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Gastric mucosal cytoprotection in the rat by naftidrofuryl oxalate

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Abstract—Ischaemic gastric mucosal injury was assessed in the rat by measurement of the area of the injury produced after 6 h by reserpine (5 mg kg^{-1} i.p.) or 5-hydroxytryptamine (5-HT) (50 mg kg^{-1} i.p.). Pretreatment with naftidrofuryl 1 mL, 1% by gavage significantly ($P < 0.001$) protected the rat stomach against the reserpine (24 ± 2.7 mm^2 vs 40 ± 4.7 mm^2, mean ± s.e.m., n = 10) and 5-HT injury (11.4 ± 1.7 mm^2 vs 27 ± 4.1 mm^2, mean ± s.e.m., n = 10). Naftidrofuryl 1 mL 2% by gavage was more effective ($P < 0.001$) in this respect and mucosal injury only developed in 50% of rats injected with reserpine (9.4 ± 1.1 mm^2) and 30% of those injected with 5-HT (3.2 ± 0.4 mm^2). Administration of naftidrofuryl 1 mL 5% by gavage completely protected the rat against both the reserpine- and 5-HT-induced acute gastric mucosal injury. This protection was not associated with any significant influence on the H^+ output.

Clinical and laboratory experience has confirmed the role of ischaemia as a mechanism of acute gastric mucosal injury (Lucas et al 1971; Ritchie 1975). Naftidrofuryl oxalate is a vasodilator (Fontaine et al 1968, 1969) which directly enhances tissue oxidative metabolism by activation of succinic dehydrogenase (Eichhorn 1969; Meynaud et al 1973). This action prompted investigation of the effect of the drug on ischaemic injury of the rat gastric mucosa induced by reserpine (Salim 1987) or 5-hydroxytryptamine (5-HT) (Ferguson et al 1973).

Materials and methods

Animals. Groups of ten Sprague-Dawley rats of either sex, 220–280 g, were fasted for 24 h before experimentation. Animals were housed in cages with wide mesh wire bottoms to prevent coprophagy.

Source and preparation of drugs. Solutions of naftidrofuryl oxalate (Praxilene, Lipha Ltd, West Drayton, Middlesex, England) were prepared by dissolving the powder in physiological saline. All other drugs were supplied by Sigma (St. Louis, MO, USA). A 1 mg mL^{-1} solution of reserpine was prepared by dissolving 80 mg crystalline powder in 0·3 mL glacial acetic acid (BP) and the volume made up to 80 mL with double distilled water. 5-Hydroxytryptamine powder was dissolved in double distilled water to prepare a 10 mg mL^{-1} solution. Injections were administered intraperitoneally into the left iliac fossa using a 25 G needle and gavage was undertaken under light ether anaesthesia using a 6 FG Infant's Feeding Tube 400/420 (Portex Ltd, Hythe, UK). Solutions were freshly prepared each day.

Surgery. Animals were anaesthetized by inhalation of diethyl ether or by intraperitoneal injection of 25 mg kg^{-1} pentobarbitone (Sagatal, May and Baker, Dagenham, England) into the left iliac fossa. When indicated, supplementary doses of pentobarbitone were given to maintain narcosis. The pyloric sphincter was ligated then the abdomen was closed as described by Salim (1988a). Tracheostomy was done as detailed elsewhere (Salim 1988b) to overcome respiratory distress from intubation.

Experimental design. One mL of 1,2 or 5% naftidrofuryl oxalate or 1 mL saline was instilled into the stomach by orogastric intubation. Animals were allowed to recover from anaesthesia then were injected with reserpine (5 mg kg^{-1}), 5-HT (50 mg kg^{-1}), or saline (5 mL kg^{-1}). Five h later, they were anaesthetized with pentobarbitone, submitted to tracheostomy and orogastric intubation with a 6 FG tube. The gastric fasting secretion was recovered by slowly instilling 1 mL of double distilled water and recovering all gastric contents. The gastric secretion was then recovered every 15 min for 1 h and the H^+ output (μmol h^{-1}) determined by titration to pH 7·0 with 0·1 M NaOH using an automatic titrator (Radiometer, Copenhagen). At the end of this hour animals were killed by ether overdose and the stomach removed and opened along the greater curvature. After washing with a direct stream of cold water the stomachs were pinned out and independently examined for the presence of mucosal injury macroscopically, each injury being measured as the maximum length and width, and the surface area (mm^2) calculated. The total injury score was obtained for each animal and the mean injury score calculated for each study group. Sections of injured and apparently uninjured gastric mucosa were examined microscopically.

