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Prostaglandin E_2-mediated Stimulation of Mucus Synthesis and Secretion by Rhein Anthrone, the Active Metabolite of Sennosides A and B, in the Mouse Colon

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Abstract—Rhein anthrone, the active metabolite of sennosides A and B, stimulated PGE_2 release into the mouse colonic lumen. At 6·24 mg kg^{-1}, it decreased net water and Na^+ absorption significantly in the case of water, but could not reverse the net absorption in mouse ligated colon, although it enhanced net K^+ secretion. Pretreatment with indomethacin diminished the effects of rhein anthrone except on K^+ net secretion. Rhein anthrone or PGE_2 markedly stimulated mucus secretion and synthesis in mouse ligated colon. The enhanced mucus secretion and synthesis induced by rhein anthrone were significantly suppressed by pretreatment with indomethacin. Our results have shown that the colonic secretion of water and electrolytes mediated by PGE_2 is partly involved in the rhein anthrone-induced diarrhoea but that in mice, the mucoid diarrhoea induced by rhein anthrone results mainly from PGE_2-mediated mucus synthesis and secretion in the colon.

Previously, we reported that rhein anthrone, the ultimate intraluminal active metabolite of sennosides A and B, stimulated the biosynthesis of prostaglandin(PG)E-like material in mouse colon but not in the caecum when administered directly. Both the purgative and large intestinal propulsive effects of rhein anthrone injected intracaecally were found to be reduced by the PG biosynthesis inhibitor indomethacin and the PGE_2 antagonist SC-19220, which suggests that these effects in mice are mediated by the formation and release of the PGE-like material (Yagi et al 1988).

Diarrhoea is characterized by the secretion of water, electrolytes and mucus by the intestine. In diarrhoea induced by rhein anthrone, mice excrete mucoid faeces with a dose of 6·24 mg kg^{-1}, corresponding to 10 mg kg^{-1} of sennosides A and B. The present study was undertaken to elucidate the mechanism of the onset of mucoid diarrhoea induced by rhein anthrone.

Materials and Methods

Materials and chemicals

Rhein anthrone was prepared as described previously (Yagi et al 1988).

The following chemicals were purchased: PGE_2 (Funakoshi Pharmaceutical Co. Ltd, Tokyo, Japan), indomethacin (Wako Pure Chemical Industries Ltd, Osaka, Japan), orcinol (E. Merck, Darmstadt, FRG), D-[U-^{14}C]galactose (Amersham International plc, Buckinghamshire, UK) and urethane (Katayama Chemical, Osaka, Japan).

Rhein anthrone was dissolved in 2% sodium bicarbonate solution and diluted with Tyrode solution immediately before use. PGE_2 was dissolved in a small amount of ethanol and then diluted with Tyrode solution. Indomethacin was suspended in a small amount of Tween 80 and diluted with water.

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Animals

Female albino mice of the Jcl:ICR strain (CLEA, Japan, Inc., Tokyo), 26–32 g, were kept at an ambient temperature of 22–25 °C and given pelleted MF diet (Oriental Yeast Co. Ltd, Tokyo, Japan) and tap water. The animals were deprived of food overnight before the ligated colon experiments.

Determination of PGE_2 released into the colonic lumen

Mice were anaesthetized with urethane (2·25 g kg^{-1} i.p.) and the entire colon was rinsed with 5 mL warm saline (0·15 M NaCl) in-situ. After 30 min, 0·5 mL of Tyrode solution alone or containing 6·24 mg kg^{-1} of rhein anthrone, was injected and the colon was ligated at the end (Forth et al 1966). After 60 min, a small sample of colonic fluid (about 0·2 mL) was withdrawn as described by Beubler & Kollar (1985) and adjusted to pH 3·0 with 1 M HCl. PGE_2 was extracted twice with 5 mL of ethyl acetate and the combined ethyl acetate solution was evaporated under vacuum. The residue was dissolved in 15% ethanol, adjusted to pH 3·0 with 0·1 M HCl and purified using a Bond Elute C_{18} cartridge according to the modified method of Kiyomiya & Oh-ishi (1985). This material was dissolved in buffer solution (10 mM sodium phosphate buffer at pH 7·0, 0·1 M NaCl, 1 mM $MgCl_2$, 0·1% NaN_3 and 0·1% ovalbumin) and determined against PGE_2 by enzyme immunoassay according to the modified method of Shono et al (1988).

