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The influence of peripheral or central administration of ondansetron on stress-induced gastric ulceration in rats

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Abstract. Ondansetron (0.08, 0.15 or 0.3 mg/kg) injected s.c., every 12 h with the fourth dose given 0.5 h before experiments, dose-dependently lessened gastric glandular mucosal ulceration produced by cold-restraint stress for 2 h. When given intracerebrally (i.c.) (0.1, 0.5 or 1 μg), using the same treatment regimen, infusion of ondansetron 1 μg into the nucleus amygdaloideus centralis decreased stress-evoked ulcers; in contrast, injection of the same dose into the nucleus accumbens intensified these lesions. The associated stress-induced stomach wall mast cell degranulation was unaffected by all s.c. or i.c. doses of ondansetron. Pretreatment with disodium cromoglycate i.p. alone, or concurrently with ondansetron s.c., prevented not only ulceration but also mast cell degranulation. 5-Hydroxytryptamine$_3$ receptor antagonism appears to inhibit stress-evoked ulcers mainly by blocking the peripheral effects of the amine after its release from the gastric mucosal mast cells.
Key words. Ondansetron; 5-hydroxytryptamine$_3$ receptors; cold-restraint stress; mucosal ulcers; mast cells; rat stomachs.

Ondansetron is a selective 5-hydroxytryptamine$_3$ (5-HT$_3$) receptor antagonist[1,2]. Drugs with this property appear to be highly effective against nausea and vomiting produced by cytotoxic agents and radiotherapy[2]; they are thought to act by blocking the 5-HT$_3$ receptors found centrally in the chemoreceptor trigger zone and peripherally on the vagus nerve endings which form the afferent pathway of the vomiting reflex. Evidence suggests that anticancer agents release 5-HT from enterochromaffin cells in the gastrointestinal mucosa; the amine then activates 5-HT$_3$ receptors on the vagal afferent nerve endings to stimulate the vomiting centre[1,2]. Patients suffering from cancer are usually under stress, which itself releases 5-HT from mast cell degranulation[3]; the amine has also been shown to possess mucosal ulcerogenic properties[4]. This study examines the central and peripheral actions of ondansetron on stress-induced gastric mucosal damage, and attempts to throw more light on the mechanism of the antiulcer action of the drug.

Materials and methods

Female Sprague-Dawley rats, weighing 160–180 g, were housed in an air-conditioned room with constant temperature (22 ± 1 °C) and humidity (65–70%). They were fed a standard pellet (Ralston Purina Company) diet and drank tap water. Solid food was withdrawn 24 h before experiments, but free access to a solution of 8% sucrose in 0.2% NaCl w/v was permitted; this drinking solution was removed 1 h before gastric ulcer induction by cold and restraint[5]. Ethical approval had previously been obtained for cold-restraint stress experiments and the stereotaxic surgical method.

Stereotaxic surgery. Rats were anaesthetised with chloral hydrate (100 mg/kg s.c.) and sodium pentobarbitone (60 mg/kg i.p.). They were placed in a stereotaxic frame (SR-5, Narishige) and kept warm with a heating lamp. An established and widely-accepted stereotaxic technique[6] was used to implant (chronically) bilateral guide cannulae, consisting of stainless steel tubing (0.65 mm diameter) in perspex holders, in the brains of the animals. Coordinates were selected for drug delivery to the centre of the nucleus amygdaloideus centralis (ACE) (anterior: 5.8, vertical: +3.1, lateral: ±4.5)[7] and the nucleus accumbens (ACB) (anterior: 9.6, vertical: 3.5, lateral: ±1.6)[7]. The guide cannulae were fixed to the skull using retaining screws and acrylic cement, and kept patent by inserting stainless steel stylets (0.3 mm diameter) which extended 0.5 mm beyond the guide tips. The animals did not show any signs of discomfort or loss of normal activity during the 10-day observation period following stereotaxic surgery.

Intracerebral injections. The rats were gently restrained manually during the drug delivery procedure. Guide cannulae stylets were first removed and blunt stainless steel injection needles (0.3 mm diameter), connected to Agla micrometer syringes containing the drug solutions, inserted bilaterally into the cannulae. The needle ends extended 5.0 mm and 3.5 mm below the guide cannuale tips for injections into the ACE and ACB, respectively.

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Drugs or their vehicle were administered bilaterally in volumes of 1 µl over a 10 s period. The injection needles were left in place for a further 50 s after drug delivery, before being removed and replaced immediately by the stylets. Animals were used 10 days after surgery and on one occasion only.

