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INHIBITION OF GASTRIC SECRETION IN RATS BY SOME QUATERNARY DERIVATIVES OF ATROPINE

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The increasing use of quaternary amines as inhibitors of gastric secretion suggested a study of some quaternary derivatives of atropine. Visscher, Seay, Tazelaar, Veldkamp, and Vander Brook (1954) have reported that hyoscine methyl bromide is a potent inhibitor of gastric secretion in rats. The reduction of gastric secretion produced by the synthetic quaternary ammonium compounds methantheline bromide (" Banthine ") and propantheline bromide (" Probanthine ") is said to be due to an atropine-like action—at least in the doses used clinically (Johnson and Wood, 1954).

During the war a study (Ing, Dawes, and Wajda, 1945; Ing, 1946) of synthetic atropine analogues was undertaken, but it did not extend to substances containing the tropine nucleus. This was because the synthesis of atropine described by Robinson (1917) was still impracticable, owing to the cost of one of the starting substances. Newer methods of obtaining one of these substances, succinaldehyde, from furan are now available.

The present work compares the effects of atropine sulphate and some quaternary atropine derivatives on gastric secretion in rats with the pylorus tied. The drugs used were atropine methyl iodide, atropine ethyl bromide, atropine propyl iodide, atropine benzyl chloride, and atropine *p*-nitrobenzyl chloride. Atropine propyl halide and *p*-nitrobenzyl halide do not seem to have been described before. The drugs were kindly supplied by Drs. R. G. Johnston and C. G. Haining, of Messrs. T. & H. Smith, Ltd., Edinburgh.

METHODS

The method used is a modification of that of Visscher *et al.* (1954). Male albino rats (160 to 260 g. body weight) were used. They were all taken from the same colony, as rats of some strains have a poor spontaneous secretion. The animals were housed at 70° F., and fed on a pelleted diet. They were fasted in cages containing groups of six to twelve. The cages had wide-meshed wire bases to minimize coprophagy.

The pre-operative fast from solid food lasted 48 hr. During the first 24 hr. of this period the animals had a choice of two bottle-feeds, one of skimmed fresh milk to which had been added 5% glucose and 0.4% NaCl, the other of water. During the next day, the animals had a choice of 5% glucose, 0.4% NaCl, and plain water. On the night before operation the animals had a choice of three drinks—0.4% NaCl, 0.2% NaCl, and water. The rats were shaved on the day before the fast, and were weighed at the end of the fasting period.

Under methyl-*n*-propyl ether and ethyl ether anaesthesia, a 4 cm. midline abdominal incision was made, and the wound held open with self-retaining eyelid retractors. A ligature was placed around the duodenum near the pylorus, in such a way as to avoid trauma to the vessels. Five ml. of 0.8% NaCl was placed in the peritoneal cavity after the closure of the muscle layer with three silk sutures. The skin wound was closed with Michel clips and painted with collodion. The operation usually lasted 3 min.

The drug, in aqueous solution, was injected intramuscularly or intraduodenally in volumes of 0.1 or 0.2 ml./100 g. body weight. The rats were deprived of water and confined in separate cages during the collection period, which was 3 hr. both with the intramuscular and intraduodenal injections. The animals were then killed with chloroform, the abdomen was opened, and the stomach was removed. Gastric fluid was placed in a graduated centrifuge tube, and the fluid volume measured after centrifugation. Gastric fluid is defined as the total gastric fluid volume. There was sometimes contamination with faeces and blood, and the fluid must consist in part of saliva. Nevertheless, the control fluid had usually a *p*H in the range 1.4 to 2.2.

The volume of the gastric fluid in the treated animals, at several dose levels, was compared with that found in simultaneously operated and sham-injected controls. Groups of at least four rats were used at each dose level ; the mean number of rats at each dose level was about six.

RESULTS

Intramuscular Injections.—The dose-response lines in Fig. 1a and b relate the dose given by intramuscular injection to the mean gastric fluid volume

