EFFECTS OF ADRENERGIC BLOCKING AGENTS ON GASTRIC SECRETION AND STRESS-INDUCED GASTRIC ULCER IN RATS

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Recently interest in investigations of adrenergic agents (stimulants and inhibitors) on gastric secretion, motility, and ulceration in experimental animals has increased (1-7). Pradhan and Wingate (6) have demonstrated that phentolamine and tolazoline enhanced the spontaneous gastric secretion in dogs while phenoxybenzamine and dichloroisoproterenol showed inhibitory effects on spontaneous as well as induced secretion by food, bethanechol, and histamine. Bass and Patterson (3) have shown that both adrenergic and adrenergic blocking agents (including phentolamine and tolazoline) possessed anti-secretory properties on rat gastric secretion. Hence, if gastric acid is a primary element in experimental ulcer, adrenergic and adrenergic blocking agents which have antisecretory effect should inhibit gastric ulceration. However, phentolamine, MJ 1999, and isoproterenol did not affect the incidence of histamine-induced ulcer in guinea pigs (6). Moreover, propranolol and phenoxybenzamine increased the incidence of ulceration in fasted rats immobilized for 8 hours (7). In this study, therefore, an attempt was made to investigate the effect of several adrenergic blocking agents on gastric secretion and stress-induced ulcer in rats and to clarify a part of the mechanism of gastric ulceration by stress.

METHODS

Gastric secretion studies: Male Donryu strain rats (200-230 g) were used in the following experiments. The animals were deprived of food for 24 hours in individual cages prior to the experiment but were allowed free access to water. Under ether anesthesia, the abdomen was incised and the junction between the pylorus and duodenum was ligated, as described by Shay et al. (8). Four hours later, the animals were killed by a blow on the head and the stomach was removed. Gastric juice was collected in a graduated test tube and the total acidity was determined by titration with 0.1 N NaOH using phenolphthalein as an indicator. The pH was determined with a glass electrode.

Stress ulcer studies: As described previously by Takagi and Okabe (9), animals (235-255 g) were placed in a stress cage and immersed into a water bath (23°C) for 7 hours to the level of xiphoid process. At the end of the stress, the animals were killed by a blow

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on the head, the stomach was removed, inflated with 1% formalin solution and placed into 1% formalin solution for 5 minutes. Then, the stomach was cut open along the greater curvature and examined grossly for lesions (mucosal ulcer and mucosal hemorrhage) in the glandular portion. The ulcer index was calculated as the sum of the length of each lesion in the stomach. Preventive ratio is calculated as follows,

$$\text{Preventive ratio (\%)} = \frac{\text{Ulcer index (control)} - \text{Ulcer index (drug)}}{\text{Ulcer index (control)}} \times 100$$

The mucosal hemorrhage which was confirmed by histological study, and was graded from 0 to 3 according to its number and severity (superficial intensity of hemorrhagic region) regardless of existence or absence of mucosal ulcer; 0, no hemorrhagic region in the mucosa, 1, one or two of hemorrhagic regions showing weak intensity of reddish color, 2, three to five of hemorrhagic regions showing moderate intensity of the color, 3, more than six of hemorrhagic regions showing severe intensity of the color. In the tables, the values following $\pm$ sign are the standard error of the mean. Level of significance was calculated using Student's *t*-test.

Drugs: The drugs used were: phentolamine (Regitine), tolazoline (Priscoline), dibenamine hydrochloride, ergotamine tartrate, yohimbine hydrochloride, propranolol hydrochloride, MJ 1999, and alprenolol hydrochloride (Aptin). Dibenamine hydrochloride, ergotamine tartrate, and yohimbine hydrochloride were suspended in 0.5% carboxymethyl-cellulose solution and propranolol hydrochloride, MJ 1999, and alprenolol hydrochloride were dissolved in 0.9% saline solution. All the agents were injected subcutaneously 30 minutes before stress or immediately after pylorus ligation. Control animals received the corresponding solvent.

RESULTS

Effects of alpha-adrenergic blocking agents on gastric secretion in pylorus-ligated rats (Table 1)

Phentolamine 10 mg/kg significantly reduced the secretory volume, total acid output

TABLE 1. Effects of alpha-adrenergic blocking agents on the gastric secretion in pylorus-ligated rats.

