incubation period may bring this concentration into relevance for the pathophysiological hyperinsulinaemia in some syndromes of type 2 diabetes. Thus in the presence of a high concentration of insulin, it appears that metformin can enhance glycogenesis by an isolated soleus muscle preparation independently of a change in insulin receptor binding. This suggests that the drug may act at postreceptor sites of the insulin effector pathway.

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A study on the aetiology of reserpine ulceration and the antiulcer action of solcoseryl in rat stomach

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The aetiology of reserpine-induced gastric ulcer formation and the antiulcer effects of solcoseryl were studied in rats. Intraperitoneal injection of reserpine produced severe ulceration, as well as mast cell and histamine depletion, in the gastric glandular mucosa. Mepyramine and cimetidine markedly antagonized the gastric lesions, but did not influence the reduced mast cell count; atropine pretreatment significantly inhibited both parameters. Intramuscular injection of solcoseryl lessened ulcer severity and prevented the decreased mast cell counts and histamine levels in reserpine-treated rats. However, the same dose of solcoseryl injected intraperitoneally was ineffective. Solcoseryl, irrespective of the route of administration, did not influence the gastric secretory activities of reserpine. It is concluded that resperine ulceration is both cholinergic- and histamine-mediated, and that the antiulcer effects of solcoseryl appear to be due to prevention of histamine depletion in the gastric mucosa.

Cholinergic activation and consequent mast cell degranulation in gastric glandular mucosae have been shown to contribute largely to ulceration (Ogle & Cho 1977a, 1978, 1979). However, the ulcerogenic mechanisms due to mast cell degranulation are not clear. A similar sequence of events has been observed in stressed animals where significant falls in stomach wall mast cell counts are causally related to increases in gastric histamine release (Cho & Ogle 1978, 1979; Ogle & Cho 1977b). Solcoseryl, a non-protein extract from calf serum has been shown to hasten the healing of skin ulcers (Barre & Alechinsky 1963) and to prevent stress gastric ulceration (Barre & Alechinsky 1963; Debray et al 1972; Jaeger et al 1979). It is, therefore, conceivable that solcoseryl may antagonize ulceration and prevent changes in gastric glandular mucosal histamine levels caused by reserpine. In this study, the aetiology of reserpine ulceration is further investigated by direct measurement of the gastric mucosal histamine content in an attempt to relate any changes to mast cell degranulation in the same region of the stomach. The effects of histamine H_1- and H_2-receptor antagonists are also examined in order to determine the importance of the amine in the pathogenesis of reserpine ulceration. The influence of first pass inactivation of solcoseryl through the liver is also examined by comparing the effects of intramuscular and intraperitoneal routes of its administration.

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Table 1. Effects of mepyramine, cimetidine and atropine treatment (given 30 min before) on ulceration and changes in mast cell counts in the gastric glandular mucosa of rats killed 4 h after reserpine administration.

	Pretreatment	Treatment (i.p.)	Glandular ulcer index (mm)	Glandular mast cell count in 40 o.i.f.	
				Mucosa	Submucosa
A.	Saline (1 ml kg^{-1} i.m. + i.p.)	Saline (5 ml kg^{-1})	0·06 ± 0·04	91·8 ± 6·7	50·6 ± 2·8
B.	Saline (1 ml kg^{-1} i.m. + i.p.)	Reserpine (5 mg kg^{-1})	7·07 ± 0·93††	36·4 ± 4·5††	37·5 ± 3·1†
C.	Mepyramine (12·5 mg kg^{-1} i.m.)	Reserpine (5 mg kg^{-1})	1·68 ± 0·40*	30·5 ± 3·1	39·4 ± 2·3
D.	Cimetidine (50 mg kg^{-1} i.p.)	Reserpine (5 mg kg^{-1})	1·75 ± 0·48*	32·4 ± 5·5	42·2 ± 2·4
E.	Atropine (0·8 mg kg^{-1} i.m.)	Reserpine (5 mg kg^{-1})	0·17 ± 0·07*	70·0 ± 5·5*	43·5 ± 3·4

Values indicate means ± s.e.m. of 10 rats. o.i.f. = oil immersion fields. †$P < 0·01$, ††$P < 0·001$ when compared with the corresponding values in group A. *$P < 0·001$ when compared with the corresponding values in group B.