Gastric ulcer production. The rats were restrained individually in close-fitting tubular wire-mesh cages and exposed to 4 °C for 2 h (stress)[5,8]. Those acting as controls were returned to their starvation cages at room temperature (22 °C). All animals were killed by a sharp blow to the head at the end of 2 h; their stomachs were immediately removed and examined for glandular mucosal lesions[8]. Following histological processing, the number of metachromatically stained (by toluidine blue) mast cells in the upper one third of the glandular mucosal layer was counted in 42 oil immersion fields[8].

Examination of brain tissue. The brains were collected after stomach removal for macroscopic and histological examination, and sections containing the ACE and ACB were preserved in buffered formalin solution. Macroscopic examination was later carried out after slicing the sections with a freezing microtome. The tracts of the guide cannulae and the locations of the injection sites were identified. Data from rats in which the cannulae ends were sited outside the ACE or ACB were excluded from the study.

Drugs used. Ondansetron (Glaxo) or disodium cromoglycate (Fisons) was freshly dissolved in a solution of 0.9% NaCl in distilled water (saline) before use. The drugs were injected every 12 h (beginning 8 days after operation), with the fourth dose administered 0.5 h before starting experiments. When given s.c. into the inner side of the thigh, this regimen with ondansetron (in doses ranging from 0.03–0.3 mg/kg) has previously been shown to provide reliable protection against stress-evoked gastric glandular mucosal ulceration[3]. Disodium cromoglycate was administered i.p.

Statistical analysis. The data of all experiments were examined statistically using the two-tailed unpaired Student *t*-test. Differences between groups were analysed by ANOVA.

Results

Nonstressed rats given vehicle or the largest dose of ondansetron or disodium cromoglycate showed comparable gastric glandular ulcer indices due to occasional petechiae, and normal mast cell counts in the glandular mucosal layer of the stomach (tables 1A and 3A). Stress for 2 h produced severe haemorrhagic lulcers in the glandular mucosa and reduced the mast cell counts in the glandular mucosal layer of the saline-treated control animals (tables 1B, 2A, 3B). Ondansetron dose-dependently [ANOVA, $F(3, 28) = 3.62$; $p < 0.05$] antagonised stress-evoked ulceration (table 1B, all $p < 0.05$), but did not influence the intensity of mast cell degranulation. Ondansetron 1 µg when injected s.c. was unable to antagonise stress-induced gastric ulceration in nonoperated rats (table 2A). Surgical implantation of guide cannulae itself did not affect stress-induced gastric ulcer formation or mast cell degranulation (table 2B, C). Injection of ondansetron 1 µg into the ACE significantly decreased (table 2B, $p < 0.05$), but administration into the ACB significantly intensified (table 2C, $p < 0.05$), stress-evoked ulcer formation (see also fig. 1) none of the doses of i.c.-injected ondansetron influenced mast cell degranulation (table 2B, C). As seen earlier, ondansetron (s.c.) alone decreased stress-evoked ulcers (table 3B, $p < 0.05$) but not mast cell degranulation. Disodium cromoglycate injected i.p. not only reduced gastric ulcer

Table 1. Effects of ondansetron (s.c. injection) on gastric ulceration and mast cell degranulation in stressed rats.

Treatment		Ulcer index (mm)	Glandular mucosal mast cell count/ 42 o.i.f.
A Unrestrained at room temperature			
Saline	2 ml/kg	0.05 ± 0.03	65 ± 8.9
Ondansetron	0.3 mg/kg	0.08 ± 0.05	63 ± 7.5
B Restrained at 4 °C for 2 h			
Saline	2 ml/kg	6.05 ± 0.74**	39 ± 4.7*
Ondansetron	0.08 mg/kg	3.83 ± 0.72**+	42 ± 4.8*
Ondansetron	0.15 mg/kg	3.50 ± 0.58**+	37 ± 4.3*
Ondansetron	0.30 mg/kg	3.40 ± 0.57**+	38 ± 5.9*

Values are the means ± SEM of 8 rats in each group. o.i.f., oil immersion fields (1000×). *$p < 0.05$, **$p < 0.01$ when compared to its corresponding nonstressed control in A. +$p < 0.05$ when compared to its own vehicle-injected control in B.

Table 2. Effects of ondansetron (i.c. injection) on gastric ulceration and mast cell degranulation in stressed rats.

