THE ACTION OF PROPIONYL ATROPINE METHYL NITRATE ON GASTRIC FUNCTION

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Received January 22, 1958

A quaternary compound, propionyl atropine methyl nitrate, previously demonstrated to have muscarinic and ganglionic blocking properties has been shown to decrease the volume and acidity of the gastric juice in man. Using a variation of the glucose tolerance test the compound was also shown to reduce the motility of the alimentary tract. Side effects due to parasympathetic blockade were in most cases absent at the doses used. A clinical trial with 26 cases of peptic ulcer has resulted in a complete alleviation of symptoms without the production of side actions. In six cases the trial has extended for six months without the development of tolerance.

In a previous paper[1] several quaternary ammonium esters of atropine, were shown to possess both muscarinic and ganglionic blocking activity. This combination of these properties in one molecule together with the capacity to inhibit gastric secretion, in the Shay Rat preparation suggested their use for the symptomatic treatment of peptic ulcers. This paper attempts an investigation of the effect of one of these esters on the gastric secretion and gastric motility in man. The results were compared with those obtained with atropine, atropine methyl bromide and hyoscine methyl bromide. The trial substance propionyl atropine methyl nitrate (PAMN) checked both secretion and motility with an almost complete absence of side actions.

Screening a potential drug for the treatment of peptic ulcer is fraught with difficulties. This applies both to the measurement of the secreted acid and the motility of the alimentary canal. The problem is discussed by Bachrach and others[2]. In view of the difficulties we decided on simplicity of method. The histamine-stimulated secretion test was rejected as this procedure could give erroneous results, since the physical discomfort produced by histamine or the apprehension of an injection in untrained volunteers could result in a variable inhibition of gastric secretion. We therefore decided to use the alcohol test meal.

The direct measurement of motility is even more unsatisfactory, especially without the services of a gastroenterological unit. The dependence on volunteers makes it necessary to employ an indirect method which will cause little or no discomfort. Indirect methods are discussed by Bachrach and others but as none appeared to offer any advantages the following method was devised.

On the assumption that the stomach itself absorbs little or no sugar the initial rise of blood sugar after the ingestion of a large amount of glucose or sucrose depends upon the ingested sugars reaching the duodenum. Any lack of motility of the stomach would thus delay the rate of rise of blood sugar. Similarly a lessening of intestinal motility would be expected to enhance this effect. A flattening of the blood sugar curve would

also be expected since the slower rate of absorption would permit of a more effective control by the insulin secretory mechanism of the endocrine system.

To demonstrate that the slower rate of sucrose absorption was not solely due to a decrease in acid hydrolysis, or to a decrease in the enzymatic splitting activity of the gastric mucosa, both glucose and sucrose tests were made. The results were more consistent than might be expected and we find that the glucose tolerance test could be extended to yield evidence of alimentary movement with a minimum of discomfort to the subject.

TABLE I

THE pH OF INDIVIDUAL GASTRIC SAMPLES AFTER SINGLE ORAL DOSES

Subject I.

