Changes in Cyclic AMP Content of Rat Gastric Mucosa Induced by Ulcerogenic Stimuli — In Relation to the Antiulcer Activity of Irsogladine Maleate

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ABSTRACT—Changes in the cyclic AMP (cAMP) content of the gastric mucosa induced by ulcerogenic stimuli were investigated in rats. Ligation of the pylorus for 5 hr produced no glandular mucosal lesion, but increased the cAMP content in the fundus and antrum. Aspirin produced glandular mucosal lesions in the pylorus-ligated rats and caused an increase of the cAMP content in the fundus and a decrease in the antrum. Irsogladine maleate (IM), an antiulcer agent, inhibited both the changes in the cAMP content and the mucosal damage induced by aspirin. IM increased the cAMP content in both regions, especially the antrum, in normal rats. Dibutyryl cAMP (dbcAMP) given orally prevented the gastric mucosal lesions induced by aspirin without affecting gastric secretion. These results suggest that 1) the changes in the cAMP content of the fundus and antrum induced by aspirin may be associated with the formation of glandular mucosal damage, 2) the antiulcer activity of IM may be related to an increase of the cAMP content in mucous cells, and 3) dbcAMP given orally may penetrate into the surface mucous cells and activate defensive functions. Thus, cAMP in the mucous cells may protect the gastric mucosa.

Cyclic AMP (cAMP) plays an important role in the intracellular signal transduction of various hormones (1) or as a modulator in the cell cycle (2) and in intercellular communication (3). In the stomach, cAMP acts as a second messenger of hormones and causes secretion of gastric acid and pepsinogen, which are aggressive factors of peptic ulcer, from parietal and chief cells (4–6).

Dibutyryl cAMP (dbcAMP) is commonly used to study the intracellular action of cAMP. It is well-known that dbcAMP accelerates both the secretion of gastric acid in isolated parietal cells (7) and the synthesis of mucus in isolated mucous cells (8). These re-

sults in vitro indicate that dbcAMP acts aggressively as well as defensively in the gastric mucosa. It has been reported that dbcAMP in a dose of 0.2 mg/kg, p.o., inhibits the formation of mucosal lesions induced by aspirin without affecting gastric secretion (9), although in the large dose of 30 mg/kg, i.v., it activates gastric acid secretion (10).

Irsogladine maleate [IM, 2,4-diamino-6-(2,5-dichlorophenyl)-s-triazine maleate], an antiulcer agent, increases the accumulation of cAMP in cultured rabbit gastric epithelial cells in the presence of a phosphodiesterase inhibitor, and the cAMP content of the mucous cells in stressed rats (11). The increasing ac-

tion of IM on cAMP in the surface mucous cells may be associated with its antiulcer and cytoprotective actions (12–17).

In this paper, we investigated the relationship between cAMP content and gastric mucosal damage in pylorus-ligated and aspirin-treated rats and the effect of IM. In addition, we examined the effect of dbcAMP on the mucosal lesions induced by aspirin and gastric secretion in pylorus-ligated rats.

MATERIALS AND METHODS

Male Sprague-Dawley rats, 8–9 weeks of age, were purchased from Shizuoka Laboratory Animal Center and fasted for 24 hr prior to the experiments, unless otherwise mentioned. There were 10 rats in each group. Radioimmunoassay kits for cAMP were obtained from Amersham. IM (Nippon Shinyaku Co., Ltd.) was suspended in saline and aspirin (Wako Pure Chemical Industries, Ltd.), in saline containing 0.5% methylcellulose. dbcAMP (Sigma) was dissolved in saline.

Determination of cAMP in gastric mucosa

Pylorus-ligated, aspirin-treated rats: Immediately after ligation of the pylorus under ether anesthesia, IM (1–10 mg/kg) was administered intraduodenally (i.d.) and aspirin (200 mg/kg) given intragastrically (i.g.). Five hours later, the animals were anesthetized with pentobarbital Na (30 mg/kg, i.v.), and the stomachs were removed quickly and gently with handling of only the stumps of the esophagus and duodenum. Within 5 sec, the stomachs were irradiated with microwaves at 5 kW for 1.5 sec with a microwave applicator (Metabostat, NJE 2601, Shin Nihon Musen, Tokyo, Japan). The denatured stomachs were opened along the greater curvature, and the damage index in the glandular mucosa was graded as normal (–), mild hemorrhage (+) or severe damage (++). Then the antral and fundal mucosa was scraped-off on ice and homogenized with 1 and 2 ml of 5% trichloroacetic acid (TCA), respectively; 1-ml portions of the homogenates were centrifuged at 10,000 rpm

for 3 min at 4°C. The supernatants and pellets were used for the assays of cAMP and protein content, respectively. After washing of 0.5 ml of the supernatants with 3 ml of water-saturated ether 5 times to remove TCA, the cAMP content was determined by the radioimmunoassay method of Narumi et al. (18). The pellets were dissolved in 0.5 ml of 10% NaOH, and the protein content was determined by the method of Lowry et al. (19) with bovine serum albumin as the standard.