Net water flux and electrolyte transport

Sixty min after 0·5 mL of Tyrode solution containing rhein anthrone was injected into the mouse ligated colon, the mice were killed by exposure to chloroform. The colon was removed and weighed. The colonic fluid was withdrawn carefully with a hypodermic needle and the empty colon was

weighed again. The colonic fluid was centrifuged at 14000 *g* for 20 min at 5°C. The supernatant was decanted into a test tube and the precipitate weighed. Net water transport was estimated by subtracting the weight of the empty colon, the weight of the precipitate and 0·5 g (0·5 mL of the initial instillate) from the weight of the filled colon, and expressed as mL g^{-1} tissue (Beubler & Juan 1979). The supernatant was analysed for sodium and potassium by atomic absorption spectrometry. Electrolyte transport was expressed as μmol g^{-1} tissue. A negative value denotes net absorption and a positive value net secretion.

Mucus secretion

Colonic mucus secretion was estimated by measuring the output of total protein-bound hexose (TPBH) in the colonic fluid. A modified method of Winzler (1955) was applied using orcinol as a reagent. After rhein anthrone or PGE_2 was injected into the mouse ligated colon, the colonic fluid was obtained as described above. The mucus was dispersed by ultrasonication (45 kHz, 60 W) and precipitated from the colonic fluid using 10% trichloroacetic acid. The precipitate was washed with 95% ethanol and dissolved in 1 mL of 0·1 M sodium hydroxide solution. Orcinol reagent (8·5 mL) was added and the TPBH content was determined photometrically using galactose as a standard at 540 nm and expressed as mg TPBH g^{-1} tissue.

Mucus synthesis

Colonic mucus synthesis was estimated by the method of Farack et al (1985). Three h after [^{14}C]galactose (2 μCi per animal) in water was injected intravenously into the mouse, rhein anthrone or PGE_2 was injected into the ligated colon. After 60 min, the colonic fluid was withdrawn and the mucus was precipitated as described above. ^{14}C-Radioactivity was measured in the precipitate dissolved in 1 mL of 1 M sodium hydroxide solution and expressed as d min^{-1} TPBH g^{-1} tissue. The ^{14}C-radioactivity of plasma samples taken by cardiac puncture at the end of an experiment was about 20000 d min^{-1} mL^{-1}.

Statistical evaluation

The results were expressed as mean value ± standard error of mean. Statistical significance was assessed using Student's *t*-test.

Results

Effect of rhein anthrone on PGE_2 release into mouse colonic lumen

Table 1 shows that rhein anthrone (6·24 mg kg^{-1}) significantly stimulated PGE_2 release into the colonic lumen. The enhanced release of PGE_2 was markedly reduced by pretreatment with indomethacin (3·0 mg kg^{-1} p.o.). Indomethacin given to control mice decreased the PGE_2 release into the colonic lumen.

Effect of rhein anthrone on water flux and electrolyte transport in mouse colon

Rhein anthrone (6·24 mg kg^{-1}) reduced the net absorption of water and Na^+, with a statistically significant result for water. Net K^+ secretion was slightly, but significantly increased by rhein anthrone. The effects of rhein anthrone on water and Na^+ transport were inhibited by indomethacin (3·0 mg kg^{-1}), while the effect on K^+ transport was not affected by indomethacin. In control mice, pretreatment with indomethacin significantly stimulated net absorption of both water and Na^+ (Table 2).