Time minutes	Control no drug	Atropine 1·3 mg.	Propionyl atropine methyl nitrate				Valeryl atropine methyl bromide		Atropine methyl bromide 8 mg.	Hyoscine methyl bromide 8 mg.
			2 mg.	4 mg.	6 mg.	8 mg.	5 mg.	8 mg.		
Fasting	1·47	1·82	1·88	2·19	1·74	1·32	2·16	1·45	1·51	1·54
0	?	2·27	2·37	2·36	2·43	2·62	2·49	2·48	2·65	2·30
15	1·47	1·58	1·75	1·72	1·72	1·49	1·33	1·57	1·48	1·55
30	1·21	1·48	1·40	1·63	1·40	1·34	1·25	?	1·35	1·38
45	1·20	2·02	1·47	1·58	1·38	1·42	1·24	1·46	1·47	1·31
60	1·30	2·38	1·42	1·40	1·66	2·0	1·59	1·55	2·60	1·54
75	1·53	2·74	1·48	3·26	4·42	6·4	1·95	2·0	2·84	1·95
90	1·55	2·81	1·44	3·92	6·40	6·53	2·06	3·2	3·16	3·7
105	1·40	3·19	1·57	4·16	5·73	6·14	2·08	5·42	2·48	2·89
120	1·47	2·3	1·75	2·34	3·48	6·25	1·50	4·79	2·23	2·30
135		2·3		1·95	2·68	3·84	2·79	5·20	2·50	2·15
150		2·0			3·20	2·55	2·82	3·60	2·00	2·30
TOTAL ACID (ML. OF 0·1 N NaOH REQUIRED TO NEUTRALIZE 100 ML. OF STOMACH CONTENT)										
Fasting	4·36	58·5	37·6	45·6	43·0	98·8	20·0	52·6	51·4	50·6
0	?	9·6	10·0	10·0	52·0	5·0	3·5	7·8	5·2	10·6
15	50·4	58·0	36·0	49·6	40·6	44·0	60·5	37·0	42·4	38·6
30	50·0	26·2	68·4	67·4	64·8	70·8	79·0	—	63·0	58·2
45	54·2	25·6	78·6	76·0	49·0	67·6	—	52·0	51·6	70·6
60	55·4	24·0	78·0	52·4	3·2	40·0	44·2	44·0	37·0	49·6
75	68·0	29·0	75·4	11·2	8·2	5·0	10·2	—	16·0	31·0
90	61·8	36·0	49·6	11·4	3·2	5·0	25·8	7·6	15·6	—
105	48·2	41·8	58·0	9·2	6·4	8·4	14·8	8·8	23·0	16·6
120	60·6	40·2	42·0	16·8	11·6	5·6	56·6	11·6	26·4	24·0
135				23·6	15·8	15·6	12·8	10·0		—
150						20·4	15·6	15·0		70·0

METHODS

Acid secretion. The antisecretory effect of a single oral dose on the secretion of the stomach in response to an alcohol test meal was estimated. The procedure was as follows.

All subjects fasted at least 8 hours previous to the test. Because of large individual variations in response to an alcohol test-meal each subject acted as his own control. The fasting content of the stomach was completely aspirated through a Rehfuss tube and any residual matter washed out with 20 ml. of distilled water at 37°. Immediately after this, the warmed test meal, with or without dissolved drug, was passed down the tube. A 10 ml. sample was then immediately withdrawn and thereafter a further 10 ml. every 15 minutes for 2 to 2½ hours. Estimations of free acidity, total acidity and pH were made on all samples including the

fasting sample. The acidities were obtained by titration with a 0·1N solution of NaOH using appropriate indicators; free acidity using Töpfer's reagent (0·5 per cent dimethylaminoazobenzene, end point approximately pH 2·8) and total acidity using phenolphthalein (end point approximately

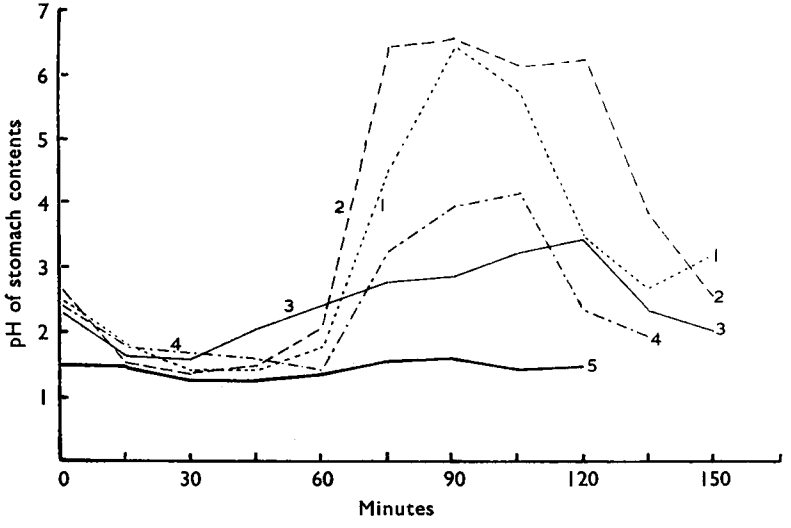

FIG. 1. Effect of various doses of PAMN of pH of human gastric contents. 1. PAMN 6 mg. 2. PAMN 8 mg. 3. Atropine 0·3 mg. 4. PAMN 4 mg. 5. Control.