To minimize day-to-day variation in response to treatment, the study was conducted over several days and animals were allocated to the control and all of the treatment groups on each experimental day.

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Statistical analysis. Results are expressed as mean ± s.e.m. The statistical significance ($P < 0.05$) of observed differences between groups was determined using the Mann-Whitney U test for non-parametric data.

Results

Table 1 presents the results of determining the H^+ output over 2 h in pylorus-ligated rats receiving single doses of naftidrofuryl oxalate. No significant difference from the control value of 293 ± 24 μmol was observed. Table 2 presents the results obtained with secretory and mucosal integrity studies.

The H^+ output of the rat stomach over 1 h was not significantly influenced by 1 mL of 1, 2 or 5% naftidrofuryl oxalate. Six hours after reserpine (5 mg kg^{-1}) or 5-HT (50 mg kg^{-1}) treatment, the H^+ output of the rat stomach over 1 h was significantly ($P < 0.001$) depressed relative to control values; pretreatment with 1 mL of 1, 2 or 5% naftidrofuryl oxalate had no significant influence on this depression.

Stomachs treated by gavage with naftidrofuryl oxalate then injected with saline showed no gastric mucosal injury and were microscopically similar to control stomachs.

Table 1. Effect of naftidrofuryl oxalate on the H^+ output of the pylorus-ligated rat (n = 10).

Experimental group	μmol H^+ output over 2 h (mean ± s.e.m.)
Saline	239 ± 24
1% Naftidrofuryl	214 ± 19
2% Naftidrofuryl	223 ± 21
5% Naftidrofuryl	235 ± 17

Saline or naftidrofuryl solution (1 mL) was instilled into the stomach by orogastric intubation.

All rats in the reserpine or 5-HT alone groups developed oval or round mucosal injury confined to the glandular stomach and of no constant relationship to rugal crests. Pretreatment with 1 mL of 1% naftidrofuryl oxalate significantly ($P < 0.001$) protected the rat stomach against the reserpine and 5-HT injury. However, 1 mL of 2% naftidrofuryl oxalate was more effective ($P < 0.001$) in this respect and mucosal injury developed in only 50% of rats injected with reserpine and 30% of those injected with 5-HT. Administration of 1 mL of 5% naftidrofuryl oxalate completely protected the rat stomach against both the reserpine- and 5-HT-induced acute gastric mucosal injury.

Microscopic study showed that the mucosal injury produced by reserpine (5 mg kg^{-1}) was similar to that produced by 5-HT (50 mg kg^{-1}) and consisted of partial or full depth (including the muscularis mucosae) mucosal necrosis and loss. The submucosal and mucosal blood vessels were severely constricted. The muscularis propria was intact and no pathological changes were detected in the forestomach or antrum. The injury developed intramurally in the submucosa and mucosa as foci of necrosis or haemorrhage, which were directly related to constricted or disrupted blood vessels and expanded to communicate with the lumen.

Discussion

The pharmacological actions of naftidrofuryl oxalate, an acid ester of diethylaminoethanol, remain poorly understood. The drug's clinical effects are attributed to its actions on cellular metabolism and regional blood flow. Clinical trials and in-vitro and in-vivo studies of animals have indicated that naftidrofuryl oxalate may directly enhance tissue oxidative metabolism by activation of succinic dehydrogenase (Eichhorn 1969; Meynaud et al 1973).

The drug is an original spasmolytic, papaverinic, non-atropinic substance with obvious vascular tropism (Fontaine et

Table 2. Effect of naftidrofuryl oxalate on reserpine- and 5-hydroxytryptamine-induced acute gastric mucosal injury (n = 10).

