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Vagal Influence on Gastric Secretion of Hydrochloric Acid and Sialic Acid-containing Mucoproteins in the Rat on Provocation of Gastric Ulceration

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ABSTRACT

The influence of the vagus nerve on the gastric secretion of HCl- and sialic acid-containing mucoproteins was studied in rats subjected to provocation of gastric ulceration by pyloric ligation simultaneous with and 2 and 4 weeks after vagotomy. The vagotomy comprised stripping of the muscular layer in the distal part of the oesophagus. The outputs of HCl and sialic acid were determined and also the tissue concentration of sialic acid in the stomach wall. Histochemical tests for the presence of sialic acid and acid sulphate-containing mucoproteins were also made. The results confirm that ulcer provocation by pyloric ligation gives a large increase of both the HCl and sialic acid output. The HCl increase is inhibited by vagotomy. The simultaneous HCl and sialic acid determinations indicate, further, that the vagal influence is of importance not only for HCl production but also for the production of sialic acid-containing mucoproteins. A decreased vagal tone appears to give an immediate increase of the mucous secretion in the stomach but later on a decreased mucus production.

INTRODUCTION

It is now generally conceded that the pathophysiology of the stomach is dependent upon its secretion of both hydrochloric acid and mucus. The effect of the vagus nerve on the secretion of hydrochloric acid has been the subject of several clinical and experimental studies, while its influence on the secretion of mucus is still essentially unknown. Reports on studies on this matter are both sparse and partly contradictory.

The aim of the present investigation was to study simultaneously the gastric secretion of hydrochloric acid and mucus under different experimental conditions with respect to the vagus nerve, on provocation of gastric ulceration in otherwise intact rats.

MATERIAL AND METHODS

Adult male albino rats (Sprague-Dawley) were used. They were fed on a standardized food of full dietary value and weighed about 250 g. Before the experiment they were fasted for 18 hours but given free access to water. The following experimental procedures were carried out.

Vagotomy

The animal was anaesthetized with ether and the abdomen opened by a midline incision, after which 5–8 mm of the distal part of the oesophagus was stripped close to the mucosa. Pyloroplasty was performed.

Pyloric ligation

Peptic ulcer was provoked by means of pyloric ligation according to the method of Shay, a silk ligature being tied around the pylorus. This was also performed under ether anaesthesia and through a midline incision. In certain groups the ulcer provocation was potentiated by tube deposition into the stomach of calcium acetylsalicylate in a single dose of 250 mg/kg body weight, at the same time as the pyloric ligation. No regard was taken of the possible presence of swallowed saliva during the Shay experiment.

Four hours after the pyloric ligation the animal was again anaesthetized with ether, the abdomen was opened, the cardia ligated and the stomach extirpated. The gastric volume was measured and the contents analysed for sialic acid and hydrogen ions. The stomach was then opened along the greater curvature and half of the glandular portion was analysed wth respect to the tissue concentration of sialic acid in the stomach wall, and the other half was stretched out on a cork plate and fixed in 10% formalin for histochemical examination.

Table I. *Gastric volume and secretion of HCl in the different groups*

For abbreviations, see text

Group	Gastric content volume (ml)	HCl	
		Conc. (μEq/l)	Output (μEq)
S	7.6±1.2 $n=12$	104.3±3.1 $n=12$	866.9±123.6 $n=12$
SV	4.7±0.5 $n=13$	80.1±8.4 $n=12$	497.3±77.3 $n=12$
SV II	5.1±0.6 $n=10$	68.1±10.2 $n=7$	417.9±88.5 $n=7$
SV IV	6.3±0.8 $n=7$	96.3±9.6 $n=7$	594.9±93.3 $n=7$
SV II S	5.9±1.5 $n=10$	67.8±7.2 $n=10$	473.7±158.8 $n=5$
SV IV S	9.3±1.2 $n=10$	73.8±5.8 $n=10$	698.7±138.1 $n=9$

Chemical analytical methods

1 ml of the gastric content was titrated for hydrogen ions against 0.1 N NaOH, with Töpfer's reagent as indicator. Chloride ions were titrated by the method of Brun (2). Sialic acid, as a measure of mucoproteins, was determined by the direct Ehrlich method as modified by Odin & Werner (9) and Carlborg (3).

