secretion is increased or passive flux changes due to changes in water permeability by a given asymmetric membrane result in net secretion, it seems reasonable to investigate each single mechanism. In this investigation, the influence of rhein on Na^+, K^+-ATPase activity and on hydraulic permeability of the isolated colonic mucosal membrane of rats was measured.

Methods

Experimental Design. Stripped descending colon specimens from Wistar rats (body weight 150–200 g) were prepared as an everted sac preparation analogous to the method by Parsons and Paterson [1965].

The sacs were tied at one end to a glass tube and immersed in 50 ml electrolyte solution at 37 °C. The sacs were filled through this tube. Hydrostatic pressure gradients were established by filling the sacs and the tubes to 2 and 7 cm water above the fluid level of the beaker in which the sacs were immersed.

Composition of Solution. The mucosal and serosal solution at the beginning of the experiments had the following composition (in mmol/l): NaCl 109; KCl 4.5, NaH_2PO_4 0.2; Na_2HPO_4 1.8; $CaCl_2$ 2.5; $MgSO_4$ 2.5; $NaHCO_3$ 25; glucose 12.2. In the experiments to measure ATPase activity, the composition of the solution differed as follows (in mmol/l): Na^+ 113; K^+ 4.5; Cl^- 92.5; HCO_3^- 25; glucose 12.2. The solution of the serosal side contained in addition 5 g/l polyethylene glycol (MW 4,000) and 5 µCi/l ^{14}C-PEG 4000. In the experiments to determine paracellular permeability, 10 µCi ^{14}C-erythrol and 5 g/l mesoerythrol were used. The pH of all solutions was 7.4 by 95% O_2–5% CO_2 saturation at 37 °C.

Net water and electrolyte transfer were calculated from changes in ^{14}C-PEG and electrolyte concentrations at the beginning and the end of the experiments. Values are expressed as $\bar{x} \pm$ SE per gram fat-free dry weight and 2 h incubation.

Paracellular Permeability. This was determined by the transfer of erythrol from the serosal solution to the mucosal solution. For comparison reasons, the transfer into the mucosal solution under control conditions at 2 cm water pressure was defined 100%.

Material and Analysis. Protein was measured by the method of Coomassie brilliant blue [Sedmak and Grossberg, 1977]. ATPase activity was measured as described by Schiffl and Loeschke [1977]. ^{14}C activity was measured by a liquid scintillation counter. In the rhein experiments it was corrected by a quench curve obtained from different rhein concentrations. Rhein was obtained from Roth, Karlsruhe, FRG, or kindly supplied by Lehner AG, Muttenz, Switzerland; PEG 4000 was obtained from Schuchardt, Munich, FRG; mesoerythrol from Merck AG, Darmstadt, FRG, and ^{14}C-PEG and ^{14}C-erythrol from New England Nuclear Chemicals, Dreieich, FRG.

Statistical comparison was made by the unpaired t test.

Results

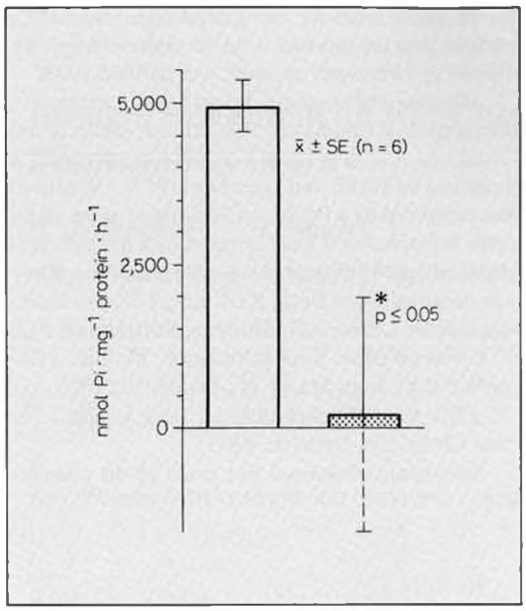
Water and Electrolyte Transfer

Under control conditions with 2 cm water pressure on the serosal side, the colonic mucosa absorbs water and electrolytes.