Methods

Male, Sprague-Dawley rats, 230–250 g, were fasted for 24 h before treatment with reserpine (Ciba) (5 mg kg^{-1} i.p.). Gastric secretory parameters were studied in rats in which the pylorus was ligated by the method of Shay et al (1945) before reserpine injection. All animals were killed at the end of each experiment.

Rats were treated with mepyramine maleate (M & B) (12·5 mg kg^{-1} i.m.), cimetidine HCl (SK & F) (50 mg kg^{-1} i.p.) or with atropine sulphate (E. Merck) (0·8 mg kg^{-1} i.m.), expressed as their salts, 30 min before reserpine administration. Control animals were given 0·9% NaCl w/v (saline; 1 ml kg^{-1} i.m. and i.p.). Solcoseryl (Solco, Basle) (2·5 ml kg^{-1} i.m. or i.p.) or saline (2·5 ml kg^{-1} i.m. and i.p.) was injected once daily 48, 24 and 0·5 h before reserpine treatment. All drugs were freshly prepared in saline before use. The animals were killed 4 h after reserpine injection and their stomachs were removed. After grading ulcer severity (Cho & Ogle 1978), the glandular segments of the stomachs were histologically processed for mast cells to be counted in the mucosal and submucosal layers (Cho & Ogle 1977). In the solcoseryl-pretreated groups and their controls, part of the gastric glandular mucosa was scraped off, by means of a glass slide, and homogenized by a polytron (TR 50) in the presence of 10% trichloroacetic acid (2·5 ml 100 mg^{-1} wet weight of tissue). After centrifugation at 3000 rev min^{-1} for 15 min, 1 ml of the supernatant was used for histamine determination (Håkanson et al 1972); the residue was measured for its protein content (Lowry et al 1951).

In the second experiment, rats were treated with either solcoseryl or normal saline. The animals were then pylorus-ligated immediately before reserpine treatment; they were killed 4 h later and their stomachs removed for collection of gastric secretion. Gastric acidity was measured by titration to pH 7·4 with 0·01 M NaOH and pepsin determined by the method of Berstad (1970).

The data were analysed for significance by Student's *t*-test.

Results and discussion

Reserpine injection produced severe haemorrhagic

Table 2. Effects of solcoseryl pretreatment (once daily for 3 days) on ulceration and changes in mast cell counts and histamine levels in the gastric glandular mucosa of rats killed 4 h after reserpine administration.

	Pretreatment	Treatment (i.p.)	Glandular ulcer index (mm)	Glandular mast cell count in 40 o.i.f.		Histamine (μg mg^{-1} protein)
				Mucosa	Submucosa	
A.	Saline (2·5 ml kg^{-1} i.m. + i.p.)	Saline (5 ml kg^{-1})	0·01 ± 0·10	99·5 ± 8·7	54·3 ± 3·8	0·58 ± 0·04
B.	Saline (2·5 ml kg^{-1} i.m. + i.p.)	Reserpine (5 mg kg^{-1})	9·20 ± 1·39††	40·1 ± 3·8††	34·7 ± 2·9†	0·31 ± 0·01†
C.	Solcoseryl (2·5 ml kg^{-1} i.m.)	Reserpine (5 mg kg^{-1})	4·93 ± 1·17*	63·2 ± 4·2**	40·7 ± 3·5	0·43 ± 0·01***
D.	Solcoseryl (2·5 ml kg^{-1} i.p.)	Reserpine (5 mg kg^{-1})	8·41 ± 1·33	35·6 ± 4·2	38·1 ± 3·1	0·31 ± 0·05

Values indicate means ± s.e.m. of 7 rats. o.i.f. = oil immersion fields. †$P < 0·01$, ††$P < 0·001$ when compared with the corresponding values in group A. *$P < 0·02$, **$P < 0·01$, ***$P < 0·001$ when compared with the corresponding values in group B.