Experimental group	Animals showing injury (%)	Injury area in mm^2 after 6 h (mean ± s.e.m.)	H^+ output in μmol h^{-1} (mean ± s.e.m.)
Saline 1 mL i.g. saline 5 mL kg^{-1} i.p.	0	0	16·1 ± 0·7
1% Naftidrofuryl 1 mL i.g. saline 5 mL kg^{-1} i.p.	0	0	15·4 ± 0·6
2% Naftidrofuryl 1 mL i.g. saline 5 mL kg^{-1} i.p.	0	0	14·9 ± 0·7
5% Naftidrofuryl 1 mL i.g. saline 5 mL kg^{-1} i.p.	0	0	15·7 ± 0·4
Saline 1 mL i.g. reserpine 5 mg kg^{-1} i.p.	100	40 ± 4·7	3·5 ± 0·5*
1% Naftidrofuryl 1 mL i.g. reserpine 5 mg kg^{-1} i.p.	100	24 ± 2·7*	3·9 ± 0·6*
2% Naftidrofuryl 1 mL i.g. reserpine 5 mg kg^{-1} i.p.	50	9·4 ± 1·1*	4 ± 0·5*
5% Naftidrofuryl 1 mL i.g. reserpine 5 mg kg^{-1} i.p.	0	0*	3·2 ± 0·7*
Saline 1 mL i.g. 5-HT 50 mg kg^{-1} i.p.	100	27 ± 4·1	3·4 ± 0·4*
1% Naftidrofuryl 1 mL i.g. 5-HT 50 mg kg^{-1} i.p.	100	11·4 ± 1·7*	3·9 ± 0·6*
2% Naftidrofuryl 1 mL i.g. 5-HT 50 mg kg^{-1} i.p.	30	3·2 ± 0·4*	3·1 ± 0·5*
5% Naftidrofuryl 1 mL i.g. 5-HT 50 mg kg^{-1} i.p.	0	0*	4·1 ± 0·7*

i.g.: instilled into the stomach by orogastric intubation.
i.p.: intraperitoneal injections.
* $P < 0.001$ Mann-Whitney U test comparing control with treatment groups.

al 1968). This drug is a vasodilator both peripherally and in the brain (Fontaine et al 1968, 1969). The vascular effects, as shown in general rotameter type tests, may be explained by sympathetic blockade at the ganglionic and, perhaps, axonal level, so that the product resembles the sympathoplegics (Fontaine et al 1968).

Parenteral administration of large doses of the vasoconstrictor reserpine in experimental animals produces acute gastric mucosal injury (Haverback & Bogdanski 1957; Blackman et al 1959). 5-HT also produces acute gastric mucosal injury in rats due to intense vasoconstriction, which results in areas of focal ischaemia (Ferguson et al 1973). The reserpine- and 5-HT-induced acute gastric mucosal injury were reproduced in this study and their association with mucosal ischaemia confirmed. Six h after intraperitoneal reserpine or 5-HT gastric mucosal injury confined to the glandular stomach developed in all animals (Table 2). This injury initiated intramurally as foci of haemorrhage or necrosis and was associated with sub-mucosal and mucosal vasoconstriction and significant depression of H^+ output. This depression is consistent with the knowledge that marked inhibition of mucosal blood flow will diminish acid secretion (Jacobson et al 1966).

Naftidrofuryl had no signficant effect on the H^+ output of the rat with or without pylorus ligation (Tables 1, 2) and protected the rat stomach against ischaemic injury without significantly influencing the H^+ output (Table 2). The action of naftidrofuryl indicates cytoprotection as defined by Robert et al (1979), the mechanism of which is unknown. It does not appear to involve increased mucosal blood flow, since the protection against the ischaemic injury was not associated with increased H^+ output (the mucosal blood flow and H^+ output are directly related). The possibility exists that cytoprotection by naftidrofuryl is achieved by enhancing tissue oxidative metabolism and the gastric mucosal blood flow.

Gastric mucosal ischaemia in both man and animals is an essential pre-requisite for stress-induced acute gastric mucosal injury (Skillman & Silen 1972). Whether naftidrofuryl protects man against stress injury of the gastric mucosa has still to be assessed.

In conclusion, naftidrofuryl protects the rat gastric mucosa against the ischaemic injury produced by reserpine or 5-HT without influencing the H^+ output, i.e. it gives cytoprotection.

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