A comparative study of the gastric ulcerogenic effects of stress and reserpine in rats with decreased stomach wall mast cell populations

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Summary. Stress-induced gastric glandular ulcers in rats appeared less severe than those evoked by reserpine, although glandular mucosal mast cell counts were equally decreased. Prior depletion of the glandular mucosal mast cell population confirmed the hypothesis that an additional mechanism contributes to reserpine ulceration.

Histamine and possibly 5-hydroxytryptamine, both released by gastric mast cell degranulation, appear to be the main cause of stomach glandular ulceration evoked by stress[1–3] or reserpine[4–6] in rats. However, experimental evidence suggests that reserpine-induced gastric lesion formation may be due partly to other factors besides those attributable to the ulcerogenic mechanism involving mast cell degranulation[6]. The influence of stomach wall mast cell depletion on stress- and reserpine-induced gastric ulcers in rats was, therefore, examined.

Materials and methods. Female Sprague-Dawley rats (150–250 g) were housed in a room with controlled temperature (22±1 °C, room temperature) and humidity (65–70%) where all experiments, except those requiring stress, were carried out.

Depletion of the mast cells in the mucosal layer of the glandular wall of the stomach was effected by the method of Räsänen[7], with modifications. Dexamethasone acetate (Sigma) 0.4 mg/kg (expressed as the base) was injected i.p. twice daily and the 7th dose (4th day) was administered 24 h before the animals were stressed or injected with reserpine on the 5th day. Controls received a similar volume of vehicle (1 ml/kg), a solution of ethyl alcohol diluted v/v to 25% with NaCl 0.9% w/v (saline), by the same route. Food was withdrawn 48 h before starting the experiments, but the rats had free access to sucrose 8% in NaCl 0.2% w/v which was removed 1 h before gastric ulcer induction by stress or reserpine.

Animals to be stressed were put into close-fitting tubular restraint cages of wire mesh and exposed to 4 °C; controls were left in their starvation cages at room temperature. After 2 h, all were killed by a sharp blow on the head. In the case of reserpine ulcer induction, reserpine (Ciba) 5 mg/kg was injected i.p.; controls were given a similar volume of its vehicle (5 ml/kg) i.p. All were killed 4 h later.

Stomachs were removed after completion of the experiments, opened along the greater curvature and examined for mucosal lesions using an illuminated magnifier (×3). Lesions were measured (mm) and expressed as the mean ulcer index[8]. The number of metachromatically stained mast cells in 42 adjacent oil immersion fields (magnification × 1000) was counted in the various layers of

Table 1. The effects of dexamethasone pretreatment (0.4 mg/kg given twice daily; ×7 doses) on gastric mucosal ulceration and stomach wall mast cell counts in stressed rats

Pretreatment (i.p.)	Dose	No. of rats	Glandular ulcer index (mm)	Mast cell count/42 o.i.f. Glandular segment Mucosa	Submucosa	Muscle	Rumenal segment Submucosa	Muscle
A. No stress (unrestrained at room temperature for 2 h)								
Dexamethasone vehicle	1 ml/kg	12	0.16±0.06	75.2±3.1	48.3±4.2	5.9±1.6	57.5±4.7	5.8±1.8
Dexamethasone	0.4 mg/kg	12	0.20±0.09	3.6±1.0[a]	50.4±3.8	7.1±1.9	50.8±5.0	4.8±2.1
B. Stress (restrained at 4 °C for 2 h)								
Dexamethasone vehicle	1 ml/kg	12	5.88±0.74[c]	42.8±5.0[c]	44.2±3.5	6.9±2.0	56.8±5.0	6.8±2.2
Dexamethasone	0.4 mg/kg	9	0.83±0.29[a,b]	2.8±1.0[a]	51.9±5.1	10.7±2.7	57.2±3.6	5.7±2.1

The values shown are the means± SEM. [a]$p < 0.001$, compared with its own control pretreated with dexamethasone vehicle. [b]$p < 0.05$; [c]$p < 0.001$, compared with the corresponding non-stressed group (A). o.i.f. = oil immersion field (× 1000).