Table 3. Effects of solcoseryl pretreatment (once daily for 3 days) on changes in gastric acid and pepsin secretion in pylorus-ligated rats killed 4 h after reserpine administration.

	Pretreatment	Treatment (i.p.)	Gastric secretion		
			Volume (ml h^{-1}/100 g)	Acidity (μ Eq h^{-1}/100 g)	Pepsin (μg h^{-1}/100 g)
A.	Saline (2·5 ml kg^{-1} i.m. + i.p.)	Saline (5 ml kg^{-1})	0·46 ± 0·07	46·2 ± 6·0	143·7 ± 12·2
B.	Saline (2·5 ml kg^{-1} i.m. + i.p.)	Reserpine (5 mg kg^{-1})	0·62 ± 0·10	71·7 ± 10·0†	245·7 ± 20·5††
C.	Solcoseryl (2·5 ml kg^{-1} i.m.)	Reserpine (5 mg kg^{-1})	0·54 ± 0·09	68·4 ± 8·0	262·7 ± 33·2
D.	Solcoseryl (2·5 ml kg^{-1} i.p.)	Reserpine (5 mg kg^{-1})	0·53 ± 0·03	69·9 ± 8·1	266·1 ± 21·3

Values indicate means ± s.e.m. of 7 rats. †$P < 0·05$, ††$P < 0·01$ when compared with the corresponding values in group A.

glandular ulceration of the stomach. Glandular mucosal mast cell counts were markedly decreased (Tables 1 & 2), and this was accompanied by a significant reduction in histamine content in the same region of the stomach (Table 2). These findings suggest that stomach mast cell degranulation following reserpine administration is likely to be causally related to the histamine released from the gastric mucosa. Degranulation of gastric mast cells, and consequent histamine liberation, are thought to be cholinergically mediated (Cho & Ogle 1977, 1979). The observed depletion of mast cells in the gastric glandular mucosae which was blocked by atropine (Table 1), or by vagotomy (Ogle & Cho 1979), indeed supports this idea.

The importance of the ulcerogenic actions of histamine, through its adverse effects on the musculovascular tissues in the stomach (Guth & Code 1978; Schwartz 1971), is generally accepted. The relation between these actions and reserpine ulceration is confirmed by the protective action of mepyramine and cimetidine against lesion formation (Table 1).

Solcoseryl, injected daily for 3 days, does not by itself affect gastric mucosal histamine levels (Cho, unpublished findings). However, it antagonized significantly reserpine-induced stomach ulcers (Table 2). The reduction in gastric mucosal histamine levels and mast cell degranulation by reserpine were also prevented by solcoseryl administration. The mechanism for the inhibition of these effects is unknown. Jaeger et al (1979) have suggested that solcoseryl may improve oxygenation and energy supply to the gastric glandular mucosa; such an action could prevent the pathological changes induced by reserpine. It is also conceivable that the non-protein calf serum extract may have lessened mast cell degranulation by a membrane-stabilizing action, similar to that of disodium cromoglycate which has been shown to inhibit reserpine-induced depletion of these cells in the gastric glandular mucosa (Ogle & Lau 1979). Intramuscular injection of solcoseryl, however, did not affect the increases in gastric acid and pepsin secretion produced by reserpine (Table 3). It is possible that elevation of gastric acid and pepsin secretion by reserpine injection could largely be dependent on activation of the cholinergic system rather than on histamine release (Ogle & Cho 1977). This idea is supported by the observation that although solcoseryl depressed histamine liberation by reserpine, it did not inhibit gastric secretory stimulation by the alkaloid. The inability of intraperitoneal injection of the non-protein calf serum extract to prevent the gastric effects of reserpine (Table 2) is likely to be due to first pass inactivation of the former by the liver.

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