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Luminal acid in stress ulceration and the antiulcer action of verapamil in rat stomachs

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The role of luminal acid and the influenee of the antisecretory action of verapamil in stress ulcer prevention in rat stomachs have been studied. Intraperitoneally injected verapamil, 4 mg kg^{-1}, inhibited gastric acid secretion and ulcer formation, however, a 2 mg kg^{-1} dose, which did not significantly influence acid output, also had an antiulcer effect. Intraperitoneal injection of bethanechol, 1·2 or 3·6 mg kg^{-1}, increased gastric acid output, but did not influence stress-induced ulcer formation. Oral administration of HCl, 25 or 50 μequiv, aggravated stress ulceration in a dose-dependent manner; this lesion-worsening effect was prevented by pretreatment with verapamil or bethanechol. The gastric luminal acid content in 2 h pylorus-ligated rats was similar in the groups given either bethanechol or HCl. These findings indicate that the antisecretory action of verapamil may not account for its antiulcer effect. It is suggested that endogenous and exogenous luminal acid may have different influences on stress ulcer formation.

Verapamil, a calcium channel blocker, has been shown to prevent stress ulceration (Ogle et al 1985a, b). Its antiulcer effects are thought to be due to inhibition of mast cell degranulation (Ogle et al 1985a) and of gastric motility (Ogle et al 1985b). However, the relationship of its antisecretory action to stress ulcer prevention remains undefined. Although the presence of acid seems to be a prerequisite for ulcer formation (Mersereau & Hinchey 1973; Ritchie 1975), there are reports indicating that gastric acid may play only a minor role in glandular mucosal lesion formation in stressed rats (Takagi & Okabe 1970; Cho & Ogle 1979). The present study examines the effects of luminal acid, elevated either by bethanechol injection or by oral administration of HCl, on stress-induced gastric ulceration. The possible role of the antisecretory action of verapamil in antagonizing stress-induced lesion formation in rat stomachs has also been evaluated.

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Methods

Female Sprague-Dawley rats (170–200 g) were reared on a balanced laboratory diet (Ralston Purina Co.) and given ordinary tap water to drink. They were housed in a room with controlled temperature (22 ± 1 °C) and humidity (65–70%). Animals were deprived of food for 48 h before use, but allowed free access to a solution of 8% w/v sucrose in 0·2% w/v NaCl (Cho & Ogle 1979) which was removed 1 h before experimentation.

Rats were prepared for pyloric occlusion by the method of Dai & Ogle (1972), 7 days before experimentation. Thirty minutes before pylorus occlusion, the animals were injected i.p. with 0·9% w/v NaCl solution (saline), 2 ml kg^{-1}, or a similar volume of verapamil HCl (Knoll), 2 or 4 mg kg^{-1}, or of bethanechol chloride (MSD Labs), 1·2 or 3·6 mg kg^{-1}; the weights of the drugs are expressed as their salts. The pylorus was then occluded by gently pulling the two externally exposed sections of wire at the flanks. Separate groups of i.p. pretreated animals were also given 0·5 ml of either distilled water or HCl, 25 or 50 μequiv, orally (p.o.) by a stainless steel intragastric tube immediately after pylorus occlusion. The rats were then returned to their cages and were killed by a sharp blow on the head at the end of a 2 h observation period. Stomachs were removed and their contents collected for measurement of volume of secretion and total titratable acid. The gastric mucosae were then examined for ulcers.

Groups of rats were injected i.p. with saline, 2 ml kg^{-1}, or with the same doses of verapamil or bethanechol used in the pylorus occlusion experiments. These groups were given 0·5 ml of either distilled water or HCl, or 25 or 50 μequiv, p.o. by a stainless steel intragastric tube 30 min later and then subjected to cold-restraint stress for 2 h. Ulcer severity in the gastric glandular segment was measured at the end of the stress period.

Acid determination and ulcer index measurement. The total gastric acid was determined by titration with 0·01 M NaOH to pH 7·4, using an autotitration system (Radiometer Model TTT 80). Ulcer severity was assessed, after opening each stomach along the greater curvature, by measuring (mm) each lesion along its greatest length; five petechiae were taken as equivalent to a 1 mm ulcer. The sum of the lesions in each group was divided by its number of animals and expressed as the ulcer index (Cho & Ogle 1978a).