Table 2. The effects of dexamethasone pretreatment (0.4 mg/kg given twice daily; ×7 doses) on gastric mucosal ulceration and stomach wall mast cell counts in reserpine-injected rats

Pretreatment (i.p.)	Dose	No. of rats	Glandular ulcer index (mm)	Mast cell count/42 o.i.f. Glandular segment Mucosa	Submucosa	Muscle	Rumenal segment Submucosa	Muscle
A. Reserpine vehicle 5 ml/kg i.p. (4 h effect)								
Dexamethasone vehicle	1 ml/kg	10	0.05±0.03	87.9±10.9	53.1±3.0	9.7±3.0	55.8±3.7	4.0±1.0
Dexamethasone	0.4 mg/kg	10	0.12±0.06	3.6±1.9[a]	52.9±4.6	6.8±2.6	50.1±4.5	4.9±1.6
B. Reserpine 5 mg/kg i.p. (4 h effect)								
Dexamethasone vehicle	1 ml/kg	10	7.25±0.85[c]	44.8±6.8[b]	55.8±3.8	11.8±2.4	58.3±4.4	6.3±1.8
Dexamethasone	0.4 mg/kg	10	2.10±0.21[a,c,d]	2.4±0.5[a]	54.2±4.6	10.7±2.0	53.8±3.2	7.4±2.0

The values shown are the means± SEM. [a]$p < 0.001$, compared with its own control pretreated with dexamethasone vehicle. [b]$p < 0.01$; [c]$p < 0.001$, compared with the corresponding reserpine vehicle-injected group (A). [d]$p < 0.01$, compared with the stressed group pretreated with dexamethasone (table 1, B). o.i.f. = oil immersion field (× 1000).

the glandular and rumenal segments of the stomach wall; rumenal mucosal counts were omitted because of the inconsistent presence of a few cells in this region[8–10]. Data were analyzed using Student's t-test.

Results. Nonstressed (table 1, A) or reserpine vehicle-injected (table 2, A) groups pretreated with either dexamethasone vehicle or dexamethasone showed low ulcer indices due to occasional petechiae found only in the gastric glandular mucosa. Dexamethasone pretreatment markedly depleted the mast cell population only in the mucosal layer of the stomach glandular wall of both nonstressed and reserpine vehicle-injected rats; the magnitude of dexamethasone-induced mast cell depletion was statistically comparable in the 2 groups.

Stress (table 1, B) or reserpine (table 2, B) produced a high ulcer index in dexamethasone vehicle-treated animals; the lesions in either group presented as grossly hemorrhagic ulcers confined to the glandular mucosa. Dexamethasone pretreatment effectively reduced both types of ulceration, but the mean ulcer index after stress (0.83 mm) was significantly smaller than that following reserpine (2.10 mm). Residual stress lesions were mainly petechiae, whereas those after reserpine were largely hemorrhagic ulcers. In the controls pretreated with dexamethasone vehicle, stress or reserpine significantly decreased the mast cell count only in the glandular mucosal layer of the stomach. Neither ulcer-producing method lowered dexamethasone-reduced mucosal mast cell counts much further.

Discussion. The etiology of stress- or reserpine-induced gastric glandular ulcer formation, primarily related to vagal overactivity, which mainly provokes mast cell degranulation in the mucosal layer of the stomach glandular wall to release ulcerogenic agents, has been discussed elsewhere[3,5,6,8–13].

The present comparative study revealed that, although both ulcer-inducing methods produced statistically similar reductions in mast cell counts in the glandular mucosal layer (stress, 43%; reserpine, 49%), glandular ulceration by reserpine was greater. A difference was also seen in the dexamethasone-pretreated rats, where the glandular mucosal mast cell population was almost completely depleted before ulcer induction. The significantly larger residual ulceration after reserpine appears, therefore, not to result from stomach mast cell degranulation. Since 5-hydroxytryptamine receptor blockade with methysergide in rats prevents reserpine-evoked gastric ulcers more strongly than those induced by stress (unpublished findings), it is possible that the residual lesions are chiefly produced by a nonvagal-mediated action of reserpine which releases 5-hydroxytryptamine directly from stomach storage sites[14,15].

