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Effect of an anti-acetylcholine drug, methscopolamine bromide, on ulcer formation and gastric mucus

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A 30-hr period of restraint in rats was followed by the appearance of ulcers in the gastric corpus coincidental with a marked reduction of gastric juice volume, acid and also of hexosamine which was used as an estimation of mucus content. Methscopolamine (Pamine), an anti-acetylcholine drug, prevented ulcer formation, reduced further volume and acid output but produced a 3-4 fold increase in hexosamine concentration. Tissue (corpus and antrum) hexosamine was moderately reduced by restraint. In the corpus, this was counteracted by methscopolamine but antrum hexosamine was not influenced by this drug. The anti-ulcer property of methscopolamine may be due not only to its effect on acid secretion but also to the rise in gastric mucus concentration that it produced.

ATROPINE-LIKE drugs are widely used for peptic ulcer therapy on the assumption that the reduction in acid secretion produced by these substances favours the healing of ulcers. Indeed, these compounds have been found to protect animals against a variety of experimentally-induced ulcers, such as those produced by fasting (Visscher, Seay, Tazelaar, Veldkamp & Vander Brook, 1954), pylorus ligation (Lehman & Stefko, 1949; Kowalewski, Mackenzie, Shnitka & Bain, 1954; Visscher and others, 1954), corticosteroids (Robert & Nezamis, 1959), histamine (Lehman & Stefko, 1949), reserpine (Blackman, Campion & Fastier, 1959; LaBarre, 1959), 5-hydroxytryptophan (Haverback & Bogdanski, 1957) and restraint (Hanson & Brodie, 1960). It must be noted that in these experiments, anti-acetylcholine drugs were given to prevent, not to cure, ulcers. There seems to be little doubt that the presence of acid in the gastric juice can hinder the healing of an ulcer. Whether hyperacidity, however, initiates peptic ulcer is debated and appears doubtful. Other factors, perhaps equally important, may be considered to increase or decrease the resistance of gastric and duodenal mucosa without much change in acid secretion. Among these, the amount and rate of secretion of gastroduodenal mucus has been implicated in the past in the natural defence against ulcerogenic agents (Hollander, 1951; Robert & Nezamis, 1959, 1963, 1964; Robert, Bayer & Nezamis, 1963).

It was thought that besides their antisecretory property, atropine-like agents might protect the stomach by increasing the mucus content of gastric juice. Actually, it was noted in an earlier study that the gastric mucosa of fasted rats treated with methscopolamine bromide (Pamine) "was covered with a thicker layer of mucus than that of control animals" (Robert & Nezamis, 1959).

We have now investigated the influence of methscopolamine bromide on the mucus content of gastric juice and tissue and on gastric juice acidity of restrained and nonrestrained rats. Restraint was previously shown to produce ulcers in rats (Rossi, Bonfils, Lieffogh & Lambling, 1956; Brodie & Hanson, 1960). A correlation was attempted of the anti-ulcer effect observed and the changes in gastric juice and tissue composition.

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Method

Separate experiments were made for gastric juice and for gastric tissue analysis. In each, 15 female Sprague-Dawley rats, weighing 190 to 205 g, were used per group.

GASTRIC JUICE

Animals were fasted at 4.00 p.m. They were first placed in individual cages for 24 hr, then transferred into stainless steel tubes during the night preceding pylorus ligation (Robert & others, 1963). After this day and a half of fasting, the pylorus was ligated under ether anesthesia, the abdominal incision closed with surgical clips and the rats returned to the metal tubes for a period of 5 hr, after which they were killed with chloroform. This procedure avoided the contamination of gastric juice by eating faeces or hair.

Other animals were fasted for the same duration but after the first night of fasting, they were immobilized on their back on linoleum boards with adhesive tape. 24 hr later, the pylorus was ligated under ether anaesthesia while the animals were still immobilised and they were killed 5 hr later with chloroform.