Statistical analysis. The data were expressed as means ± s.e.m. and analysed by the Student two-tailed *t*-test.

Results

Effects of verapamil, bethanechol or HCl on gastric acid secretion in pylorus-occluded rats. Under on-stress conditions, the small dose of i.p. administered verapamil did not significantly affect gastric secretion (Table 1); however, the higher dose, 4 mg kg^{-1}, markedly inhibited both volume of secretion and acid output in the pylorus-occluded rats. Intraperitoneal injection of bethanechol, 1·2 or 3·6 mg kg^{-1}, dose-dependently increased gastric secretion. The doses of both drugs did not significantly change the ulcer index, when compared with the non-stressed controls injected with saline. Oral administration of either 25 or 50 μequiv HCl markedly elevated the total titratable acid, but the ulcer indices of both groups did not differ significantly from that of the distilled water-treated control.

Effects of verapamil or bethanechol on the potentiating effect of exogenous HCl on stress ulceration. Intraperitoneally injected verapamil or bethanechol, or p.o. instilled HCl given alone or after drug pretreament, did not significantly affect the gastric ulcer index in non-stressed animals, when compared with the saline-pretreated and distilled water-treated controls (Table 2A). Stress-induced haemorrhagic gastric glandular ulceration was markedly aggravated by HCl, 25 or 50 μequiv, given p.o. immediately beforehand (Table 2B). Both dose levels of verapamil antagonized the potentiating effect of HCl on stress ulcer formation, as did pretreatment with bethanechol 1·2 or 3·6 mg kg^{-1}.

Discussion

The inhibitory action of verapamil on gastric acid secretion and on stress-induced ulceration (Ogle et al 1985a, b) is confirmed by the present study which also throws more light on the pathogenesis of stress ulceration and the relationship between the two inhibitory effects of the drug. It is known that cold-retraint stress depresses the volume and acidity of gastric secretion, as shown in pylorus-occluded rats (Cho et al 1976b; Dai & Ogle 1974); this could be explained either by the effect of ischaemia and hypothermia due to restraint at 4 °C

Table 1. Effects of verapamil, bethanechol (given i.p. 30 min before hand) or HCl (given 0·5 ml p.o. at 0 h) pretreatment on gastric secretion in pylorus-occluded rats (unrestrained at 22 °C for 2 h).

Pretreatment	No. of rats	Volume of secretion (ml/100 g body weight h^{-1})	Total titratable acid (μequiv HCl/100 g body weight h^{-1})	Ulcer index (mm)
Saline (i.p.) 2 ml kg^{-1}	10	0·83 ± 0·07	44·36 ± 3·79	0·04 ± 0·02
H_2O (p.o.) 0·5 ml	10	0·86 ± 0·12	37·45 ± 5·88	0·12 ± 0·08
Verapamil (i.p.) 2·0 mg kg^{-1}	12	0·75 ± 0·12	37·82 ± 3·05	0·03 ± 0·02
Verapamil (i.p.) 4·0 mg kg^{-1}	12	0·46 ± 0·08††	25·32 ± 2·17††	0·02 ± 0·01
Bethanechol (i.p.) 1·2 mg kg^{-1}	12	1·12 ± 0·05††	62·24 ± 4·18††	0·06 ± 0·04
Bethanechol (i.p.) 3·6 mg kg^{-1}	10	2·64 ± 0·15†††	132·83 ± 6·15†††	0·04 ± 0·04
HCl (p.o.) 25 μequiv	10	1·26 ± 0·13	78·54 ± 15·23†	0·32 ± 0·10
HCl (p.o.) 50 μequiv	10	1·16 ± 0·13	118·51 ± 17·63†††	0·25 ± 0·12

Values are means ± s.e.m.
†$P < 0·05$, ††$P < 0·01$, †††$P < 0·001$ when compared with its own saline-pretreated group.

Table 2. Effects of verapamil or bethanechol (given i.p. 30 min beforehand) pretreatment on HCl (given p.o. at 0 h)-provoked aggravation of 2 h stress ulceration in rats.

