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Suppression of the Purgative Action of Rhein Anthrone, the Active Metabolite of Sennosides A and B, by Indomethacin in Rats

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Abstract—Rhein anthrone (12·48 mg kg^{-1}) produces watery and mucoid diarrhoea approximately 20 min after intracaecal administration to rats. Pretreatment with the prostaglandin (PG) biosynthesis inhibitor indomethacin (10 mg kg^{-1}, i.p.) only delayed and did not completely block the onset of the induced diarrhoea. Rhein anthrone stimulated PGE_2 release into the rat colonic lumen and the increased release was depressed by indomethacin. Rhein anthrone also accelerated large intestinal transit and this acceleration could be partly inhibited by indomethacin, which was probably responsible for the delay in the onset of diarrhoea. Indomethacin prevented the enhanced water, K^+ and mucus secretion and the reduced Na^+ absorption in the colon which were induced by rhein anthrone. The net water secretion could not be reversed to net absorption and the mucus secretion was only slightly depressed by indomethacin. Thus, our findings suggest that other mechanisms, together with the PG-dependent mechanism, are involved in the purgative action of rhein anthrone in rats.

In previous studies with mice, we showed that intracaecal administration of rhein anthrone, the intraluminal active metabolite of sennosides A and B, quickly causes severe mucoid diarrhoea and that pretreatment with the prostaglandin (PG) biosynthesis inhibitor indomethacin can block the onset of this diarrhoea. Both the large intestinal propulsive and colonic mucus secretory effects of rhein anthrone injected intracaecally were found to be suppressed by indomethacin. In addition, observations of the intestinal action of rhein anthrone demonstrated a significant increase in the biosynthesis and release of PGE_2 in the colon and their reduction by pretreatment with indomethacin. PGE_2 administered intracaecally also caused mucoid diarrhoea and enhanced colonic mucus synthesis and secretion in mice. These findings strongly suggest that the purgative action of rhein anthrone is mainly mediated by the biosynthesis and release of PGE_2 in mice (Yagi et al 1988, 1990).

Based on studies with rats, several groups also have suggested that sennosides A and B and senna preparations act by stimulating the formation and release of PGE-like material or PGE_2 (Beubler & Juan 1979; Cohen 1982; Beubler & Kollar 1985; Capasso et al 1986). Dreessen et al (1981) also reported that rhein anthrone is the active metabolite of sennosides A and B in rats. In the present investigation we have explored the suppression of the purgative action of rhein anthrone by indomethacin in rats.

Materials and Methods

Materials and chemicals

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

The following chemicals were purchased: indomethacin (Wako Pure Chemical Industries Ltd, Osaka, Japan), carmine red, orcinol (E. Merck, Darmstadt, Germany), PGE_2 (Funakoshi Pharmaceutical Co. Ltd, Tokyo, Japan) and urethane (Katayama Chemical, Osaka, Japan).

Rhein anthrone was dissolved in 2% sodium bicarbonate solution immediately before administration. For injection into the ligated colon, the rhein anthrone solution was diluted with Tyrode solution. Indomethacin was suspended in a small amount of Tween 80 and diluted with 0·9% NaCl (saline).

Animals

Female Wistar rats (CLEA, Japan, Inc, Tokyo), 100-130 g, were kept at ambient temperature (22–25°C) and fed with pelleted MF diet (Oriental Yeast Co. Ltd, Tokyo, Japan), which was withdrawn overnight before the experiments. Water was freely available.

Intracaecal cannula

The operation was carried out as described previously (Yagi et al 1988) with slight modifications. The animals with a cannula in the caecum were used in the experiments on the third day after the operation.

Purgative test

The procedure was similar to that of Yagi et al (1988), except for slight modifications. A rhein anthrone solution was injected at 5 mL kg^{-1} through the intracaecal cannula followed by 0·5 mL kg^{-1} of water to complete the injection. The rats were observed over 8 h for diarrhoea (excretion of wet or shapeless faeces with staining on the blotting paper). The purgative activity was expressed as the ratio (incidence of diarrhoea) of the number of diarrhoeal animals to the total number of test animals, or expressed as the mean of the time to onset of diarrhoea ± s.e.m. Also, diarrhoea was scored as follows: 0 = no faeces or normal faeces; 1 = moist faeces with faint staining on the under surface of blotting paper; 2 = soft faeces with staining on the blotting paper; 3 = shapeless

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sludged faeces; 4 = shapeless mucoid faeces, according to the modified method of Doherty (1981). Preliminary tests showed that 5 mL kg^{-1} of 2% sodium bicarbonate aqueous solution injected into the rat caecum had no purgative effect.