Methscopolamine bromide was administered subcutaneously at the dose of 0·5 mg/0·2 ml (2·5 mg/kg) per injection, in saline solution. A total of three injections was given: at the time of restraint, 8 hr, and 24 hr later, with the last injection made immediately before pylorus ligation. The controls, either fasted only or restrained, received saline injections. At autopsy the oesophagus was clamped, the stomach washed with deionised water, dried with a towel and the contents emptied into a graduated cylinder through an incision of the forestomach. In methscopolamine-treated animals, there was not enough gastric juice to permit chemical analysis; therefore, 2 ml of deionised water was first injected, from a calibrated syringe, through the cardia into the stomach before dissecting it. The juice of these animals was further diluted with water up to 3 ml. The stomachs were later opened along the greater curvature and examined with a 2× magnifier for the presence of ulcerations.

GASTRIC TISSUE

The conditions of the animals were identical to those for gastric juice studies except that the pylorus was not ligated. Duration of the experiment, restraint and treatment with methscopolamine, were the same. At termination, the stomach was dissected, opened along the greater curvature and washed with lukewarm running tap water and then examined for the presence of ulcerations. The fore-stomach was separated and discarded and the antrum was cut out. Both corpus and antrum were dried separately to constant weight (overnight at 75°).

CHEMICAL ANALYSIS

Hexosamine in gastric juice and tissue was determined and used as an estimation of mucus content. A modification (Robert & others, 1963)

of Boas method (Boas, 1953) was used. Results are expressed, for the juice, in mg/ml (concentration) and mg/5 hr (output) and for the tissues in mg/100 mg of dry weight. Free and total acid output was measured using Topfer's reagent and phenolphthalein and expressed in m-equiv./5 hr.

Results

GASTRIC JUICE (Table 1)

Restraint alone reduced the volume of secretion by 34%. Administration of methscopolamine to either restrained or unrestrained rats produced a reduction of 90% from the level of saline injected animals. The output/5 hr of free and total acid was decreased by about 50% after restraint and it was almost completely inhibited (83–93%) by methscopolamine in both restrained and unrestrained animals.

Hexosamine concentration was not changed by restraint but was greatly increased (3–4 fold) by methscopolamine. Total hexosamine output, on the other hand, was reduced by 38% in restrained animals. Methscopolamine produced a 51–56% reduction in output in all rats.

TABLE 1. GASTRIC JUICE HEXOSAMINE AND ACIDITY AFTER RESTRAINT. EFFECT OF METHSCOPOLAMINE

	Unrestrained		Restrained			
	I Saline	II Methscopol-amine	III Saline	IV Methscopol-amine	% Change	P*
Exp. 1						
No. of animals	14	15	13	15		
Initial weight g	199	198	198	198		
Final weight g	165	165	164	167		
Gastric juice Volume (ml)	6·7 ± 0·3	0·7 ± 0·04	4·4 ± 0·5	0·4 ± 0·03	I–II −90 III–IV −91 I–III −34	<0·01 <0·01 <0·01
Hexosamine mg/ml	0·318 ± 0·02	1·416 ± 0·07	0·316 ± 0·02	1·583 ± 0·09	I–II +345 III–IV +402 I–III −1	<0·01 <0·01 >0·05
mg/5 hr	2·110 ± 0·1	0·933 ± 0·03	1·303 ± 0·1	0·635 ± 0·04	I–II −56 III–IV −51 I–III −38	<0·01 <0·01 <0·01
Acid output (m-equiv./5 hr) Free acid	0·681 ± 0·03	0·051 ± 0·003	0·339 ± 0·04	0·031 ± 0·002	I–II −93 III–IV −91 I–III −50	<0·01 <0·01 <0·01
Total acid	0·881 ± 0·04	0·119 ± 0·006	0·458 ± 0·05	0·077 ± 0·006	I–II −87 III–IV −83 I–III −48	<0·01 <0·01 <0·01
Ulcer incidence %	0	0	64	0		

* P value, obtained from a "*t*" test comparing the means of two groups.

GASTRIC TISSUE (Table 2)

Hexosamine concentration in restrained animals was slightly decreased in the corpus (7%); the reduction was more marked in the antrum (16%).

Both changes were statistically significant. Methscopolamine exerted the opposite effect in the corpus (increase of 14%), whereas it did not affect hexosamine content of the antrum.