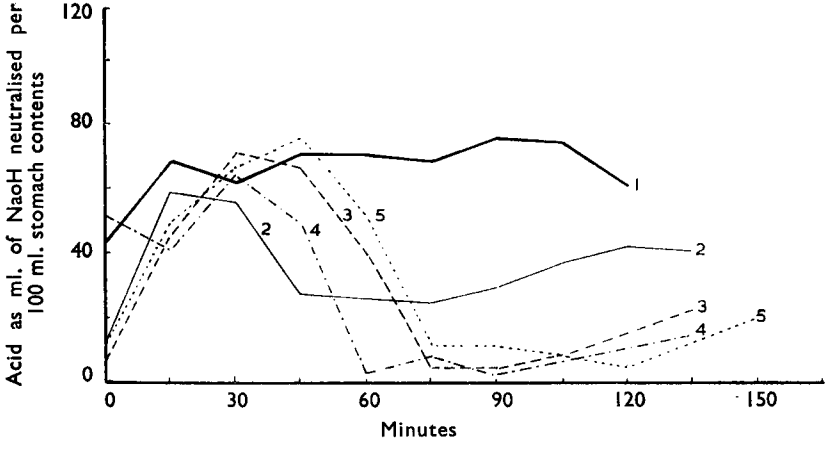

FIG. 2. Effect of various doses of PAMN on the acidity of the gastric contents (same subject as in Fig. 1). 1. Control 2. Atropine 0·3 mg. 3. PAMN 4 mg. 4. PAMN 6 mg. 5. PAMN 8 mg.

pH 7). All acid results were expressed as ml. of 0·1N NaOH required to neutralise 100 ml. of stomach content. As continuous aspiration apparatus was not available, the volume of secretion for each time interval could not be obtained.

Motility. Each subject acted as his own control, and different drugs were tested on separate days. The drugs were swallowed in tablet form with the aid of a little water 30 to 45 minutes before administration of the sugar. Immediately before the ingestion of 50 g. of the sugar (dissolved in 100 ml. of water), 0·05 ml. blood was taken from a

TABLE II

SUMMARY OF HUMAN GASTRIC SECRETION EXPERIMENTS

Total number subjects tested	Drug single oral dose	Dry mouth (side effect)	Maximum pH (average)	Average duration of maximum pH minutes	Decrease of secretion (qualitative)
6	Atropine 0·65 mg.	++	3·8	30	+ (?)
5	Atropine methyl bromide 8 mg.	++	4·5	30	++
6	Propionyl atropine methyl nitrate 8 mg.	+ (?)	4·9	60	++++
6	Hyoscine methyl bromide 8 mg.	++	3·2	30	+ (?)

finger and 5 similar samples were taken at 15 minute intervals after the ingestion of the sugar. The glucose content of the blood samples was estimated by a sensitive colorimetric method requiring small samples of blood only. This facilitated the accurate timing of the blood samples. Each sample was diluted with distilled water (5 ml.) and the protein precipitated with $Zn(OH)_2$. The samples were then centrifuged and two

TABLE III

THE EFFECT OF DRUGS ON THE UPTAKE OF GLUCOSE AND SUCROSE FROM THE ALIMENTARY TRACT (MG. GLUCOSE/100 ML. BLOOD)