Table I. Net transfer of water and sodium and established transmucosal concentration gradient for sodium at different serosal pressures (SP) in controls and under the influence of rhein ($2 \cdot 10^{-4}$ mol/l) on the mucosal side ($\bar{x} \pm$ SE; n = 8–23)

	SP 2 cm water		SP 7 cm water	
	water	sodium	water	sodium
Control, ml or mmol g^{-1} d.w. 2 h^{-1}	8.4±0.6	2.3±0.1	−1.0±0.6	1.1±0.1
Rhein ($2 \cdot 10^{-4}$ mol/l), ml or mmol/l g^{-1} d.w. 2 h^{-1}	0.8±1.3	0.2±0.3	−4.0±0.4	−0.5±0.1
Initial sodium concentration, mmol/l		136		136
Serosal sodium concentration after 2 h incubation, mmol/l				
control		154±1		144±2
rhein ($2 \cdot 10^{-4}$ mol/l)		139±1		137±1

Values are expressed per gram fat-free dry weight (d.w.) of the mucosal tissue after 2 h incubation. − = net secretion; + = net absorption.



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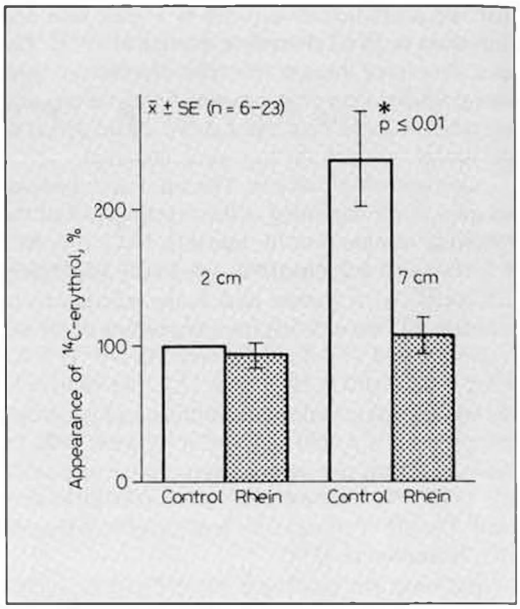

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Fig. 1. Na^+, K^+-ATPase activity in the isolated stripped rat colonic mucosa after 2 h incubation under controls (open column) and under the influence of $2 \cdot 10^{-4}$ mol/l rhein at the mucosal side (hatched column).

Fig. 2. Appearance rate of ^{14}C-erythrol activity from the serosal at the mucosal side in relation to serosal pressure of 2 cm water (left) or 7 cm water (right) and under the influence of $2 \cdot 10^{-4}$ mol/l rhein in the mucosal solution (hatched columns) vs. controls (open columns). ^{14}C activity was measured as cpm in the mucosal solution after 2 h incubation and expressed as percent of controls at 2 cm water pressure which was defined as 100%. For further details see text.

Compared with the initial solution on both sides of the membrane there is a hypertonic absorption as shown for sodium in table I. At 7 cm serosal water pressure the net water flux changes to slight secretion whereas sodium is still absorbed but absorption is reduced. The membrane patterns of hypertonic absorption do not change as shown in detail elsewhere [Wanitschke et al., 1977].

When $2 \cdot 10^{-4}$ mol/l rhein is applied on the mucosal side, net absorption of water and electrolytes is abolished. At a hydrostatic pressure of 7 cm, net secretion of water and sodium occurs in an almost isotonic fashion compared to the initial solution.

Na^+, K^+-ATPase Activity

The results are shown in figure 1. Rhein, applied on the mucosal side of the colonic tissue at a concentration of $2 \cdot 10^{-4}$ mol/l, reduces the Na^+, K^+-ATPase activity from controls (4,680 ± 420 nmol Pi/mg protein·h) to almost zero (110 ± 1,830 nmol Pi/mg protein·h).

Paracellular Permeability

In controls at 2 cm water pressure, 12 ± 4% of the total ^{14}C-erythrol activity applied to the serosal side of the colonic mucosa at the beginning of the experiment moved during 2 h to the mucosal side. This amount was

defined as 100%. The increase of hydrostatic water pressure on the serosal side to 7 cm doubled the appearance rate of ^{14}C-erythrol activity at the mucosal side (233 ± 36%, $p < 0.01$ vs. 2 cm water pressure). Under the influence of $2 \cdot 10^{-4}$ mol/l rhein there were no changes of paracellular permeability compared to controls at 2 cm serosal water pressure, or even under the higher pressure of 7 cm water (fig. 2).