Pretreatment (mg kg^{-1})	No. of rats	Teatment (p.o., 0·5 ml, μequiv)	Ulcer index (mm)
A. Non-stressed (unrestrained at room temperature for 2 h)			
Saline 2 ml kg^{-1}	10	H_2O	0·02 ± 0·01
Saline 2 ml kg^{-1}	12	HCl 25	0·06 ± 0·03
Saline 2 ml kg^{-1}	12	HCl 50	0·55 ± 0·50
Verapamil 2·0	10	H_2O	0·01 ± 0·01
Verapamil 2·0	12	HCl 25	0·02 ± 0·01
Verapamil 2·0	12	HCl 50	0·05 ± 0·03
Verapamil 4·0	10	H_2O	0·22 ± 0·10
Verapamil 4·0	12	HCl 25	0·10 ± 0·09
Verapamil 4·0	12	HCl 50	0·08 ± 0·05
Bethanechol 1·2	10	H_2O	0·04 ± 0·02
Bethanechol 1·2	12	HCl 25	0·08 ± 0·04
Bethanechol 1·2	12	HCl 50	0·06 ± 0·03
Bethanechol 3·6	10	H_2O	0·01 ± 0·01
Bethanechol 3·6	12	HCl 25	0·03 ± 0·01
Bethanechol 3·6	12	HCl 50	0·05 ± 0·03
B. Stressed (restrained at 4 °C for 2 h)			
Saline 2 ml kg^{-1}	10	H_2O	5·53 ± 0·81*
Saline 2 ml kg^{-1}	12	HCl 25	10·26 ± 3·53*
Saline 2 ml kg^{-1}	12	HCl 50	18·60 ± 1·47*
Verapamil 2·0	10	H_2O	3·27 ± 0·55*†
Verapamil 2·0	12	HCl 25	3·64 ± 0·90*
Verapamil 2·0	12	HCl 50	9·35 ± 2·80*††
Verapamil 4·0	10	H_2O	1·72 ± 0·52*†††
Verapamil 4·0	12	HCl 25	2·99 ± 1·04*
Verapamil 4·0	12	HCl 50	5·93 ± 1·69*†††
Bethanechol 1·2	10	H_2O	5·65 ± 1·17*
Bethanechol 1·2	12	HCl 25	6·38 ± 1·73*
Bethanechol 1·2	12	HCl 50	9·87 ± 2·15*††
Bethanechol 3·6	10	H_2O	5·92 ± 1·54*
Bethanechol 3·6	12	HCl 25	6·25 ± 0·97*
Bethanechol 3·6	12	HCl 50	8·02 ± 1·80*†††

Values are means ± s.e.m.
†$P < 0·05$, ††$P < 0·01$, †††$P < 0·001$, when compared with its own saline-pretreated group.
*$P < 0·001$, when compared with the corresponding non-stressed group in A.

(Hottenrolt et al 1978; Koo et al 1985) or by acid back-diffusion (Davenport 1968; Skillman & Silen 1970). The latter action has been thought to result in stress ulcer formation, but this has not been demonstrated experimentally. Depressed gastric acid secretion by stress is likely to be due to hypofunction of the parietal cells, as a consequence of ischaemia (Cho & Ogle 1979). An observation which argues against the possibility of significant acid involvement is that complete neutralization of luminal acid by antacids does not prevent lesion formation in stressed animals (Takagi & Okabe 1970; Cho & Ogle 1979). Recent studies (Koo et al 1986; Ogle et al 1985b) indicate that verapamil may antagonize stress ulceration chiefly through its ability both to reduce gastric smooth muscle contractions and to permit the mucosal mucus layer to remain intact. Thus, evidence points to the likelihood that acid reduction by verapamil may not contribute significantly to its antiulcer action.

The finding that the smaller dose of verapamil antagonizes stress ulceration but is unable to inhibit gastric secretion indeed indicates that the antiulcer effect of the drug may not be wholly related to inhibition of acid output. Similar observations have been made in metiamide- or cimetidine-pretreated animals, where non-acid-inhibiting doses have prevented stress ulceration (Bugajski et al 1976; Cho & Ogle 1979; Okabe et al 1977a,b). The present results with bethanechol are in accord with these reports. Although vagal activation is responsible for ulcer formation in stressed rats (Cho et al 1976a, 1985), bethanechol injection, which markedly elevates luminal acid content, does not worsen stress-induced lesion formation. This observation also suggests that further stimulation of the gastric muscarinic receptors (due to the cholinergic effects of bethanechol being added to those of vagal stimulation) does not potentiate stress ulceration.