Table 1. Effect of rhein anthrone (6·24 mg kg^{-1}) on PGE_2 release into mouse ligated colon without and with pretreatment with indomethacin (3 mg kg^{-1}) given orally 90 min before treatment.

	PGE_2 released (ng g^{-1} tissue)	
	Control	Rhein anthrone
Without indomethacin	12·88 ± 1·21	22·49 ± 2·32**
With indomethacin	8·02 ± 0·60††	10·95 ± 1·60††

Each value is the mean ± s.e.m. of 7 experiments.
** $P < 0.01$ compared with the control group.
†† $P < 0.01$ compared with the respective groups without indomethacin.

Effect of rhein anthrone on colonic mucus secretion in mice

Table 3 shows that rhein anthrone (6·24 mg kg^{-1}) markedly stimulated mucus secretion and indomethacin (3·0 mg kg^{-1}) suppressed the enhanced mucus secretion induced by rhein anthrone. In comparative experiments, PGE_2 (0·2 mg kg^{-1}) caused similarly enhanced mucus secretion.

Effect of rhein anthrone on colonic mucus synthesis in mice

Rhein anthrone (6·24 mg kg^{-1}) significantly promoted colonic mucus synthesis. The accelerated synthesis induced by rhein anthrone was depressed to the control level by pretreatment with indomethacin (3·0 mg kg^{-1}). PGE_2 (0·2 mg kg^{-1}) also stimulated colonic mucus synthesis (Table 4).

Discussion

Cohen (1982), Capasso et al (1986) and Yagi et al (1988) have emphasized the possibility that senna preparations and rhein anthrone act via increased production of PGE-like material in the rat and mouse colonic tissue. However, Beubler (1988) has pointed out that they could not use measurement of PGs in the tissue to prove the involvement of PGs in a secretory effect and that PG release into the lumen was closely correlated with the secretory effect. This prompted us to measure PGE_2 release into the colonic lumen by enzyme immunoassay. We found that rhein anthrone stimulated PGE_2 release in the ligated colon and the PGE_2 release was inhibited by indomethacin. The enzyme immunoassay used in this experiment showed a high sensitivity and the anti-PGE_2 antibody exhibited a high degree of specificity with respect to cross-reactivity with PGE_1.

In our previous study, we could not observe the colonic fluid accumulation induced by rhein anthrone in mice because the amount of accumulated fluid was too small to measure volumetrically. In the present study the amount of colonic fluid accumulated in the ligated colon was estimated gravimetrically. Rhein anthrone decreased the net absorption of colonic water and Na^+, with statistical significance in the case of water, and enhanced K^+ net secretion. Pretreatment with indomethacin diminished the effects of rhein anthrone except on K^+ net secretion. These experiments

Table 2. Effect of rhein anthrone (6·24 mg kg^{-1}) on net water flux and electrolyte transport in mouse ligated colon without and with pretreatment with indomethacin (3·0 mg kg^{-1}) given orally 90 min before treatment.

		Net water flux (mL g^{-1} tissue)	Na^+ transport (μmol g^{-1} tissue)	K^+ transport (μmol g^{-1} tissue)
Without indomethacin	Control	−0·18 ± 0·05	−61·81 ± 10·88	9·17 ± 1·28
	Rhein anthrone	−0·04 ± 0·05*	−36·16 ± 10·08	14·28 ± 1·28**
With indomethacin	Control	−0·44 ± 0·05†††	−103·77 ± 10·00††	8·37 ± 0·99
	Rhein anthrone	−0·24 ± 0·06*††	−78·69 ± 10·69††	11·62 ± 1·36

Each value is the mean ± s.e.m. of 30 experiments.
A negative value denotes net absorption and a positive value net secretion.
* $P < 0·05$ and ** $P < 0·01$ compared with the respective control groups.
†† $P < 0·01$ and ††† $P < 0·001$ compared with the respective groups without indomethacin.