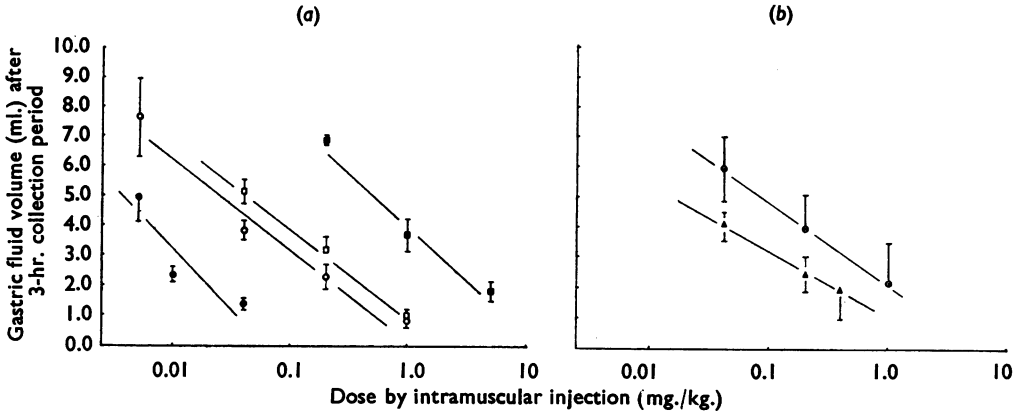

FIG. 1*a* and *b*.—Dose-response relations for inhibition of gastric secretion in rats after intramuscular injection of various atropine derivatives. The lines were drawn by eye. Vertical bars represent the standard errors of the means. Symbols: O, sulphate; ⊙, benzyl chloride; ●, methyl iodide; ▲, *p*-nitrobenzyl chloride; □, ethyl bromide; ■, propyl iodide.

at the end of a 3 hr. collection period. The vertical bars represent the standard error of the mean. The mean control secretion was 4.8 to 7.3 ml. No correction is made for the differences in molecular weight of the various derivatives.

Approximate ED50 values, calculated from these data, give the relative potencies listed in Table I, where atropine is assigned an arbitrary potency of 1.0. Atropine methyl iodide is the most potent of the drugs tested. Atropine ethyl bromide and atropine *p*-nitrobenzyl chloride are of the same order of activity parenterally as atropine sulphate, and atropine benzyl chloride and atropine propyl iodide are notably less active.

Intraduodenal Injections.—The results of the assays in which the substances were injected intraduodenally are indicated in Fig. 2a and b. If Fig. 2b is compared with Figs. 1a and 2a it will be seen that atropine methyl iodide, although exceedingly potent parenterally, is less potent than atropine sulphate by the duodenal route.

TABLE I

INHIBITION OF GASTRIC SECRETION: APPROXIMATE POTENCY OF QUATERNARY ATROPINE DERIVATIVES BY INTRAMUSCULAR INJECTION

Atropine Derivative	Approx. ED50 (μg./kg.)	Rel. Potency (Atropine = 1·0)
Sulphate	130	1·0
Methyl iodide	12·5	10
Ethyl bromide	220	0·6
Propyl iodide	2,100	0·06
Benzyl chloride	950	0·14
p-Nitrobenzyl chloride	300	0·4

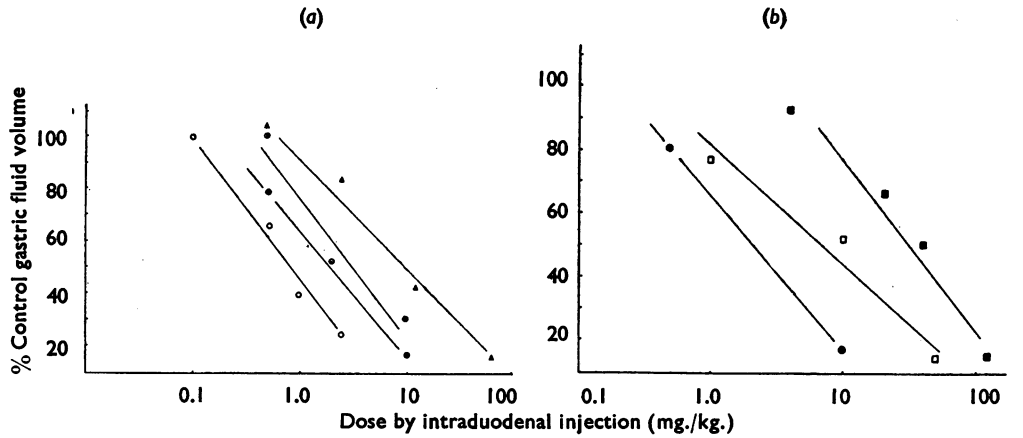

FIG. 2*a* and *b*.—Dose-response relations for inhibition of gastric secretion in rats after intraduodenal injection of various atropine derivatives. Symbols: O, sulphate; ⊙, benzyl chloride; ●, methyl iodide; ▲, *p*-nitrobenzyl chloride; □, ethyl bromide; ■, propyl iodide.

The third column of Table II shows that the ratio of the intraduodenal ED50 to the intramuscular ED50 for the methyl-, ethyl-, and propyl-quaternary derivatives of atropine is lowest for the propyl derivative and highest for the methyl derivative.

TABLE II

INHIBITION OF GASTRIC SECRETION: APPROXIMATE POTENCY OF QUATERNARY ATROPINE DERIVATIVES BY INTRADUODENAL INJECTION; RATIO OF INTRADUODENAL ED50 TO INTRAMUSCULAR ED50

Atropine Derivative	Approx. ED50 (μg./kg.)	Rel. Potency (Atropine = 1·0)	Duodenal ED50 / i.m. ED50
Sulphate	800	1·0	6·2
Methyl iodide ..	1,900	0·4	160
Ethyl bromide ..	6,500	0·1	30
Propyl iodide ..	26,000	0·03	12
Benzyl chloride ..	3,100	0·25	3·3
p-Nitrobenzyl chloride	9,800	0·1	33

DISCUSSION

The use of a preparation in which the pylorus is ligated, and the animal allowed to recover from the anaesthetic and collect juice in its stomach for a number of hours, derives from the studies of Shay, Komarov, Fels, Meranze, Gruenstein, and Siplet (1946) concerning the production of gastric ulcers in rats. Most of the literature of twenty-odd papers on the method deals with the production of ulcers or with the prevention of ulceration.