Normal rats: At given intervals after the oral administration of IM (1–10 mg/kg), the stomachs were removed under pentobarbital Na (30 mg/kg, i.v.) anesthesia and irradiated with microwaves. The cAMP and protein contents in the gastric mucosa were determined as described above.

Effect of dbcAMP on aspirin-induced mucosal lesions and gastric secretion

Aspirin-induced mucosal lesions: Animals were fasted for 48 hr. Aspirin-induced mucosal lesions were produced by the method of Best et al. (9). Five hours after the intragastric administration of aspirin (150 mg/kg), the length (mm) of the gastric mucosal lesions was measured under stereoscopic microscopy, and the total length was used as the lesion index. IM (1–10 mg/kg) was given orally 45 min before aspirin administration. dbcAMP (0.02–0.2 mg/kg) was given orally 45 min before or intravenously at the same time as aspirin administration.

Gastric secretion: After ligation of the pylorus, dbcAMP (0.02–0.2 mg/kg) was administered orally, intraduodenally or intravenously. Five hours later, the gastric content was collected and centrifuged. The supernatant was measured for volume and H^+ and Na^+ . The acidity was titrated with 0.01 N NaOH to pH 7.0, and Na^+ output was determined by an automatic analyzer for electrolytes (300A, Horiba, Kyoto, Japan).

Analysis of data

The significance of differences between the means was verified by the Dunnett method

(20). The significance of the differences in the damage index between the groups was assessed by the Wilcoxon rank sum test.

RESULTS

Gastric mucosal content of cAMP and lesions in pylorus-ligated rats

The cAMP content was significantly increased in both the fundus and the antrum by ligation of the pylorus (Fig. 1). Aspirin pro-

duced a marked increase in the cAMP content in the fundus and in turn, a slight decrease in the antrum. IM inhibited these changes induced by aspirin dose-dependently. No damage was induced in the glandular stomach by pylorus ligation alone. However, aspirin caused severe mucosal damage in pylorus-ligated rats (Table 1). IM inhibited the aspirin-induced gastric mucosal damage in a dose-dependent manner.

Fig. 1. Effect of irsogladine maleate (IM) on gastric mucosal cAMP contents in pylorus-ligated, aspirin-treated rats. IM was administered intraduodenally simultaneously with aspirin (200 mg/kg, i.g.) to the pylorus-ligated rats. Each column with bar represents the mean and S.E. of 10 rats. NC: non-treated control. PC: pylorus-ligated control, PAC: pylorus-ligated, aspirin-treated control. *, Significantly different from NC at $P < 0.01$. §, Significantly different from PC at $P < 0.05$. * and **, Significantly different from PAC at $P < 0.05$ and $P < 0.01$, respectively.

Table 1. Effect of irsogladine maleate (IM) on gastric mucosal damage induced by aspirin (200 mg/kg, i.g.) in pylorus-ligated rats

Treatment	Dose of IM (mg/kg, p.o.)	Rats used	No. of rats with damage index of			P value as compared with pylorus-ligation and aspirin-treated control
			—	+	++	
Non-treatment	—	10	10	0	0	$P < 0.01$
Pylorus-ligation	—	10	10	0	0	$P < 0.01$
Pylorus-ligation and aspirin	1	10	0	2	8	—
	3	10	3	5	2	NS
	10	10	5	4	1	$P < 0.05$
			8	2	0	$P < 0.01$

NS, not significant.

Fig. 2. Effect of oral administration of irsogladine maleate (IM) on gastric mucosal cAMP content in normal rats. Each point with bar represents the mean and S.E. of 10 rats. *, Significantly different from the control at $P < 0.05$. (●) control; (○) IM, 1 mg/kg; (□) IM, 3 mg/kg; (△) IM, 10 mg/kg.

Effect of IM on cAMP content in gastric mucosa of normal rats

IM had little effect on the fundal cAMP content, but in a dose of 10 mg/kg, it significantly increased the antral cAMP at 2 and 4 hr after administration (Fig. 2).

Effect of dbcAMP on aspirin-induced mucosal lesions and gastric secretion

Aspirin-induced mucosal lesions: Oral administration of dbcAMP decreased the lesion index dose-dependently (Table 2). However, intravenous dbcAMP had no effect. IM given orally also decreased the lesion index dose-dependently.

Gastric secretion: dbcAMP given orally, intraduodenally or intravenously had no effect

on the volume of gastric juice or the output of H^+ and Na^+ (Table 3).

