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POLYAMINE INHIBITION OF GASTRIC ULCERATION AND SECRETION IN RATS

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Abstract—The effect of polyamines on gastric ulceration and secretion in rats was studied. Stress-induced gastric ulceration and ulceration in pylorus-ligated rats were inhibited by subcutaneous or oral administration of spermine; spermidine's inhibitory effect was somewhat less. Histamine-induced ulceration was also inhibited by the subcutaneous injection of spermine. In addition, the daily oral administration of spermine for 10 days was therapeutic against an acetic acid-induced ulcer (chronic ulcer). Gastric secretion in pylorus-ligated rats and in rats with fistulae and stimulated by histamine injection was decreased by the subcutaneous injection of spermine.

An interaction between polyamines and membranes has been suggested from the observation that polyamines can prevent the lysis of *Micrococcus luteus* with lysozyme [1] and can change the activity of microsomal enzymes involved in estradiol metabolism [2] and lipid peroxidation [3, 4]. We have recently shown that the synthesis of PGE_2 and 6-keto-$PGF_{1\alpha}$ was inhibited by spermine through the latter's interaction with phospholipids [5]. Since PGE_1, PGE_2 [6, 7], and probably PGI_2 [8] are triggers of polyamine biosynthesis via an increase in cAMP content, the inhibition of PGE_2 and 6-keto-$PGF_{1\alpha}$ synthesis by spermine may be a kind of "feedback inhibition". Therefore, we have examined the possibility that polyamines can substitute for some of the functions of PGE_2 or PGI_2. In this communication, we show that spermine has an anti-ulcerogenic action similar to that exhibited by PGE_2 [9] and PGI_2 [10]. During our studies, it has been reported that polyamines are inhibitors of gastric acid secretion [11].

MATERIALS AND METHODS

Stress-induced gastric ulceration. Male Wistar rats, weighing 180–220 g and fasting from food for 18 hr but with free access to water, were given orally or subcutaneously polyamines dissolved in 0.9% NaCl. Saline (0.9% NaCl solution) was administered to the control animals either orally or subcutaneously. The animals were then placed in a stress cage and immersed in a water bath (23°) up to the level of the xiphoid process for 7 hr [12]. At the end of the stress, the animals were killed by a blow on the head and the stomach was removed and treated according to the method of Okabe *et al.* [13]. The area of each lesion in the stomach was expressed in terms of the ulcer index (mm^2).

Gastric ulceration in pylorus-ligated rats (Shay rats). After the rats were fasted for 48 hr with free access to water, the junction between the pylorus and duodenum was ligated by the method of *Shay et al.* [14]. Polyamines were administered orally or subcutaneously immediately after ligation. The control rats received 0.9% NaCl instead of polyamines dissolved in 0.9% NaCl. The animals were killed 18 hr after the operation and the stomach was removed. The ulcer index was determined as described above.

Gastric secretion in pylorus-ligated rats (Shay rats). The antisecretory effect of spermine was tested on pylorus-ligated rats 6 hr after ligation, since the antisecretory effect may dissipate by 18 hr. The operative procedure was the same as that described above except that the fasting period was 18 hr. Gastric juice was collected in a graduated test tube and the total acidity was determined by titration with 0.1 N NaOH.

Histamine-induced gastric ulceration and secretion. After rats were fasted for 18 hr with free access to water an ulcer was induced by intraperitoneal injection of histamine (300 mg/kg). The ulcer index was determined 4 hr after injection. Spermine was administered orally or subcutaneously 3 hr before the histamine injection. The control rats received 0.9% NaCl instead of spermine dissolved in 0.9% NaCl.

For a collection of gastric juice, an abdominal fistula was made in the rats by the methods of Ishii [15]. Spermine was administered subcutaneously 1 hr after the operation and histamine (5 mg/kg) was administered subcutaneously 3 hr later. The gastric juice was washed out with 10 ml of saline every hour and the washings were titrated with 0.02 N NaOH.