Table 3. Effect of rhein anthrone (6·24 mg kg^{-1}) and PGE_2 (0·2 mg kg^{-1}) on mucus secretion in mouse ligated colon without and with pretreatment with indomethacin (3·0 mg kg^{-1}) given orally 90 min before treatment.

	Mucus secretion (mg TPBH g^{-1} tissue)		
	Control	Rhein anthrone	PGE_2
Without indomethacin	0·53 ± 0·03 (n = 16)	1·20 ± 0·07*** (n = 16)	1·02 ± 0·07*** (n = 15)
With indomethacin	0·50 ± 0·04 (n = 15)	0·84 ± 0·05***††† (n = 16)	—

Each value is the mean ± s.e.m.
*** $P < 0·001$ compared with the respective control groups.
††† $P < 0·001$ compared with the rhein anthrone group without indomethacin.

Table 4. Effect of rhein anthrone (6·24 mg kg^{-1}) and PGE_2 (0·2 mg kg^{-1}) on mucus synthesis in mouse ligated colon without and with pretreatment with indomethacin (3·0 mg kg^{-1}) given orally 90 min before treatment.

	Mucus synthesis (d min^{-1} TPBH g^{-1} tissue)		
	Control	Rhein anthrone	PGE_2
Without indomethacin	1524·2 ± 106·5 (n = 16)	2275·1 ± 124·8*** (n = 16)	2468·2 ± 194·7*** (n = 10)
With indomethacin	1215·9 ± 98·6† (n = 16)	1540·8 ± 98·5*††† (n = 16)	—

Each value is the mean ± s.e.m.
* $P < 0·05$ and *** $P < 0·001$ compared with the respective control groups.
† $P < 0·05$ and ††† $P < 0·001$ compared with the respective groups without indomethacin.

were carried out using a closed system, the ligated colon, to minimize atmospheric oxidation of rhein anthrone to rhein.

In mice, colonic water flux and electrolyte transport are apparently only slight and furthermore rhein anthrone could not reverse the net water and Na^+ absorption at a dose of 6·24 mg kg^{-1}. The colonic mucus secretion appears to play an important role in the onset of mucoid diarrhoea. The mucus secretion was markedly enhanced by rhein anthrone or PGE_2, but this enhancement with rhein anthrone could be significantly suppressed by pretreatment with indomethacin. PGE_2 is thought to be involved in this enhancement. There is the possibility of mucus secretion due to the forced detachment of mucus from the intestinal surface by rhein anthrone; decreased enhancement in the presence of indomethacin, however, suggests the contribution from that source to be minor.

We examined whether rhein anthrone could intracellularly stimulate mucus synthesis in the mouse colon. We found that colonic mucus synthesis could be increased by rhein anthrone or PGE_2 whereas indomethacin reduced the enhanced mucus synthesis induced by rhein anthrone to the control level. Although a dose of 0·2 mg kg^{-1} PGE_2 was much larger than the amount of PGE_2 released in the mouse colonic lumen by the intracolonic injection of 6·24 mg kg^{-1} of rhein anthrone, it caused diarrhoea of similar severity to that induced by 6·24 mg kg^{-1} rhein anthrone when administered intracaecally (Yagi et al 1988). The mechanism of the promotion of mucus synthesis in the colon by PGE_2 is not known.

Beubler & Kollar (1985) have suggested that senna preparation exerts its purgative action at least partially via stimulation of colonic fluid and electrolytes secretion, and that this secretion is mediated by stimulation of endogenous PGE_2 formation. Our results have shown that the colonic secretion of water and electrolytes mediated by PGE_2 is indeed partly involved in the rhein anthrone-induced diarrhoea. However, our findings would also lead to the conclusion that the mucoid diarrhoea induced by rhein anthrone in

mice results mainly from PGE_2-mediated colonic mucus synthesis and secretion.

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