EFFECT OF SAS (A NEW 10-N-ACYLAMINOPHENOTHIAZINE) ON GASTRIC SECRETION AND ULCERATION IN RATS

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The antiulcer and antisecretory activity of 2-chloro-10-[4′(*N*-β-hydroxyethyl) piperazinyl-1′] acetylphenothiazine (SAS) has been investigated. At 10 and 20 mg/kg (s.c.) the drug was found to possess potent antigastric secretory and antiulcer activity (both in shay and stress ulcers) and did not exhibit any peripheral parasympathetic blocking activity. The pronounced antispasmodic activity of SAS was nonspecific rather than a specific parasympatholytic effect.

Introduction Most of the antiulcer drugs in use today, lack specificity of action and efforts continue to find new antiulcer drugs devoid of undesirable secondary effects. Amongst the phenothiazines, chlorpromazine in high doses (20 mg/kg) has been reported to possess antigastric secretory and antiulcer activity (Radwan & West, 1971). However, at such high doses, many other effects are produced and the antiulcer activity is not specific. Secergan, a new quaternary acylamino phenothiazine derivative, has been reported to be an effective antiulcer and antispasmodic drug (Tomenius, 1957). However, quaternary compounds are less predictable in action than tertiary analogues (Anichkov & Zavodskaya, 1968) and introduction of a tertiary amine function in the side chain, has yielded compounds which produce selective parasympathetic blockade (Pardo, Vargas, Cato & Laguna, 1956). Hence on theoretical grounds, acylaminophenothiazines, with a tertiary amino group in the side chain, should be preferable to quaternary compounds like Secergan. With this in view, a series of 10-*N*-acylaminophenothiazines were synthesized (Sharma, Banerjee, Sharma & Mital, 1970) and the present communication deals with the observed antigastric secretory and antiulcer activity of one of these compounds *viz* 2-chloro-10-[4′-(*N*-β-hydroxyethyl) piperazinyl-1′] acetylphenothiazine (SAS).

Methods The gastric antisecretory effect of SAS was determined by the method of Meyer, Cummings, Bass & Collier (1965) in 48 h fasted, 4 h ligated rats of either sex (180–200 g) of the Haffkin strain. The test compound, dissolved in distilled water was given subcutaneously immediately after ligation to groups of 10 rats. Four hours after ligation, the rats were killed and the stomach contents were removed and centrifuged, at 2000 rev/min for 10 min and subsequently were assessed for volume, pH and total acidity. The samples (0.1 ml) of gastric juice were titrated against 0.01N NaOH using phenolphthalein as indicator. The results were expressed in mEq/l of total acids.

Two methods for producing gastric lesions in rats were used: (a) Temporary pylorus ligation: groups of ten female rats (120–150 g) were ligated at the pyloric sphincter by the method of Shay, Komarov, Fels, Merange, Gruenstein & Sipiet (1945) as modified by Yasuo Ishi (1969). Briefly, rats were housed individually and fasted for 24 h and the pylorus ligated under light ether anaesthesia. After suturing the wound, SAS or an equivalent volume of 0.9% w/v NaCl solution (saline) was injected subcutaneously. In one group, SAS was also administered orally at a dose of 20 mg/kg. After 18 h, the mortality in the groups, if any, was noted and then the stomachs of all the surviving rats were removed, cut along the greater curvature, and examined visually for the degree of ulceration using a score from 1 to 5 depending upon severity of ulceration (Adami, Marazzi-Uberti & Turba, 1964). Mean ulcer score was then calculated from the individual scores. (b) Immobilization of rats (Adami *et al.*, 1964): rats of either sex, fasted for 24 h, were immobilized by wrapping them tightly with a flexible wire mesh sheet. The test compound or control vehicle was injected subcutaneously 30 min before immobilization and 12 h after the first dose. After 24 h of immobilization, stomachs were removed and gastric mucosal damage was assessed as described for the Shay rats.

The antispasmodic activity was tested on rabbit isolated ileum preparations. Tyrode solution containing 1% barium chloride was used to maintain a constant spasm of the ileum. The spasmolytic activity of SAS, was compared with papavarine as reference standard in a series of six experiments.

The spasmolytic, antiacetylcholine and antihistamine activity of SAS was studied on the guinea-pig isolated ileum preparation allowing a 2 min drug contact time. Barium chloride (2 mg/ml), acetylcholine (1 μg/ml) and histamine (10 μg/ml) were used as spasmogens and activities were compared with papavarine hydrochloride, atropine sulphate and diphenhydramine respectively. The ED_{50}

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for SAS as well as for the standard drugs was calculated by graphic interpolation and the activity ratios, with respect to the appropriate agonists, were calculated.

The effects of SAS on blood pressure, respiration and on intact intestine was studied on dogs anaesthetized with pentobarbitone sodium (30 mg/kg). Up to 20 mg/kg of the test compound was used to study its antagonism to acetylcholine, adrenaline, noradrenaline, isoprenaline, histamine and dimethylphenylpaperazinium iodide (DMPP). The effect of a 1% solution of SAS on iris muscle was also studied in a group of six rabbits and compared with that of 0.1% atropine sulphate.

Results and Discussion SAS in doses of 10 mg/kg and 20 mg/kg, greatly reduced the volume and total acidity of gastric secretion in 4 h pylorus ligated rats. A significant increase in pH of the gastric contents of drug-treated rats was also observed (Table 1).