Acetic acid-induced gastric ulcer (chronic ulcer). An ulcer was induced by the injection of 0.02 ml of 20% acetic acid solution into the rat stomach wall according to the method of Takagi *et al.* [16]. Spermine was given orally once a day for 10 days. The control rats received 0.9% NaCl instead of spermine dissolved in 0.9% NaCl. The animals were killed 20 hr after the last administration of spermine and an ulcer index was determined.

Table 1. Effect of polyamines on stress-induced gastric ulceration in rats

Exp. No.	Polyamine	Dose (mg/kg)	No. of animals	Ulcer index (mm^2)	Inhibition (%)
I	Control		11	1.38 ± 0.29[a]	
	Spermine	12.5 (P.O.)	8	0.89 ± 0.21	34
		25 (P.O.)	8	0.05 ± 0.02**	96
		50 (P.O.)	8	0.12 ± 0.08**	91
	Spermidine	12.5 (P.O.)	8	0.64 ± 0.18	53
		25 (P.O.)	8	0.70 ± 0.17	48
		50 (P.O.)	8	0.09 ± 0.05**	93
II	Control		8	1.42 ± 0.30	
	Spermine	50 (S.C.)	8	0.16 ± 0.08**	88
	Spermidine	50 (S.C.)	8	0.45 ± 0.12*	68

[a] Mean ± S.E.
* Asterisk indicates a significant difference from control (* $P < 0.05$, ** $P < 0.01$, calculated according to Student's *t*-test).

RESULTS

Effect of polyamines on ulceration in rats

As shown in Table 1, stress-induced gastric ulceration in rats was inhibited by spermine and to a lesser degree by spermidine. The oral administration of 12.5, 25 and 50 mg/kg spermine inhibited ulcer formation 34, 96 and 91%, respectively. Gastric ulceration in pylorus-ligated rats was also inhibited by spermine and to a lesser degree by spermidine (Table 2). However, the ability of spermine to inhibit ulceration in pylorus-ligated rats was less than that in stress-induced gastric ulceration. A subcutaneous injection was more effective than oral administration in inhibiting gastric ulceration in pylorus-ligated rats. As shown in Table 3, a histamine-induced ulceration was inhibited by the subcutaneous injection of spermine but not by oral administration. However, the subcutaneous injection of spermine was less efficient in inhibiting histamine-induced ulceration than gastric ulceration in pylorus-ligated rats.

The effect of spermine on acetic acid-induced gastric ulcer (chronic ulcer) was then tested (Table 4). Acetic acid-induced gastric ulcers were reduced by the oral administration of spermine.

Effect of spermine on gastric secretion

In pylorus-ligated rats and in rats containing a fistula and stimulated by histamine injection, gastric secretion was inhibited significantly by the subcutaneous injection of spermine (50 mg/kg) (Table 5 and Fig. 1). This suggests that the antiulcerogenic action of polyamines may be at least partially due to the inhibition of gastric secretion.

DISCUSSION

The data presented show that polyamines, especially spermine inhibited gastric ulceration and secretion in rats. Among the three ulcers, the degree of inhibition by spermine was in the order stress-induced ulcer > ulcer in pylorus-ligated rats > histamine-induced ulcer. Since the oral administration of spermine was not effective on the inhibition of histamine-induced ulceration and this type of administration was less effective than the subcutaneous injection in limiting ulceration in pylorus-ligated rats, enteral absorption of spermine may not be effective so much.

Spermine was also found to inhibit acetic acid-induced ulcers, a form of chronic ulcer. It has been reported that drugs which are known to promote mucosa regeneration and formation of granulation in gastric tissue showed a significant acceleration on the healing in acetic acid-induced ulcers [17]. This suggests that the inhibitory action of spermine may be due to the inhibition of gastric secretion and the stimulation of the formation of glycosaminoglycans

Table 2. Effect of polyamines on gastric ulceration in pylorus-ligated rats

Exp. No.	Polyamine	Dose (mg/kg)	No. of animals	Ulcer index (mm^2)	Inhibition (%)
I	Control		8	8.6 ± 2.0[a]	
	Spermine	50 (P.O.)	8	3.3 ± 0.8*	62
	Spermidine	50 (P.O.)	8	5.8 ± 1.4	33
II	Control		9	25.4 ± 5.0	
	Spermine	12.5 (P.O.)	8	20.8 ± 5.7	18
		25 (P.O.)	8	10.4 ± 4.5*	59
		50 (P.O.)	9	9.1 ± 1.8**	64
III	Control		7	5.0 ± 2.0	
	Spermine	50 (S.C.)	8	0.1 ± 0.1*	98
	Spermidine	50 (S.C.)	8	1.8 ± 0.4	64

[a] Mean ± S.E.
* $P < 0.05$, ** $P < 0.01$, calculated according to Student's *t*-test.