These results indicate that reserpine, in contrast to stress, activates an additional mechanism which plays a small but significant role in its gastric ulcerogenicity in rats.

1 T. Räsänen, in: Peptic Ulcer, p.237. Ed. C.J. Pfeiffer. Munksgaard, Copenhagen 1971.
2 J.C. Schwartz, in: Peptic Ulcer, p.190. Ed. C.J. Pfeiffer. Munksgaard, Copenhagen 1971.
3 C.W. Ogle and C.H. Cho, Pharmac. Res. Commun. *9*, 679 (1977).
4 T. Räsänen and E. Taskinen, Acta physiol. scand. *68*, 360 (1966).
5 C.W. Ogle and C.H. Cho, IRCS Med. Sci. *5*, 119 (1977).
6 C.W. Ogle and C.H. Cho, Pharmac. Res. Commun. *10*, 325 (1978).
7 T. Räsänen, Acta path. microbiol. scand. suppl. *154*, 201 (1962).
8 C.H. Cho and C.W. Ogle, Eur. J. Pharmac. *48*, 97 (1978).
9 C.W. Ogle and C.H. Cho, IRCS Med. Sci. *7*, 152 (1979).
10 H.K. Lau and C.W. Ogle, Pharmac. Res. Commun. *11*, 253 (1979).
11 C.H. Cho and C.W. Ogle, Experientia *34*, 1294 (1978).
12 C.H. Cho and C.W. Ogle, Eur. J. Pharmac. *55*, 23 (1979).
13 C.W. Ogle and C.H. Cho, IRCS Med. Sci. *5*, 536 (1977).
14 K.S. Kim and P.A. Shore, J. Pharmac. exp. Ther. *141*, 321 (1963).
15 P.A. Shore, Fedn Proc. *24*, 1322 (1965).

A selective concentration-dependent dysrhythmogenic and antidysrhythmic action of prostaglandins E_2, $F_{2\alpha}$ and I_2 (prostacyclin) on isolated rat hearts

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Summary. Prostaglandins (PGs) E_2, $F_{2\alpha}$ and I_2 were examined for their effects on the electrical and mechanical activities of isolated rat, rabbit and guinea-pig hearts. All PGs produced dysrhythmias in rat hearts at low concentrations only, while higher concentrations were antiarrhythmic. Guinea-pig hearts were less responsive while rabbit hearts were completely resistant.

PG release from myocardium, particularly that subjected to hypoxic stress, has been demonstrated by various workers[2,3]. In man the primary PGs released are I_2, E_2 and $F_{2\alpha}$[4]. Although these substances are known to influence various parameters of cardiac activity there is substantial uncertainty regarding their exact function.

PGs have been described as endogenous antiarrhythmic factors released by the hypoxic or ischemic myocardium[5]. They have been demonstrated to have antiarrhythmic properties in a variety of experimental situations[5–8]. Some protection against ventricular arrhythmias has been demonstrated with $PGF_{2\alpha}$ in man[8]. It has however been suggested that PGs released by the myocardium may be contributing factors for arrhythmogenesis[9] and some PGs have been shown to cause rhythm disturbances in clinical and experimental situations[10–12]. We therefore examined the effects of PGE_2, $PGF_{2\alpha}$ and PGI_2 on the electrocardiogram (EKG) of isolated rat, rabbit and guinea-pig hearts and report that these PGs cause EKG disturbances in rat hearts at low concentrations while demonstrating antiarrhythmic properties at high levels.

Methods. Male Sprague-Dawley rats (average weight 250 g), albino guinea-pigs (500 g) and New Zealand rabbits (2.5 kg) were utilized in this investigation. The animals were sacrificed either by decapitation or cervical dislocation (rabbits) and their hearts were rapidly excised and placed in ice-cold buffer until contractions ceased. They were then mounted by the aorta and perfused at a constant