Treatment	Dose (mg/kg)	No. of animals	Secretory volume (ml)	pH	Total acid output ($\mu\text{Eq/hr}$)
			Mean $\pm$ S.E.		Mean $\pm$ S.E.
Control (0.9% saline)		15	6.1 $\pm$ 0.5	1.2	153.6 $\pm$ 18.1
Phentolamine	10	9	1.4 $\pm$ 0.1**	1.9	24.8 $\pm$ 2.1**
Tolazoline	2	10	4.8 $\pm$ 0.5	1.1	120.4 $\pm$ 16.4
	10	10	3.4 $\pm$ 0.4**	1.5	91.4 $\pm$ 5.6*
Control (0.5% CMC)		10	6.5 $\pm$ 0.4	1.0	158.7 $\pm$ 10.5
Dibenamine	50	10	3.5 $\pm$ 0.7**	1.4	79.4 $\pm$ 9.5**
Ergotamine	10	10	2.9 $\pm$ 0.2**	1.3	50.9 $\pm$ 6.4**
Yohimbine	1	10	5.3 $\pm$ 0.4	1.1	122.0 $\pm$ 10.1*
	5	10	3.6 $\pm$ 0.3**	1.1	79.1 $\pm$ 10.3**
	10	10	3.0 $\pm$ 0.3**	1.1	64.6 $\pm$ 7.5**

* $P < 0.05$, ** $P < 0.01$ compared with control group.

($P < 0.01$) and slightly raised the pH value. Tolazoline 2 and 10 mg/kg also reduced the volume and acid output but the reduction was significant only at 10 mg/kg of this agents. Dibenamine 50 mg/kg, ergotamine 10 mg/kg and yohimbine 5, 10 mg/kg also significantly reduced the volume and total acid output ($P < 0.01$). However, yohimbine 1 mg/kg slightly reduced the total acid output. These agents exerted no or little influence on the pH value.

Effects of beta-adrenergic blocking agents on the gastric secretion in pylorus-ligated rats (Table 2)

All of the three agents used apparently reduced the secretory volume but the significance of the reduction was recognized at 20, and 50 mg/kg of propranolol, at 50 mg/kg of MJ 1999, and at 40 mg/kg of alprenolol ($P < 0.01$). Alprenolol 40 mg/kg extensively reduced the secretory volume (control, 6.1 ml, alprenolol, 1.3 ml). Propranolol 20, 50 mg/kg and alprenolol 40 mg/kg significantly reduced the total acid output ($P < 0.01$) and MJ 1999 50 mg/kg also significantly reduced the acid output ($P < 0.05$). These agents exerted no or little influence on the pH value.

TABLE 2. Effects of beta-adrenergic blocking agents on the gastric secretion in pylorus-ligated rats.

Treatment	Dose (mg/kg)	No. of animals	Secretory volume (ml)	pH	Total acid output (μ Eq/hr)
			Mean $\pm$ S.E.		Mean $\pm$ S.E.
Control (0.9% saline)		15	6.1 $\pm$ 0.5	1.2	153.6 $\pm$ 18.1
MJ 1999	10	9	5.7 $\pm$ 0.2	1.1	148.2 $\pm$ 7.4
	50	10	3.6 $\pm$ 0.5**	1.3	86.4 $\pm$ 16.5*
Propranolol	20	10	4.0 $\pm$ 0.4**	1.1	98.4 $\pm$ 11.9**
	50	9	2.6 $\pm$ 0.6**	1.1	74.8 $\pm$ 18.1**
Alprenolol	10	9	4.3 $\pm$ 0.6	1.0	121.7 $\pm$ 12.8
	40	10	1.3 $\pm$ 0.1**	1.6	26.8 $\pm$ 3.3**

* $P < 0.05$, ** $P < 0.01$ compared with control group.

Effects of alpha-adrenergic blocking agents on the stress-induced gastric ulcer and the hemorrhage (Table 3)

TABLE 3. Effects of alpha-adrenergic blocking agents on the gastric ulceration and mucosal hemorrhage produced by water-immersing stress in rats.