The role of luminal acid in the pathogenesis of stress ulcers is elucidated by the experiment with p.o. administered acid. Oral administration of 25 or 50 μequiv HCl increased the luminal acid content (Table 1); this exogenous acid did not produce any mucosal damage in non-stressed animals, but it markedly aggravated stress-induced lesions. It is likely that under non-stress conditions the gastric mucosa is resistant to high concentrations of acid, and that in stress the mucosal defensive mechanism is weakened by increased contractions of the gastric musculature and depletion of mucus lining the glandular mucosa (Cho & Ogle 1978b; Graef 1971; Ogle et al 1985b). Defective mucosal defence would then permit H^+ back-diffusion into the gastric mucosa, in conditions when high levels of acid are present in the stomach, to result in ulceration (Himal et al 1975).

Stress ulcer aggravation by acid administration was prevented by bethanechol pretreatment. The mechanism for this antiulcer effect is unclear. Increased H^+ back-diffusion is required for the development of acute gastric stress ulceration. It has been shown that activation of the gastric mucosa by a secretogogue decreases H^+ diffusion from the lumen into the mucosa (Kivilaakso et al 1978), and thereby prevents ulceration. Thus, it is possible that bethanechol could possess such actions to antagonize ulceration; also its ability to increase stomach mucus and bicarbonate secretion (Takagi & Okabe 1970; Williams & Turnberg 1980) could contribute significantly to the observed antiulcer effects.

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Tissue cholinesterase inhibition by propranolol and related drugs

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The effect of (±)-propranolol and some related drugs have been investigated on the cholinesterase (ChE) enzyme activity of heart and brain tissues of the rat. Brain homogenates hydrolysed more methacholine than benzoylcholine and the reverse was true for the heart tissue. In-vitro, (±)-, (+)- and (−)-propranolol, as well as its quaternary analogue, UM-272, all significantly inhibited heart and brain ChE. Timolol and sotalol, however, were less potent. In-vivo, (±)-propranolol (30 μmol kg^{-1}) significantly inhibited brain ChE activity in rats when compared with saline controls. It is inferred that propranolol inhibits brain and heart ChE enzyme in a non-stereoselective manner and that this cholinomimetic action could be involved in the mediation of some of its therapeutic effects.

The β-adrenergic blocking agent propranolol has been one of the most widely used drugs in disorders of the cardiovascular system. It has also been found to be useful in conditions related to the eye such as wide angle glaucoma and the central nervous system like psychosis, tremor and alcohol withdrawal states. However, many of the drug's therapeutic and pharmacological actions cannot be explained by its ability to block β-adrenoceptors; other ancillary properties have been proposed to explain these effects. Because of the overlapping of cholinergic and adrenergic innervation in many systems, drugs acting on one system are known to modify the activity of the other; thus it is likely that propranolol could modulate cholinergic activity, and this is supported by recent evidence from our laboratory. Bilateral vagotomy has been reported to attenuate the antiarrhythmic action of propranolol in dogs (Alkondon et al 1984). Also, the anticholinergic agent, atropine, has been shown to antagonize the ocular hypotensive effect of propranolol in rabbits (Alkondon et al 1986), reduce the antiaggressive effect of this drug in rats (Ray et al 1984) and inhibit the increase in airway resistance of normal and asthmatic subjects (MacDonald et al 1967). All this evidence indicates involvement of a cholinergic mechanism in propranolol pharmacodynamics. Recent observations from our laboratory also suggested the ability of propranolol to inhibit the cholinesterase (ChE) enzyme of human plasma and red blood cells (Alkondon et al 1983). The present study is an attempt to evaluate the effect of propranolol and some related drugs on the ChE activity of heart and brain tissues with the aim of exploring the nature of cholinergic mediation in the cardiovascular and central nervous system effects of propranolol.

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Methods

Wistar rats (150–200 g) of either sex had free access to food and water until the morning of the day of the experiment when they were killed by cervical dislocation, the heart and the brain removed immediately, washed in ice cold phosphate buffer (pH 8·0) and separately homogenized in 7 ml of buffer. The homogenates were centrifuged at 10 000 rev min^{-1} at 0 °C for 10 min. The supernatant was tested for cholinesterase enzyme activity, which was determined photometrically (Pilz 1974). The protein content was also estimated (Lowry et al 1951). Methacholine