Determination of PGE_2 released into the colonic lumen

PGE_2 released into the colonic lumen was determined by the method of Yagi et al (1990) with slight modifications. Rats were anaesthetized with urethane (1·25 g kg^{-1} i.p.) and the entire colon was rinsed cautiously with a syringe of 20 mL warm 0·15 M NaCl (saline) in-situ. After 30 min, 2 mL Tyrode solution containing 12·48 mg kg^{-1} of rhein anthrone, or none for the control, was injected and the colon was ligated at the end (Forth et al 1966). After 60 min, a small sample of colonic fluid (about 0·5 mL) was withdrawn as described by Beubler & Kollar (1985). PGE_2 was extracted from the colonic fluid, purified according to the modified method of Kiyomiya & Oh-ishi (1985), determined by enzyme immunoassay using a modification of the method of Shono et al (1988), and expressed as ng (g tissue)$^{-1}$.

Measurement of large intestinal transit

Carmine red (5 mg in 0·2 mL water per animal) as a colour marker was injected into the caecum through the intracaecal cannula immediately after the intracaecal administration of rhein anthrone or 2% sodium bicarbonate solution as the control according to the modified method of Leng-Peschlow (1986). The time to the first excretion of coloured faeces was measured. Also, the incidence of the colour marker excretion was expressed as the ratio of the number of animals which excreted coloured faeces within 8 h to the total number of test animals.

Net water flux and electrolyte transport

Sixty minutes after 2 mL Tyrode solution containing rhein anthrone, or none for the control, was injected into the rat ligated colon, the rats were killed by exposure to chloroform. The colon was removed and the total colonic fluid was withdrawn and centrifuged at 100 000 *g* for 20 min at 5°C. According to the modified method of Beubler & Kollar (1985), net water flux and electrolyte transport were estimated and expressed as mL water (g tissue)$^{-1}$ and μmol (g tissue)$^{-1}$, respectively (Yagi et al 1990).

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 the rhein anthrone solution was injected into the rat ligated colon, the mucus was precipitated using 10% trichloroacetic acid from the colonic fluid and the precipitate was washed with 95% ethanol. The TPBH content was determined photometrically using galactose as a standard at 540 nm and expressed as mg TPBH (g tissue)$^{-1}$, according to the method of Yagi et al (1990).

Statistical evaluation

The results were expressed as mean values ± s.e.m. Statistical significance was assessed using Student's *t*-test.

Results

Effect of indomethacin on rhein anthrone-induced diarrhoea

Rhein anthrone produced watery and mucoid diarrhoea in all of the rats approximately 20 min after intracaecal administration at a dose of 12·48 mg kg^{-1} corresponding to 20 mg kg^{-1} of sennosides A and B. Indomethacin (10 mg kg^{-1}) administered intraperitoneally 90 min before rhein anthrone delayed the onset of diarrhoea, which was observed with a similar mean score approximately 60 min later (Table 1).

Effect of indomethacin on the PGE_2 release into colonic lumen induced by rhein anthrone

Rhein anthrone (12·48 mg kg^{-1}) significantly increased PGE_2 release into the colonic lumen. This enhanced release could be markedly reduced by pretreatment with indomethacin (10 mg kg^{-1}). In control rats, indomethacin also decreased the PGE_2 release into the colonic lumen (Table 2).

Effect of indomethacin on the large intestinal transit

In control animals, coloured faeces were excreted approximately 7 h after intracaecal administration of the marker substance. Rhein anthrone (12·48 mg kg^{-1}) produced marked reduction in the large intestinal transit time. Pretreatment with indomethacin (10 mg kg^{-1}) significantly prolonged the transit time reduced by rhein anthrone, which was still far below the normal transit time. Indomethacin also clearly depressed the normal large intestinal transit (Table 3).

Table 1. Effect of indomethacin on diarrhoea induced by rhein anthrone.