SAS in doses up to 5 mg/kg intravenously, was found to have no effect on the blood pressure and respiration of anaesthetized dogs. However, a dose-dependent, transient fall in blood pressure was observed at 50 mg/kg, the blood pressure recovering in less than one minute. Doses of 20 mg/kg SAS did not alter the blood pressure responses elicited by acetylcholine, histamine, noradrenaline, adrenaline, isoprenaline and DMPP. A 1% solution of the test compound did not show any mydriatic activity in the rabbit eye.

The gastric anti-secretory activity of SAS is not due to peripheral parasympathetic blockade, as the drug even in large doses does not antagonize the blood pressure responses to acetylcholine and DMPP and elicits no mydriatic activity. Failure to block the compensatory reflexes triggered by the stimulation of carotid and aortic baroreceptors also indicate that the drug has no parasympatholytic activity. Although several compounds, which are devoid of any anti-acetylcholine or ganglion blocking activity, are known to possess antisecretory action, the exact mechanism of such a non-specific action is not clear. SAS is structurally analogous to chloracizine, a phenothiazine antidepressant (Lappin & Shchelkunov, 1963), and indeed antidepressants like imipramine possess antisecretory effects. The antidepressant effects of SAS have recently been observed (unpublished data) but whether this is responsible for the observed antisecretory activity is debatable.

In Shay rats, gastric lesions were observed mainly in the rumenal area and the control group showed 35% mortality. However, in SAS-treated Shay rats, the mortality (25%) and severity of ulcers were greatly reduced even at 10 mg/kg ($P < 0.05$). The protection against mortality (10%) and gastric mucosal damage was more marked at 20 mg/kg subcutaneously while at 20 mg/kg orally the mortality was nil and the mean

Table 1 Effect of 2-chloro-10-[4'(*N*-β-hydroxyethyl) piperazinyl-1'] acetylphenothiazine (SAS) on gastric secretion, total acidity, pH and ulceration in rats‡

Dose mg/kg s.c.	Mean volume ± s.e.	Total acidity ± s.e.	pH ± s.e.	% Ulceration Shay rats	% Ulceration Restrained rats	Mean Ulcer Score ± s.e. Shay rats	Mean Ulcer Score ± s.e. Restrained rats	% Mortality Shay rats	% Mortality Restrained rats
Control	5.8 ± 1.2	68.1 ± 13.7	2.1 ± 0.35	85	75	4.3 ± 0.5	3.8 ± 0.3	35	—
5	4.0 ± 0.3	48.2 ± 16.5	3.6 ± 0.2**	60	—	3.1 ± 0.3	—	25	—
10	2.2 ± 0.7**	18.4 ± 4.7**	4.0 ± 0.1***	22	40	2.1 ± 0.4**	1.4 ± 0.3**	25	—
20	1.1 ± 0.2***	13.1 ± 2.4***	5.8 ± 0.2***	12	15	1.1 ± 0.1***	0.6 ± 0.2***	10	—
20*	—	—	—	15	—	0.8 ± 0.2***	—	Nil	—

‡ each group contained 10 rats; * Given orally; ** $P < 0.05$; *** $P < 0.01$.

ulcer score was only 0.8 ± 0.2 ($P < 0.01$). This protection offered by SAS against ulceration and mortality, could be due to its effect in reducing volume and acidity of gastric secretion. The local anaesthetic activity of SAS (Sharma, Mital, Banerjee & Sharma, 1971) might also have played some role in this protective action, either by blocking gastrin release from the gastric antrum or by reducing ulcer pain (Posey, Boler & Posey, 1969). However, conclusive evidence on this point is lacking at this stage.

The preventive effect of SAS against the severity and incidence of immobilization stress-induced gastric lesions was significant ($P < 0.01$) at 20 mg/kg subcutaneously (Table 1). Immobilization results in ulcers which are mainly neurogenic in origin. Hence the protective effect of SAS against restraint-induced ulcers indicate that its anti-secretory effect may not be the sole factor responsible for its antiulcer activity. Some central action might also be involved. Although at effective dose levels, peripheral parasympatholytic or sympatholytic activities are lacking in SAS, the exact mechanism for the observed antiulcer activity could not be ascertained.

The test compound produced a general spasmolytic effect as it reversibly antagonized acetylcholine, histamine and $BaCl_2$-induced contractions of the guinea-pig isolated ileum. The ED_{50}, which inhibits maximum contraction by 50%, was found for SAS to be 0.9 μg/ml, 0.6 μg/ml and 8 μg/ml compared with the ED_{50} of standard antagonists: 81 ng/ml (diphenhydramine) 4 ng/ml (atropine) and 3.5 μg/ml (papavarine) respectively. On the rabbit isolated ileum it produced relaxation and inhibited pendular movements. In the intact intestine, in anaesthetized dogs, prolonged relaxation was observed on intravenous administration (2 mg/kg and more) of SAS.

The pronounced antispasmodic effects of SAS are consistent with reports that acylamino phenothiazines (Millot & Guillot, 1965) and some other 10-*N*-esteralkylaminophenothiazines (Dahlbom & Ekstrand, 1952) possess spasmolytic activities. Here again, the observed antispasmodic action does not seem to be a specific parasympatholytic effect as the antagonistic action of the drug against $BaCl_2$-induced spasm is also pronounced. This musculotropic action could also be due to its local anaesthetic activity (Sharma *et al.*, 1971) which may cause a nonspecific relaxant action, though several antispasmodics like adiphenine and amprotropin which possess local anaesthetic properties have no demonstrable effects on gastric secretion.

Our findings are therefore consistent with the views expressed by Gordon (personal communication, 1970) as well as by Anichkov & Zavodskaya (1968) that nonquaternary antiulcer and antisecretory agents should be preferable to the quaternary ones because of their lesser peripheral cholinergic involvement.

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