Treatment	Dose (mg/kg)	No. of animals	Ulcer index (mean $\pm$ S.E.)	Preventive ratio (%)	Mucosal hemorrhage rating			
					0	1	2	3
Control (0.9% saline)		15	20.7 $\pm$ 2.5		0	5	4	6
Phentolamine	10	10	34.9 $\pm$ 2.3	-76.1**	10	0	0	0
Tolazoline	2	10	30.7 $\pm$ 2.7	-48.3**	2	4	4	0
	10	10	32.3 $\pm$ 1.9	-56.0**	5	5	0	0
Control (0.5% CMC)		20	18.5 $\pm$ 1.7		1	1	13	5
Dibenamine	50	10	15.0 $\pm$ 2.5	18.9	1	5	3	1
Ergotamine	10	10	22.4 $\pm$ 2.8	-21.1	0	0	5	5
Yohimbine	1	10	20.0 $\pm$ 1.7	-8.1	1	5	3	1
	5	10	14.1 $\pm$ 2.4	23.8	9	1	0	0
	10	10	7.0 $\pm$ 1.5	62.2**	10	0	0	0
	25	7*	4.7 $\pm$ 1.7	74.6**	7	0	0	0

* Three out of 10 animals died during the stress. ** $P < 0.01$ compared with control group.

Phentolamine 10 mg/kg and tolazoline 2, and 10 mg/kg significantly increased the ulcer index compared with control group ($P<0.01$). Ergotamine 10 mg/kg also increased the ulcer index but was not significant. Dibenamine 50 mg/kg slightly reduced the ulcer index. On the other hand, higher doses of yohimbine resulted in the marked reduction of the ulcer index. At 10, and 25 mg/kg of the agent the inhibition of ulceration was significant ($P<0.01$); the preventive ratio was 62.2% and 74.6%, respectively. However, of 10 animals receiving yohimbine (25 mg/kg) 3 died during the stress. Concerning the mucosal hemorrhage, phentolamine, tolazoline, dibenamine and yohimbine markedly prevented the appearance of the hemorrhage. However, ergotamine did not influence the hemorrhage at all.

Effects of beta-adrenergic blocking agents on the stress-induced gastric ulcer and the hemorrhage (Table 4)

Three agents used showed an apparent inhibition of gastric ulceration at the higher dose levels. MJ 1999, 10 mg/kg did not exert any influence on the stress-induced ulcer but at 50 mg/kg gastric ulcer was significantly inhibited ($P<0.01$); the preventive ratio was 78.7 %. Propranolol 20, 50 mg/kg significantly prevented the ulceration ($P<0.01$); the preventive ratio was 62.3% and 94.2%, respectively. Alprenolol, 10 mg/kg, slightly reduced the ulceration while at 40 mg/kg the reduction was significant ($P<0.01$); the preventive ratio was 64.3%. Concerning the hemorrhage, propranolol 20 and 50 mg/kg slightly reduced the appearance. However, alprenolol exerted very weak inhibitory effect on the hemorrhage. Although the effect of MJ 1999 on the hemorrhage was not determined qualitatively, the hemorrhage was inhibited in parallel to the inhibition of gastric ulceration.

TABLE 4. Effects of beta-adrenergic blocking agents on the gastric ulceration and mucosal hemorrhage produced by water-immersing stress in rats.

Treatment	Dose (mg/kg)	No. of animals	Ulcer index (mean $\pm$ S.E.)	Preventive ratio (%)	Mucosal hemorrhage rating			
					0	1	2	3
Control (0.9% saline)		15	20.7 $\pm$ 2.5		0	5	4	6
MJ 1999	10	10	20.9 $\pm$ 4.4	1.0				
	50	9*	4.4 $\pm$ 1.7	78.7**				
Propranolol	20	10	7.8 $\pm$ 2.2	62.3**	0	5	2	3
	50	10	1.2 $\pm$ 0.3	94.2**	6	3	0	1
Alprenolol	10	10	18.2 $\pm$ 2.6	12.1	0	2	2	6
	40	10	7.4 $\pm$ 1.6	64.3**	1	2	4	3

* One out of 10 animals died during the stress. ** $P<0.01$ compared with control group.