Pretreatment	Incidence of diarrhoea	Time to onset of diarrhoea (min) mean ± s.e.m.	Mean diarrhoea score
None (control)	9/9	17·1 ± 1·8 (n = 9)	3·4 (n = 9)
Indomethacin	10/10	60·1 ± 9·8* (n = 10)	3·4 (n = 10)

Rhein anthrone (12·48 mg kg^{-1}) was administered intracaecally. Indomethacin (10 mg kg^{-1} i.p.) was given 90 min before rhein anthrone administration. Diarrhoea was scored as follows: 0 = no faeces or normal faeces; 1 = moist faeces with faint staining on the under surface of blotting paper; 2 = soft faeces with staining on the blotting paper; 3 = shapeless sludged faeces; 4 = shapeless mucoid faeces. *$P < 0·001$ compared with the control group.

Table 2. Effect of indomethacin on normal and rhein anthrone-stimulated PGE_2 release into rat ligated colon.

	PGE_2 released ng (g tissue)$^{-1}$	
	Control	Rhein anthrone
Without indomethacin	2·74 ± 0·39 (n = 8)	14·36 ± 1·72** (n = 8)
With indomethacin	1·22 ± 0·20† (n = 9)	4·13 ± 0·73*†† (n = 9)

Each value is the mean ± s.e.m. Rhein anthrone (12·48 mg kg^{-1}) was injected into the ligated colon. Indomethacin (10 mg kg^{-1}, i.p.) was given 90 min before chemical administration. *$P < 0·01$ and **$P < 0·001$ compared with the respective control groups. †$P < 0·01$ and ††$P < 0·001$ compared with the respective groups without indomethacin.

Table 3. Effect of indomethacin on normal and rhein anthrone-stimulated large intestinal transit.

Administration		Incidence of	Transit time (min)
First	Second	marker excretion	mean ± s.e.m.
—	2% $NaHCO_3$	9/9	440·7 ± 20·8 (n = 9)
—	Rhein anthrone	9/9	15·8 ± 1·5* (n = 9)
Indomethacin	2% $NaHCO_3$	5/9	—
Indomethacin	Rhein anthrone	9/9	64·1 ± 9·4† (n = 9)

Rhein anthrone (12·48 mg kg^{-1}) or 2% $NaHCO_3$ solution was administered intracaecally. Carmine red marker solution, 0·2 mL, was injected into the caecum immediately after chemical administration. Indomethacin (10 mg kg^{-1}, i.p.) was given 90 min before chemical administration. Incidence of marker excretion was expressed as the ratio of the number of animals which had excreted carmine red marker within 8 h to the total number of test animals. Transit time was expressed as the time to the first excretion of the colour marker. *$P < 0·001$ compared with the respective control groups. †$P < 0·001$ compared with the respective groups without indomethacin.

Table 4. Effect of indomethacin on normal and rhein anthrone-stimulated net water flux and electrolyte transport in rat ligated colon.

	Net water flux mL (g tissue)$^{-1}$	Na^+ transport μmol (g tissue)$^{-1}$	K^+ transport μmol (g tissue)$^{-1}$
Without indomethacin			
Control (n = 20)	−0·30 ± 0·06	−127·63 ± 10·52	9·46 ± 0·76
Rhein anthrone (n = 20)	0·67 ± 0·03*	−32·87 ± 12·98*	24·05 ± 1·03*
With indomethacin			
Control (n = 20)	−0·61 ± 0·06††	−215·13 ± 12·16††	15·82 ± 2·29†
Rhein anthrone (n = 19)	0·34 ± 0·05*††	−80·32 ± 9·77*††	17·15 ± 1·36††

Each value is the mean ± s.e.m. Rhein anthrone (12·48 mg kg^{-1}) was injected into the ligated colon. Indomethacin (10 mg kg^{-1}, i.p.) was given 90 min before chemical administration. A negative value denotes net absorption and a positive value net secretion. *$P < 0·001$ compared with the respective control groups. †$P < 0·05$ and ††$P < 0·001$ compared with the respective groups without indomethacin.

Table 5. Effect of indomethacin on normal and rhein anthrone-stimulated mucus secretion in rat ligated colon.

	Mucus secretion mg TPBH (g tissue)$^{-1}$	
	Control	Rhein anthrone
Without indomethacin	0·24 ± 0·02 (n = 14)	1·83 ± 0·07* (n = 14)
With indomethacin	0·19 ± 0·02 (n = 13)	1·63 ± 0·05*† (n = 14)

Each value is the mean ± s.e.m. Rhein anthrone (12·48 mg kg^{-1}) was injected into the ligated colon. Indomethacin (10 mg kg^{-1}, i.p.) was given 90 min before chemical administration. TPBH = total protein bound hexose. *$P < 0·001$ compared with the respective control groups. †$P < 0·05$ compared with the rhein anthrone group without indomethacin.