Discussion

Rhein is an active metabolic product derived from colonic microbial fermentation of the natural occurring sennoside laxatives [Breimer and Baars, 1976; Lemli and Lemmens, 1980]. When applied locally into the lumen of the small and large intestine it reduces net absorption and induces net secretion of electrolytes and water depending on its concentration at the mucosal side [Lemmens and Borja, 1976; Ewe, 1980; Leng-Peschlow, 1980]. Despite clear evidence that anthraquinones as well as their agluconic active compounds induce motor changes in the colon resulting in a faster passage of feces [Okada, 1940; Hardcastle and Wilkins, 1970; Garcia-Villar et al., 1980; Leng-Peschlow, 1986], this clinically important finding cannot explain net secretion.

In accordance with results from Chignell [1968] that 1,8-dihydroxyanthraquinone inhibits the Na^+, K^+-ATPase activity in rat enterocytes, rhein reduces, in this study, the Na^+, K^+-ATPase activity to almost zero in the isolated stripped rat colonic mucosa. As shown elsewhere [Wanitschke, 1980], $2 \cdot 10^{-4}$ mol/l rhein exposed to the mucosal side has a maximal effect which corresponds to a 75% inhibition of net absorption of sodium, chloride, and water. The Na^+, K^+-ATPase which contributes to transfer processes of electrolytes and water is particularly active on the basolateral cell membrane of enterocytes [Skou, 1965]. To differentiate whether passive changes of the membrane may contribute to changes in net ion and water flux, measurements of the paracellular permeability are needed. Erythrol with a molecular radius of 3.2 Å is a suitable marker to assess changes in paracellular permeability [Nell et al., 1977].

In contrast to other laxative agents such as deoxycholic acid or diphenols where paracellular permeability determined by the same method markedly increases [Karbach and Wanitschke, 1984], rhein does not change intestinal permeability and behaves like a secretagogue acting primarily on cellular transport mechanisms. If one looks at hydraulic permeability changes of the stripped rat colonic mucosa, secretagogues can also be differentiated [Karbach and Wanitschke, 1984] in those that primarily decrease the diffusional resistance of the paracellular pathway (like bile acids, diphenols, ethacrynic acid) and those that do not change permeability patterns. In accordance with the results of the transmucosal erythrol movement, rhein does not increase membrane permeability of stripped rat colonic mucosa but makes it even tighter.

In conclusion, rhein acts primarily on a cellular level. It reduces net absorption of sodium, chloride, and water by inhibiting the Na^+, K^+-ATPase activity and does not increase but has a tendency to decrease diffusional resistance of the paracellular pathway. The unidirectional flux from mucosa to serosa is reduced so that the unidirectional

flux from serosa to mucosa representing physiological cellular secretion dominates. As a result, net secretion occurs. This does not rule out the possibility that rhein may, in addition, activate cellular secretion mediated by stimulation of endogenous prostaglandin E_2 formation as shown by Beubler et al. [1985] or by a Ca^{2+}-dependent process as suggested by Donowitz et al. [1984].

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Prof. Dr. Roland Wanitschke
I. Medizinische Klinik und Poliklinik
Johannes-Gutenberg-Universität Mainz
Langenbeckstrasse 1
D-6500 Mainz (FRG)

Discussion

Sund: One reason for the varying efficacy among secretagogue drugs regarding their effects on permeability could be their metabolism within the intestinal wall. Rhein seems to be a drug which is quite extensively and very rapidly metabolized in the gut mucosa, whereas bisacodyl is much less. Thus, the efficacy of a laxative may not only be a question of its inherent ('intrinsic') activity but may be determined to a large extent by local pharmacokinetic factors as well.

Wanitschke: I do not think that this is the explanation for the lack of effect of rhein in our permeability experiments, as rhein was offered in a high quantity compared to the size of the gut preparation. It seems very unlikely that rhein may extensively be changed to an inactive metabolite within our experimental period.

Beubler: Rhein reduced net absorption in your experiments, but did not induce net secretion. Is this a matter of dose?

Wanitschke: Under open-circuit conditions in vitro, net secretion never occurs in the rat colon, only under short-circuit conditions or during increased hydrostatic pressure on the serosal side of the membrane.

Beubler: Rhein inhibited completely the Na^+, K^+-ATPase, but net absorption still occurred to some 20%. How do you explain that?

Wanitschke: There must be an additional sodium uptake not controlled by the Na^+, K^+-ATPase.

De Witte: Do you have experience with rhein anthrone instead of rhein in your in vitro experiments? It might be much more active.

Wanitschke: On one hand, rhein anthrone was not available to us and it is a very labile compound. On the other hand, we know rhein very well from in vivo perfusion studies as a safe and active compound. In vitro, you need rather high concentrations, but nevertheless the integrity of the epithelium is completely preserved.