Time minutes	50 g. glucose	50 g. glucose + 8 mg. PAMN	50 g. glucose + 5 mg. pipenzolate	50 g. glucose + 15 mg. propantheline	50 g. glucose + 0·65 mg. atropine	50 g. sucrose	50 g. sucrose + 8 mg. PAMN
0	103	101	82	95	109	90	95
15	102	119	109	108	132	87	?
30	127	131	95	107	124	117	122
45	168	126	90	116	149	153	105
60	136	?	103	105	114	139	120
75	136	102	84	100	104	115	100
Maximum Rise	65	30	27	21	40	63	27

aliquots (2 ml.) of clear supernatant liquid were taken. To each aliquot, chilled in an ice bath, a 3 ml. aliquot of anthrone solution (0·02 per cent in 95 per cent sulphuric acid) was added, and the colour of the mixture developed for exactly 7 minutes in a boiling water bath. Colour intensities were compared with the colour intensity of a known standard glucose solution using a Beckman spectrophotometer (wavelength 620 mμ)[3].

RESULTS

Gastric acidity. Comparisons of the test drug with atropine, atropine methyl bromide and controls were made in 76 experiments on 6 subjects. Tests with different drugs were done on different days. The variation noted from time to time in one individual was less than the variation between different subjects.

The results of a typical experiment are given in Table I and Figures 1 and 2. The results of 76 experiments on 6 persons in Table II. All subjects in whom 0·65 mg. atropine caused a rise in gastric pH and some decrease in acid secretion showed to the quaternary derivative a more pronounced response of longer duration. At pH 4·5 or more gastric pepsin is inactive. The blocking of the proteolytic enzyme greatly aids in the healing of gastric lesions. In most of the experiments in which the quaternary derivative was given, difficulty in obtaining some of the later 10 ml. samples was noted, indicating a qualitative decrease in secretion. This was not noted in either the control or atropine experiments.

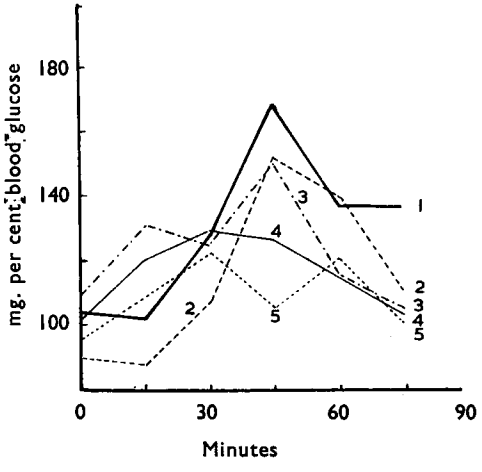

FIG. 3. Effect of PAMN on the uptake of glucose and sucrose from the human alimentary canal. 1. Glucose control. 2. Sucrose control. 3. Glucose × 0·65 mg. of atropine. 4. Glucose + 8 mg. of PAMN. 5. Glucose + 8 mg. of atropine.

Motility. 100 experiments on 8 persons were carried out. The results of a typical experiment are shown in Table III and Figure 3.

PAMN proved to be active in seven of the eight subjects tested. The results obtained from the eighth subject, a diabetic, were more variable but nevertheless in the majority of cases PAMN lessened the rise in blood sugar. The same tests were made on two of the subjects after the previous administration of 0·65 mg. atropine which proved to be about two thirds as effective as PAMN in each case, i.e. the rise in blood sugar after atropine was always less than that of the control but was greater than that obtained after PAMN. Thus PAMN produced a greater immobility of the alimentary tract and there was evidence that its action outlasted that of atropine.

Effect on blood pressure and heart rate. Ten volunteers were given 6 mg. of PAMN and the blood pressure and pulse rate measured for the next 3 hours. The results were variable, however the pulse rate increased by about five per minute in a number of instances. The systolic and diastolic pressures rose in some volunteers by about 5 mm. Hg. But the same variable results were obtained when the volunteers were tested again with placebos.