Effect of indomethacin on water flux, electrolyte transport and mucus secretion in the colon

Net water absorption was observed in control rats. Rhein anthrone (12·48 mg kg^{-1}) reversed net water absorption into net secretion with significantly decreased net Na^+ absorption and increased net K^+ secretion. These effects of rhein anthrone were significantly prevented by pretreatment with indomethacin (10 mg kg^{-1}). In control rats, pretreatment with indomethacin significantly stimulated net water and Na^+ absorption and net K^+ secretion (Table 4). Furthermore, as shown in Table 5, rhein anthrone (12·48 mg kg^{-1}) significantly stimulated mucus secretion. Indomethacin (10 mg kg^{-1}) slightly but significantly depressed the enhanced mucus secretion.

Discussion

Our present results show that indomethacin could only delay and not completely block the onset of diarrhoea induced by rhein anthrone injected intracaecally. However, rhein anthrone was found to markedly stimulate the release of PGE_2 into the colonic lumen, and the accelerated release could be depressed to approximately the control level by indomethacin. According to Beubler (1988), PGE_2 release into the colonic lumen is correlated closely with the secretory effect. Indomethacin, which could decrease the PGE_2 release, stimulated net water and Na^+ absorption and net K^+ secretion in control rat colon and also prevented the enhanced water and K^+ secretion and reduced Na^+ absorption induced by rhein anthrone. Why indomethacin increased K^+ secretion in control rat colon is not known and remains for further study. Colonic mucus secretion was also prominently accelerated by rhein anthrone, which was only slightly depressed by indomethacin. These experiments were carried out carefully using a closed system, in this case, a ligated colon, because rhein anthrone is stable only in a reducing atmosphere and is easily oxidized to rhein in the presence of oxygen (Leng-Peschlow 1989).

In spite of these inhibitory effects of indomethacin on the secretion of water, electrolytes and mucus by the colon, the diarrhoea induced by rhein anthrone after treatment with indomethacin appeared about 40 min later than that without indomethacin. In addition, the intensity of diarrhoea was apparently similar to that without indomethacin. Such a delay in the onset of diarrhoea seems to correspond to a prolongation by indomethacin of the reduction in large intestinal transit time caused by rhein anthrone. These results generally agree with the data obtained with sennosides A and B (50 mg kg^{-1} intracaecally) of Leng-Peschlow

(1988). Since indomethacin considerably depressed normal large intestinal transit and delayed the rhein anthrone-induced accelerated transit, which was still far from normal, a local PG synthesis may partly mediate peristalsis and the propulsive effect in the colon as reported by Bennett et al (1976) for guinea-pigs and Staumont et al (1988) for dogs.

Mascolo et al (1988) demonstrated that oral administration of senna pod extract to rats maintained on essential fatty acid-deficient diet for 30–90 days, produced diarrhoea and reversed net water absorption into net secretion as in normal rats although such a diet drastically reduced the PG production in the colonic lumen both in senna-free and senna-treated rats. PG mediation did not seem to be present in essential fatty acid-deficient rats. Thus, those authors concluded that the PGs are not essential for diarrhoea induced by senna and that water secretion and PG production in the rat intestinal lumen are unrelated. In our present investigation, the diarrhoea induced by rhein anthrone appeared in indomethacin-treated rats in which the colonic PG biosynthesis was thought to be markedly reduced. The antisecretory effects of indomethacin in the rat colon were less potent than those in mice, especially in the case of mucus secretion (Yagi et al 1990). The net water secretion was not reversed to net absorption by indomethacin. The finding that indomethacin only delays the onset of diarrhoea may be explained by the suggestion that indomethacin inhibits the effect of rhein anthrone, but after the effect of indomethacin has ceased, the anthrone, still present in the gut, exerts its full action. There remains also the point of view of Rask-Madsen et al (1984) who argued that indomethacin only partly reduces local PG synthesis and the remaining amount would still result in a near maximal secretory response. However, we concluded that other mechanisms, together with the PG-dependent mechanism, are involved in the purgative action of rhein anthrone in rats unlike in mice.

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