Treatment		Number of rats	Ulcer index (mm)	Glandular mucosal mast cell count/ 42 o.i.f.
A Subcutaneous injection				
Saline	1 µl	8	6.05 ± 0.82	40 ± 6.3
Ondansetron	1 µg	8	5.83 ± 0.59	42 ± 5.2
B Intracerebral injection: nucleus amygdaloideus centralis				
Saline	1 µl	9	5.76 ± 0.90	41 ± 3.3
Ondansetron	0.1 µg	9	5.89 ± 0.89	38 ± 4.4
Ondansetron	0.5 µg	7	5.94 ± 1.24	43 ± 5.9
Ondansetron	1 µg	7	2.73 ± 0.95+	39 ± 1.3
C Intracerebral injection: nucleus accumbens				
Saline	1 µg	6	5.63 ± 0.58	40 ± 5.3
Ondansetron	0.1 µg	7	5.59 ± 0.74	42 ± 60
Ondansetron	0.5 µg	7	6.06 ± 0.76	41 ± 6.7
Ondansetron	1 µg	6	8.70 ± 1.09+	38 ± 6.2

Values are the means ± SEM. o.i.f., oil immersion fields (1000×). +$p < 0.05$ when compared to it own vehicle-injected control.

Table 3. Effects of ondansetron (s.c. injection) and disodium cromoglycate (i.p. injection) on gastric ulceration and mast cell degranulation in stressed rats.

Treatment		Number of rats	Ulcer index (mm)	Glandular mucosal mast cell count/ 42 o.i.f.
A Unrestrained at room temperature				
Saline	2 ml/kg	10	0.10 ± 0.05	64 ± 5.7
ON	0.3 mg/kg	9	0.11 ± 0.05	70 ± 7.9
DC	5 mg/kg	9	0.09 ± 0.07	68 ± 7.1
ON 0.3+DC	5 mg/kg	9	0.07 ± 0.05	67 ± 6.5
B Restrained at 4 °C for 2 h				
Saline	2 ml/kg	9	6.12 ± 0.94**	46 ± 3.8*
ON	0.3 mg/kg	8	3.55 ± 0.65**+	43 ± 4.6*
DC	5 mg/kg	9	3.21 ± 0.50**++	64 ± 5.8++
ON 0.3 + DC	5 mg/kg	8	3.25×0.62**+	65 ± 5.9++

Values are the means ± SEM. o.i.f., oil immersion fields (1000×). *$p < 0.05$, **$p < 0.01$ when compared to its corresponding non-stressed control in A. +$p < 0.05$, ++$p < 0.02$ when compared to its own vehicle-injected control in B. ON = ondansetron, DC = disodium cromoglycate.

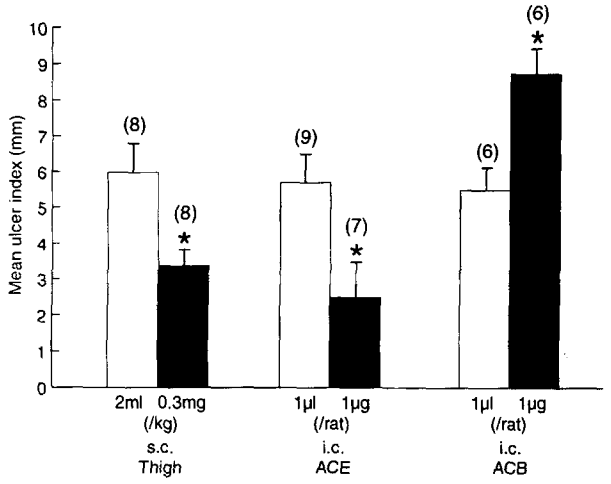

Figure 1. Comparison between the effects of s.c.- and i.c.-injected ondansetron on gastric ulceration in cold-restraint stressed rats. Values are the means ± SEM. Number of rats in each group in parentheses. □ = saline; ■ = ondansetron; s.c. = subcutaneous injection; i.c. = intracerebral injection; ACE = nucleus amygdaloideus centralis; ACB = nucleus accumbens. *$p < 0.05$ when compared to its own vehicle-injected control.

size (table 3B, $p < 0.02$) but also prevented mucosal mast cell degranulation (table 3B, $p < 0.02$) in stressed rats. Concurrent administration of these two drugs significantly antagonised lesion formation (table 3B, $p < 0.05$) but the intensity of reduction was not greater than that achieved by either drug alone; marked prevention of mast cell degranulation ($p < 0.02$) was also seen.