Table 3. Effect of spermine on histamine-induced ulceration in rats

Polyamine	Dose (mg/kg)	No. of animals	Ulcer index (mm^2)	Inhibition %
Control		8	4.1 ± 0.9[a]	
Spermine	50 (P.O.)	8	4.8 ± 1.0	−17
Control		8	4.0 ± 0.7	
Spermine	50 (S.C.)	8	0.9 ± 0.5**	78

[a] Mean ± S.E.
** $P < 0.01$, calculated according to Student's t-test.

Table 4. Effect of spermine on acetic acid-induced ulceration in rats

Treatment	No. of animals	Ulcer index (mm^2)	Curation (%)
Control (0.9% NaCl, P.O. 10 times)	10	2.4 ± 0.6[a]	
Spermine (50 mg/kg, P.O. 10 times)	9	1.0 ± 0.3†	58

[a] Mean ± S.E.
† $0.05 < P < 0.10$, calculated according to Student's t-test ($t = 2.057$).

Table 5. Effect of spermine on gastric secretion in pylorus-ligated rats

Polyamine	Dose (mg/kg)	No. of animals	Secretory volume (ml/100 g body weight)	Acid output (μEq H^+/100 g body weight)
Control		8	3.7 ± 0.2[a]	249.9 ± 20.5
Spermine	50 (S.C.)	8	1.0 ± 0.1***	45.5 ± 10.0***

[a] Mean ± S.E.
*** $P < 0.001$, calculated according to Student's t-test.

and glycoproteins, which have antisecretory and cytoprotective properties. In this connection, the stimulation of polypeptide synthesis by polyamines in eukaryotic cell-free systems has been reported previously [18–21].

The results also suggest that spermine and similar compounds may have a therapeutic potential for the treatment of hyper-acidity and peptic ulcer. For the use of spermine and similar compounds as an antiulcerogenic drug, however, devices to decrease the toxicity of polyamines and to increase its rate of enteral absorption would be necessary. The finding that a subcutaneous injection of 100 mg/kg spermine increased the amounts of albumin in urine significantly, suggests the toxicity of spermine in kidney (data not shown). Spermine which is made in the stomach and duodenum may function as an antiulcerogenic factor.

It is of interest that polyamines apparently can substitute for a part of the function of PGE_2 and PGI_2 as spermine inhibits the synthesis of PGE_2 and 6-keto-$PGF_{1\alpha}$ in a type of "feedback inhibition". This phenomenon may be rational and economical in cells. Experiments are now in progress to elucidate whether polyamines can substitute for other functions of PGE_2 and PGI_2.

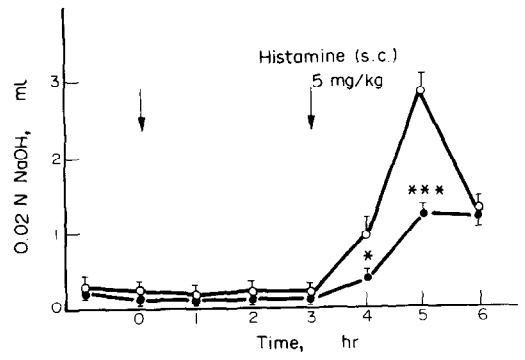

Fig. 1. Effect of spermine on acidity of gastric juice in rats containing fistula and stimulated by histamine injection. Acidity was determined by titration with 0.02 N NaOH as described in Materials and Methods. Asterisk indicates a significant difference from control (*$P < 0.05$, ***$P < 0.001$, calculated according to Student's t-test). Left arrow indicates the time when either spermine (50 mg/kg) or saline was administered subcutaneously. ○, Control; ●, spermine.

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