DISCUSSION

The present experiment confirms the antisecretory effect of several adrenergic blocking agents in rats previously described by Bass and Patterson (3). Therefore, if the stress-induced ulcer is caused by gastric acid as suggested by the prevention of ulceration with anticholinergic agents or vagotomy (9-12), these agents should inhibit the ulceration. As a matter of fact, yohimbine (alpha-blocker) and propranolol, MJ 1999, and alprenolol

(each beta-blocker) showed significant inhibition of the ulceration with increasing doses. Djahanguiri et al. (6) have reported that MJ 1999 10 mg/kg and phentolamine 2 mg/kg (i.p.) did not affect the incidence of histamine-induced ulcer in guinea pigs. However, it seemed that the dose of these agents used by Djahanguiri et al. was relatively low. In fact, it was found that MJ 1999 10 mg/kg (s.c.) did not also influence the gastric secretion and ulceration in this study. Dibenamine significantly suppressed the gastric secretion whereas the inhibition of the ulceration was weak. On the other hand, phentolamine and tolazoline significantly aggravated the stress ulceration in spite of the marked suppression of the gastric secretion. Although we did not perform the histological examination, it seemed that the aggravation was limited only within the increase of number and size of ulcerated region. In addition, ergotamine slightly aggravated the stress ulceration but the agent markedly suppressed the gastric secretion. Brodie and Valitski (13) have reported that multiple stress (cold+restraint), which is relatively similar to our method, markedly reduced both volume and gastric acidity in chronic fistula rats. From these data, it was considered that the gastric acid factor would not play a primary role at least on the stress-induced ulceration. Accordingly, it remains obscure whether the antiulcer effect of yohimbine and three beta-blockers is related to their antisecretory effect. Bass and Patterson (3) have suggested that the antisecretory properties of adrenergic agents (both stimulant and inhibitor) would be due to the increase of the biologic half-life of catecholamines. Brodie and Valitski (13) have found that adrenaline blocked the gastric hemorrhage induced by the multiple stress in rats. We also obtained an inhibitory effect of adrenaline and noradrenaline on stress-induced ulceration (unpublished data). However, phentolamine and tolazoline, which prolong the biologic half-life of catecholamines in blood and urine (14-16), aggravated the stress-induced ulceration. Thus it was considered that the effect of adrenergic blocking agents on the gastric ulceration did not relate to the biologic half-life of catecholamines. Concerning the mucosal ulceration and the mucosal hemorrhage, we have so far considered that these two phenomena were induced by the same cause since both lesions were blocked by anticholinergics and vagotomy. Guth and Hall (17) and Guth and Kozbur (18) also have shown that mucosal vascular engorgement occurred within one half hour of restraint, that is, prior to the development of mucosal ulceration and engorgement and ulceration were blocked by vagotomy. Therefore, they considered that vascular alteration was responsible for the vagal discharge and played an important role in the genesis of restraint-induced ulcer. In this experiment, yohimbine showed marked inhibition of the hemorrhage in parallel to the prevention of the ulceration, but dibenamine and three beta-blockers showed weak inhibitory effects on the hemorrhage compared with the significant prevention of the ulceration. However, phentolamine and tolazoline aggravated the ulceration and completely or almost completely inhibited the appearance of the hemorrhage. Thus it seems that the mucosal hemorrhage does not necessarily precede the stress ulceration.

SUMMARY

Effects of several alpha-, and beta-adrenergic receptor blocking agents on gastric secretion in pylorus-ligated rats and stress-induced gastric lesions were studied. Gastric lesions induced in the glandular portion of the stomach were divided into the mucosal ulcer and mucosal hemorrhage by gross observation. All adrenergic blocking agents significantly reduced the gastric secretion (both volume and total acid output). Contrary to the anti-secretory effect, phentolamine and tolazoline significantly aggravated the gastric ulceration, though these agents almost completely inhibited the appearance of the hemorrhage. Ergotamine slightly aggravated the ulceration but did not affect the hemorrhage at all. Dibenamine weakly inhibited both the ulceration and the hemorrhage. On the other hand, yohimbine markedly inhibited both the ulceration and the hemorrhage. Three beta-blockers (MJ 1999, propranolol, and alprenolol) significantly inhibited the ulceration, but weakly suppressed the hemorrhage. From these data, it was thought that gastric secretion would not play a primary role at least in the mechanism of stress ulceration in rats. Moreover, it is suggested that the mucosal hemorrhage does not causally relate to the development of the stress ulceration, that is, the hemorrhage is not a necessary condition for ulcer formation.

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