Clinical Trial. Two separate preliminary clinical trials have been made with PAMN at a dose of 6 mg. 6 hourly. No side actions were observed and no disturbances of micturition in any of the 26 patients. In both trials, one with 15, the other with 11 patients, the alleviation of symptoms has been satisfactory. Six patients have been treated for six months without any tolerance developing.

Discussion

Two tests, applicable to human volunteers, were used to evaluate a quaternary ammonium derivative of atropine as a potential therapeutic agent for the relief of distress in peptic ulceration. The two tests were the alcohol test meal and the glucose tolerance test, the latter to determine the effect on alimentary motility. These gave results which are in remarkable agreement with one another, especially when made on the same subject.

The drug reduces the volume and acidity of the gastric juice and decreases the motility of the intestine. The decrease in volume of the gastric juice was often accompanied by an increase in mucous content as well as by a considerable rise in pH, which was too great to be attributed to swallowed saliva. Normal gastric secretion consists of a mixture of three secretions obtained from three distinct types of cells which may not be equally affected by the drugs. Perhaps in our case a greater inhibition of the acid-secreting cells than of the mucin-secreting cells was obtained. The secretion of the latter, which is alkaline in nature, also acts as a buffer because of its high protein content. The only untoward effect noticed has been a slight drying of the mouth in 19 experiments out of 171. There were no disturbances of vision or micturition. The slight degree of inhibition of salivation was noticed with doses of 8 mg. when the stomach was empty, a state which would increase the absorption of the drug. It was not noticed in patients receiving normal meals and at a dose of 6 mg.

The novel feature of the tests is the use of the glucose tolerance test to measure alimentary motility. The lower blood sugar levels which were obtained consistently when atropine or its derivatives were given, strongly supported our hypothesis that these reduced levels were due to lessened alimentary activity. Similar results obtained in the sucrose tolerance tests also supported this concept and as they were little, (if at all) different from the glucose tolerance tests it would appear that acid hydrolysis of the cane sugar in the stomach contributed little to the results.

The experiments on the diabetic volunteer were also interesting. This patient was an experienced research worker who fully understood the nature of his condition and was well stabilised. Fifteen experiments (alcohol test meals and glucose tolerance tests) were carried out, and in 6 cases the volunteer experienced an hypoglycaemic attack either during, or after the performance of the test. This would strongly suggest that the PAMN was delaying the uptake of sugar, presumably by decreased motility. Such evidence provides further support for our contention that the glucose tolerance test may be used as an index of alimentary motility.

Lieber[4] has compared thoroughly the effects of atropine, propantheline (Probanthine) and oxyphenonium (Antrenyl) on the post insulin and basal gastric secretion. At the same time he has studied the antisecretory activity and the side actions due to parasympathetic inhibition. Propantheline has most effect in accelerating the pulse. The other side actions parallel the effect on the heart and in decreasing order of activity of drug are propantheline, oxyphenonium and atropine. The gastric antisecretory activity does not follow the effects on salivary secretion etc.

For comparable antisecretory effect atropine has the least side actions. As our results show that, for equivalent antisecretory effect, PAMN has less side effects than atropine one would expect that PAMN has less side actions than either propantheline or oxyphenonium. Preliminary trials bear this out.

Finally we have not as yet tried the effect of PAMN on the "basal" secretion. In many ways "basal" secretion is a logical rather than physiological concept, in that it depends upon a definition. Lieber[4] has shown that atropine lessens the basal secretion and as PAMN is a close chemical derivative of atropine and has been shown to retain the anti-muscarinic actions of atropine[1] it might be assumed that PAMN would have a similar inhibitory action on the basal secretion.

PAMN would appear to have little effect on the circulatory system, both in volunteers and peptic ulcer patients.

Acknowledgements. We wish to thank Dr. Ian Wood, Director of the Clinical Research Unit, Royal Melbourne Hospital, for the preliminary notes of the first clinical trial and Dr. S. Gershon for his assistance in the second trial.

Propionyl atropine methyl nitrate is subject to Australian Patent No. 23270/56 and other patents.

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