The basis of the method is the observation of Shay *et al.* (1946) that substantial quantities of gastric juice are secreted only if the animals are quickly operated on under light anaesthesia and are conscious during the collection period. The rate of secretion under continuous urethane anaesthesia is less than 0.1 ml./100 g. body weight/hr. (Komarov, Shay, Rayport, and Fels, 1944). Inhibition of secretion of juice following pyloric ligation under light barbiturate anaesthesia was used as a measure of enterogastrone activity by Friedman and Sandweiss (1946) and by Visscher (1948). The former used a 90-minute collection period, and the latter two hours: both gave the hormone intravenously.

Atropine and methantheline were assayed by Visscher and Tazelaar (1951) in rats in which the pylorus had been ligated under ether. The drugs were injected intravenously, and a two-hour collection period was used. In the study of hyoscine methyl bromide by Visscher *et al.* (1954), the drugs were given intravenously with collection of juice in two hours, and intraduodenally with collection of juice in three hours. After intravenous injection the action lasted only two hours, so that a longer collection period could not be used.

In the present work, the parenteral assay involved the intramuscular injection of the drug. This gives for atropine an ED50 comparable to that obtained by Visscher *et al.* (1954) by the intravenous method, and admits of the use of a three-hour collection period. Thus one avoids the prolongation of anaesthesia for a femoral vein injection, or the loss of rats from an experiment because of failures with tail-vein injections. It enables one to perform the assay with a mean control secretion of about 6 ml. from rats of about 200 g. body weight. Control secretion rates of the order of 2 ml./100 g. body weight/hr., such as are needed for the two-hour assay, are hard to obtain consistently even with rats of a good strain. In this work, as in the experiments of Visscher *et al.* (1954), some experiments have had to be excluded because the mean control secretion was less than 4 ml. This may cause serious waste of animals with some strains. Two features of the present method have helped to get over this difficulty. First, there is the use, already mentioned, of a three-hour collection period for the parenteral assay. Second, it was found that attention to the adequate nutrition and hydration of the animals during the fast from solid food greatly improves their performance. Furthermore, if rats with an average secretion of gastric juice are injected with 2.5 mg. cortisone acetate on the day of the experiment, the production of highly acid gastric juice is increased (unpublished observations); this phenomenon is being investigated and will be reported on in detail elsewhere.

Since complete vagotomy, when performed as an acute experiment, abolishes the interdigestive secretion in rats (Komarov, Shay, and Gruenstein, 1947; Harkens, 1947; Madden, Ramsburg, and Hundley, 1951), and reduces it by 80% to 90% in the chronically vagotomized preparation (Komarov *et al.*, 1947), it is clear that the secretion inhibited in this assay procedure is comparable with the night secretion of the duodenal ulcer patient. Vagotomy reduces the night secretion in these patients by 80% (Dragstedt, Woodward, Storer, Oberhelman, and Smith, 1950). This would seem to mark the procedure as one particularly relevant in the search for an agent which will be useful in controlling the hyperchlorhydria of these patients.

Ing (1946) showed that the methyl salts of the belladonna alkaloids, though intrinsically more potent than the corresponding tertiary compounds, are usually less potent locally as mydriatics. A similar impairment of activity in depressing gastric secretion in rats, apparently due to imperfect

absorption, was noted by Visscher *et al.* (1954) when hyoscine methyl bromide was given by the intestinal route.

The relative inactivity of the methyl derivative of atropine given as a mydriatic locally (as the methonitrate ; Ing, 1946) is in line with its relative inactivity by the intestinal route in depressing gastric secretion (Table II, col. iii). However, relative inactivity by the intestinal route does not seem to be a general feature of quaternary derivatives of atropine. The duodenal-intramuscular ED50 ratio of the propyl derivative is of the same order of magnitude as that obtained with atropine sulphate (Table II).

Summary

1. A method is described of studying the inhibition of gastric secretion in rats with the pylorus ligated.

2. Atropine methyl iodide, atropine ethyl bromide, atropine propyl iodide, atropine benzyl chloride, and atropine *p*-nitrobenzyl chloride were compared with atropine sulphate, by an enteral and a parenteral route on this preparation.

3. Parenterally, atropine methyl iodide is the most active derivative. Enterally, the sulphate is more active than any of the quaternary derivatives; of the latter, the methyl iodide is the most active.

4. The relative inactivity of the methyl derivative by the intestinal route is not a general characteristic of quaternary atropine derivatives.

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