Discussion

Changes in the metabolism of 5-HT[9] or in serotoninergic activation[10] occur in stress, and when injected into the CNS, 5-HT can influence gastric function[11]. Psychological stress has been shown to increase 5-HT metabolism in the ACE or ACB[12]. The ACE influences the stomach through the vagus and plays an important role in stress-induced gastric ulcer formation[13,14]. 5-HT_3 blockers antagonise the stimulation of dopamine (DA) release in the ACB[15] which protects against stress-evoked gastric ulcers[16]; inhibition of the DA pathway thus results in enhanced ulceration. The findings with ondansetron infusion in the ACB support this possibility. It was seen that ondansetron administration in the ACE or ACB did not affect gastric mucosal mast cell degranulation which is caused by a vagal-mediated cholinergic pathway[17]. The present findings suggest that 5-HT_3 receptors in the ACE and ACB have different actions on stress-induced gastric ulceration, and that neither effect involves the cholinergic pathway. As 5-HT plays a role in cold stress-induced gastric lesions, probably by potentiating the ulcerogenic activity of thyrotropin-releasing hormone[18], blocking 5-HT_3 activity in the ACE could therefore diminish lesion formation. These interesting findings on the possible ulcerogenic effects of 5-HT_3 receptors in the ACE need further study.

In the present experiments, peripheral administration of ondansetron (0.08–0.30 mg/kg) significantly reduced stress ulcer formation. This accords with the idea that stress releases 5-HT, and that 5-HT_3 receptors in the stomach account for part of the ulcerogenic action[19]. The conflicting results seen with infusion of ondansetron into the ACE and ACB suggest that its gastric effects do not reflect a CNS pathway involving a cholinergic mechanism because there was no lessening (in the presence of ulcer protection: ACE) or worsening (in the presence of ulcer aggravation: ACB) of mast cell degranulation by stress. The highest i.c. dose of 1 µg which showed gastric effects when infused into the ACE or ACB did not influence stress-evoked ulceration when given s.c.; this implies that the ulcer protection seen with ACE infusion is unlikely to be due to a peripheral action, which only much bigger doses of ondansetron exhibited. It is, therefore, possible that ondansetron could be acting on the peripheral receptors, assuming that the mechanism of its antagonistic actions is similar to that which produces its established antiemetic[2,20] and anxiolytic[21] properties (fig. 2).

Stress-induced gastric mucosal mast cell degranulation releases ulcerogenic substances including 5-HT[4,17] (see also fig. 2). Disodium cromoglycate, which prevents mast cell degranulation, antagonises stress-induced gastric ulcer formation[22]. In the current study, disodium cromoglycate pretreatment did not further reduce ulcer size even when given together with ondansetron. This suggests the gastric 5-HT release by stress originates mostly from mast cell degranulation, although the amine could also come from other sources[1,2]. It is

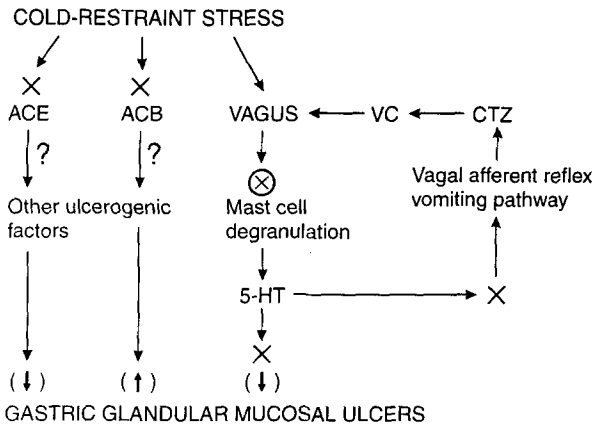

Figure 2. An outline of the possible sites of the antiulcer action of ondansetron in cold-restraint stressed rats.
X = blockade by ondansetron; ⊗ = blockade by disodium cromoglycate; ACE = nucleus amygdaloideus centralis; ACB = nucleus accumbens; CTZ = chemoreceptor trigger zone; VC = vomiting centre.

unlikely that ondansetron owes a significant antiulcer action to blockade of the 5-HT_3 receptors initiating the vagal afferent reflex of the vomiting pathway because cold-restraint stress-evoked gastric mucosal damage is mainly the consequence of centrally-originating impulses transmitted along the vagus nerve[17]. However, increased gastric 5-HT release could activate this afferent reflex pathway; 5-HT_3 receptor antagonism at this site would, therefore, partly contribute to the observed antiulcer action (fig. 2).

To summarise: the present findings suggest that 5-HT_3 receptor block in two different areas of the brain in stressed rats produces dissimilar gastric effects, but more work is required before a clearer picture emerges. In contrast, s.c. administration of effective doses of ondansetron consistently prevents stress-evoked ulceration. It is likely that 5-HT_3 receptors at a peripheral post-vagal site are mainly involved in stress-induced gastric glandular mucosal damage, and that the 5-HT causing the ulcerogenic effect originates chiefly from mucosal mast cell degranulation. Thus, a reasonable conclusion is that ondansetron exerts its antiulcer action mainly through 5-HT_3 receptor antagonism in the stomach.

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