TABLE 2. GASTRIC TISSUE HEXOSAMINE AFTER RESTRAINT. EFFECT OF METHSCOPOLAMINE

	Unrestrained		Restrained			
	I Saline	II Methscopolamine	III Saline	IV Methscopolamine	% Change	P*
Exp. 2						
No. of animals	15	15	15	15		
Initial weight g	195	195	195	196		
Final weight g	166	168	167	168		
Hexosamine mg/100 mg dry tissue						
Corpus	0·988 ± 0·02	1·129 ± 0·02	0·915 ± 0·02	1·044 ± 0·02	I–II +14 III–IV +14 I–III −7	<0·01 <0·01 <0·05
Antrum	2·202 ± 0·05	2·052 ± 0·06	1·849 ± 0·06	1·884 ± 0·06	I–II −7 III–IV +2 I–III −16	>0·05 >0·05 <0·01
Ulcer incidence	0	0	74	14		

* P value, obtained from a "*t*" test comparing the means of two groups.

ULCER FORMATION (Tables 1 and 2)

In both experiments the incidence of ulcers was about the same in restrained, untreated animals. It is noteworthy that the ulcers appeared only in the corpus, the antrum always being intact. Methscopolamine completely prevented ulcer formation in the first experiment; in the second, only 3 out of 15 rats had ulcers (20%) and these were single and very small. This confirms results obtained by Hanson & Brodie (1960) using other anti-acetylcholine agents.

Discussion

Inhibition by restraint of the volume and acidity of gastric juice observed in these experiments agrees with results obtained by Brodie, Marshall & Moreno (1962), although the change in acidity was greater in our experiments. It was not as marked, however, as that reported by Menguy (1960) (94%). Such minor discrepancies in these three studies can be ascribed to differences in techniques and include factors like sex, body weight, restraint procedure, interval between times of pylorus ligation and killing.

The most interesting finding was the marked reduction in hexosamine output in gastric juice (first experiment) in animals developing restraint ulcers. Hexosamine was used as an index of mucus content. There is no good method for measuring the amount of mucus, but hexosamine, although present in most mucoproteins, was found to relate very well with histological localisation of mucus in many organs (Robert & others, 1963). The results of the second experiment indicate that after a 30-hr

period of restraint, the mucosa itself synthesises less mucus than it normally does since concentration of hexosamine in the corpus and especially in the antrum was reduced. It is probable that the real diminution in corpus mucus was greater than that actually measured because this portion of the stomach contains abundant parietal and chief cells which are not expected to contain appreciable amounts of hexosamine. The antrum mucosa, on the other hand, is composed entirely of mucus glands. This conclusion is supported by determination of hexosamine in superficial scrapings of corpus mucosa, previously reported (Robert & others, 1963). Scrapings, composed almost exclusively of mucus cells, were found to contain 2·5 times more hexosamine than the whole wall of the corpus.

Although methscopolamine depressed secretory activity of the stomach measured by volume, acid and mucus output, it increased the concentration of hexosamine in the juice over 3 fold in unrestrained and 4 fold in restrained rats. This suggests that the ulcer-preventing property of this compound could be due not only to its acid-suppressing effect but also to its action on mucus. Methscopolamine is not a mucigogue, since it reduced the total output of mucus, but whatever juice was still being secreted was viscous and very rich in mucus. It is possible that such a thick secretion, spread over the mucosa, constituted a mucus barrier that contributed in preventing the development of ulcers. Piper, Stiel & Fenton (1962) observed a similar rise in mucus concentration in gastric juice of psychiatric patients given anti-acetylcholine drugs and undergoing insulin hypoglycaemia therapy. They found that the increase in mucus coincided with a fall in volume and acidity.

Finally, the fact that the antrum was not ulcerated following restraint is worth mentioning. The mucus (hexosamine) concentration of this portion is twice that of the corpus (Table 2). It is suggested that the two findings are related. Even though antrum hexosamine of restrained animals was reduced by 16%, so much remained in the mucosa that it may have been enough to protect it from ulcerating. A similar correlation was observed also in the case of ulcers due to prolonged fasting (Robert & others, 1963).

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