Histochemical methods

The sialic acid-containing mucopolysaccharides were stained with Alcian Blue at pH 2.5 *ad modum* Romeis (10) and sulphate-containing mucopolysaccharides with Astra Blau at pH 0.5 *ad modum* Bloom & Kelly (1).

The animals were divided into the following groups. Each group consisted of 10 animals.

Group I. Pyloric ligation by the Shay method (S).

Group II. Pyloric ligation + vagotomy (SV).

Group III. Pylorc ligation in animals vagotonized 2 weeks previously (SV II).

Group IV. Pyloric ligation in animals vagotomized 4 weeks previously (SV IV).

Group V. Pyloric ligation + deposition of calcium-acetylsalicylate in animals vagotomized 2 weeks previously (SV II S).

Group VI. Pyloric ligation + deposition of calcium-acetylsalicylate in animals vagotomized 4 weeks previously (SV IV S).

RESULTS

Table I gives the volume of the gastric content and the secretion of HCl in the different groups, and Table 2 shows the content of sialic acid in the stomach wall and gastric juice, as well as the HCl – sialic acid index. Some of these variables are also shown diagrammatically in Figs. 1–2. The Shay procedure in itself gave a considerable secretory increase of the secretion of both HCl and sialic acid, and resulted also in a high index, i.e. the HCl increased to a relatively greater extent than the sialic acid (5). The immediate effect of vagotomy was a decrease in HCl secretion and a slight increase in the secretion of sialic acid. Two weeks after vagotomy the Shay operation gave about the same effect on HCl secretion. At the same time the sialic acid concentration decreased below the level noted after pyloric ligation alone. After 4 weeks the HCl secretion increased but did not reach that after pyloric ligation alone, while the sialic acid showed a slight further decrease. Potentiation of the ulcer provocation with calcium acetylsalicylate 2 weeks after vagotomy caused no definite differences in the results of the chemical analyses. 4 weeks after vagotomy, however, there was a slight increase in HCl secretion and a marked increase in sialic acid output.

The histochemical examinations with Alcian Blue and Astra Blau revealed changes well correlated to the quantitative chemical determinations. This did not hold for the salicylate experiments, however. The staining reaction was weaker than might have been expected from the results of the chemical analyses. The staining response

Table II. *Secretory indices and content of sialic acid in the stomach wall and gastric juice*

For abbreviations, see text

Group	Sialic acid			
	Stomach wall (μg/mg)	Gastric content		Index
		Conc. (μg/ml)	Output (μg)	
S	0.352±0.01 $n=13$	210.9±9.6 $n=11$	1767.3±153.2 $n=11$	0.52±0.04 $n=11$
SV	0.383±0.01 $n=13$	472.1±11.0 $n=11$	1956.0±147.0 $n=12$	0.28±0.05 $n=12$
SV II	0.496±0.02 $n=11$	296.0±39.7 $n=7$	1517.7±280.2 $n=7$	0.34±0.005 $n=5$
SV IV	0.300±0.04 $n=7$	182.9±17.5 $n=7$	1204.7±223.7 $n=7$	0.57±0.09 $n=7$
SV II S	0.399±0.02 $n=11$	248.5±23.0 $n=11$	1491.9±349.8 $n=4$	0.41±0.01 $n=3$
SV IV S	0.381±0.02 $n=9$	234.2±16.3 $n=9$	2287.8±205.4 $n=9$	0.30±0.04 $n=9$