DISCUSSION

The fundal epithelium is composed mainly of 4 kinds of cells: parietal, chief, mucous and mucous neck cells. It is generally considered that the cAMP content in the fundus reflects the total activities of all these cells. However, in reality, the fundal cAMP content seems to be dependent on the activity of the parietal cells, since they contain the most cAMP (21). On the other hand, the antral cAMP seems to represent the activity of mucous cells, which are the most numerous epithelial cells in the antrum.

Table 2. Effects of irsogladine maleate (IM) and dibutyryl cyclic AMP (dbcAMP) on gastric lesions induced by aspirin (150 mg/kg, i.g.) in rats

Route	Drug	Dose (mg/kg)	N	Lesion index (mm)	% Decrease
p.o.	control	—	10	24.0 ± 3.2	—
		1	10	21.8 ± 4.6	9.2
	IM	3	10	13.1 ± 1.0*	45.4
		10	10	10.9 ± 2.7*	54.6
		0.02	10	19.0 ± 3.9	20.8
	dbcAMP	0.06	10	12.7 ± 0.8*	47.1
		0.2	10	9.3 ± 1.6**	61.3
i.v.	control	—	9	39.0 ± 8.3	—
		0.02	10	26.3 ± 6.5	32.6
	dbcAMP	0.06	10	37.0 ± 8.3	5.1
		0.2	10	25.7 ± 6.7	34.1

Each value represents the mean ± S.E. * and **, Significantly different from the control at $P < 0.05$ and $P < 0.01$, respectively.

Table 3. Effect of dibutyryl cyclic AMP (dbcAMP) on gastric secretion in pylorus-ligated rats

Route	Drug	Dose (mg/kg)	N	Juice (ml/kg)	H ⁺ output (μEq/kg)	Na ⁺ output (μEq/kg)
p.o.	control	—	10	32.1 ± 2.3	3243 ± 282	1328 ± 87
	dbcAMP	0.02	10	38.8 ± 2.7	3948 ± 299	1456 ± 149
		0.06	10	32.9 ± 2.8	3401 ± 392	1317 ± 95
		0.2	10	37.3 ± 3.2	3936 ± 364	1420 ± 130
i.d.	control	—	10	32.2 ± 3.3	3297 ± 354	1013 ± 128
	dbcAMP	0.02	9	24.3 ± 2.4	2435 ± 354	888 ± 65
		0.06	10	25.9 ± 2.1	2762 ± 280	895 ± 46
		0.2	10	28.4 ± 0.9	2795 ± 109	951 ± 54
i.v.	control	—	10	32.5 ± 3.0	3254 ± 355	1264 ± 118
	dbcAMP	0.02	10	34.9 ± 2.7	3573 ± 306	1367 ± 114
		0.06	10	35.1 ± 2.7	3763 ± 371	1228 ± 82
		0.2	10	24.0 ± 1.9	2335 ± 223	929 ± 65

Each value represents the mean ± S.E.

In the present study, ligation of the pylorus for 5 hr caused an increase of the cAMP content in the fundus and antrum of rats and no mucosal damage. The increase of fundal cAMP may be due to the stimulation of gastric secretion induced by ligation of the pylorus (22) and the increase of antral cAMP, to the activation of the mucous cells. These results suggest that the concomitant stimulation

of parietal and mucous cells does not cause mucosal damage.

The administration of aspirin to pylorus-ligated rats caused glandular mucosal damage. Aspirin increased the fundal and decreased the antral cAMP content. These results are in agreement with the aspirin-induced decrease of mucus in the rat fundus and antrum (23) and in the cAMP content of cultured rabbit

gastric epithelial cells (24). The increase of the fundal cAMP by aspirin may be due to the stimulation of parietal cells, since aspirin accelerates the secretion of gastric acid (25). On the other hand, the decrease of the antral cAMP content induced by aspirin may indicate depression of the mucous cells. Therefore, the production of mucosal damage by aspirin is considered to be due to the stimulation of aggressive parietal cells and inhibition of defensive mucous cells.

IM inhibited the lesion formation induced by aspirin, and both the increase of cAMP in the fundus and its decrease in the antrum induced by aspirin in pylorus-ligated rats. These results suggest that IM prevents both the depression of the mucous cells and the stimulation of the parietal cells induced by aspirin. The inhibitory effect of IM on the cAMP increase in the fundus may be associated with the weak antigastric secretory action of IM (14). In normal rats, IM increased the cAMP content in both regions, especially in the antrum. Thus, the effect of IM on the cAMP content may be due to its action in the surface mucous cells, which are most numerous in the antrum and least numerous in the fundus.

dbcAMP inhibited aspirin-induced mucosal lesions when administered orally but not intravenously, while it had no effect on gastric secretion. These findings indicate that dbcAMP may penetrate into the surface mucous cells and compensate for the aspirin-induced decrease in the cAMP content. In conclusion, cAMP in the mucous cells appears to play an important role in the protection of the gastric mucosa.

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