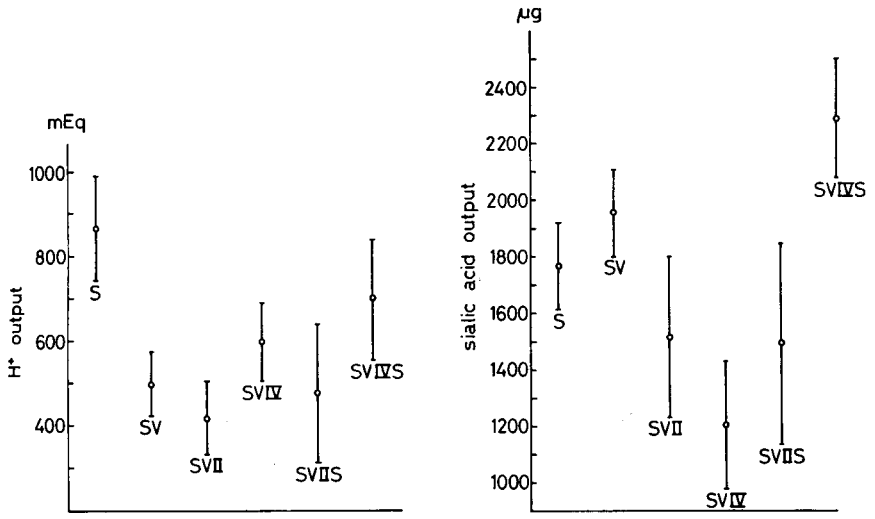

Fig. 1. Output of HCl and sialic acid. Groups: See text.

in both glandular tubules and external layers was thus enhanced by vagotomy, as a sign of an increased production of mucus. The staining with Alcian Blue was well correlated to that with Astra Blau, though the reaction to the latter stain was generally weaker.

DISCUSSION

Pyloric ligation implies provocation of gastric ulceration (11) and gives rise to strong stimulation of HCl secretion (7). At the same time an increase of the mucous secretion, estimated as sialic acid, is also obtained, as demonstrated by Carlborg et al. (5), among others. It has been discussed whether this increase in the sialic acid is a direct effect of the enhanced HCl secretion (8) or whether it is caused indirectly via vagal stimulation (6).

In our study the immediate effect of vagotomy, which comprised stripping of the muscular layer in the distal part of the esophagus, together with pyloric ligation, was an increase of the sialic acid secretion simultaneous with a reduction of the secretion of HCl, indicating stimulation of mucin production regardless of the HCl secretion in the stomach. Two weeks after vagotomy the HCl output was unchanged, while the sialic acid output decreased. 4 weeks after vagotomy there was a moderate increase in HCl secretion and a further decrease in sialic acid output. These findings do not indicate any correlation between the secretion of HCl and sialic acid-containing mucoproteins. Further, our findings indicate that the sialic acid output may be influenced by the vagus nerve and not only by the HCl production. On the other hand, Menguy (8) pointed out that the secretion of mucin in isolated antrum pockets does not increase on vagal stimulation. To our knowledge, simultaneous determinations of the

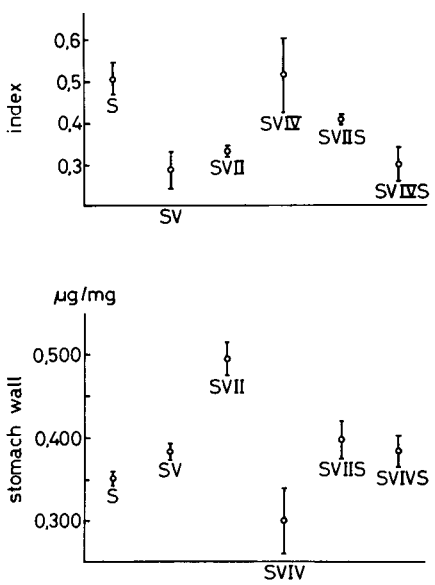

Fig. 2. Secretory index and content of sialic acid-containing mucoproteins. Groups: See text.

sialic acid and hydrochloric acid output after vagotomy and ulcer provocation in otherwise intact animals have not been made previously. Our results of such determinations indicate that a decreased vagal effect in Shay experiments would seem to have an immediately stimulating effect on the sialic acid output with a simultaneous increase of the tissue concentration. Two weeks after vagotomy there was a reduced sialic acid output accompanied by a further increase in the stomach wall concentration.

Four weeks after vagotomy there was a marked decrease both in sialic acid output and concentration in the stomach wall which may indicate a reduced mucus production.

From the point of view of gastric ulceration, reduction of the vagal influence gave, after observation intervals within 2 weeks, a favourable reduction of the HCl – sialic acid index (4), i.e. a relatively greater increase of the sialic acid than of the HCl output. Four weeks after vagotomy there was an index comparable to that after pyloric ligation alone.

With the method of chemical determination used in these experiments we were unable to verify that the mucus was reduced after administration of salicylate, but preliminary studies *in vitro* have indicated an interaction between salicylate and sialic acid in the analytical procedure, giving falsely high sialic acid values. These preliminary findings are supported by the histochemical determinations in the present study, in which a reduction of the mucus output was found after salicylate administration.

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