RESEARCH PAPERS

THE PHARMACOLOGY OF PROPIONYL ATROPINE METHYL NITRATE

BY LAURA HERMAN, F. H. SHAW AND E. I. ROSENBLUM

From the Department of Pharmacology, University of Melbourne

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A series of esters of atropine and hyoscine, both in the tertiary and quaternary form, have been examined for their muscarinic and ganglionic blocking and neuromuscular transmission inhibiting activity. In addition the effect on the gastric secretion has been studied by the Shay rat method. Figures are also given for toxicities. In general, the pharmacology of the quaternary derivatives is qualitatively and quantitatively the same as the parent substances. The quaternary compounds retain a considerable amount of their tertiary activity but in addition become relatively strong ganglionic blocking agents. Most of the compounds reduce the volume and acidity of the gastric juice.

IN recent years several new compounds have been introduced into medicine for the symptomatic relief of peptic ulcers. Most are quaternary ammonium compounds differing pharmacologically from the natural and synthetic tertiary amines in producing their effects by ganglionic blockade, whilst the tertiary amines block the effector organs innervated by post-ganglionic cholinergic nerves. In an earlier paper we have shown that the acylation of hyoscine produced esters whose action was prolonged but less marked than atropine on the central nervous system[1]. It therefore seemed of interest to prepare the atropine analogues and study their pharmacology. It was possible also to prepare the quaternary derivatives of these esters. While quaternisation would be expected to profoundly alter the pharmacological properties of the compounds, perhaps even to the extent of producing a curariform action[2], these quaternary compounds have been shown to retain some of their original pharmacological properties together with mild ganglionic blocking powers, the neuromuscular junction being only slightly affected.

METHODS

Materials used. Atropine sulphate, atropine methyl bromide (AMB), hyoscine hydrobromide, propionyl atropine methyl nitrate (PAMN), valeryl atropine methyl bromide (VAMB), hyoscine methyl bromide, *N*-butyryl hyoscine bromide, (Buscopan), oxyphenonium (Antrenyl), pipenzolate (Piptal), and pentamethonium (C5).

Isolated Intestine

The spasmolytic potency of the test drug compared with atropine in inhibiting acetylcholine-induced spasms of guinea pig isolated ileum was used as an index of post ganglionic cholinergic nerve blocking action. The dose of acetylcholine—0·1 ml., concentration range 10^{-6} to 10^{-7} w/v causing a sub-maximal contraction of the ileum was determined. For

each preparation the concentration of atropine and then test drug which when left in the bath for 30 seconds partially or completely inhibited for 15 to 25 minutes this acetylcholine induced contraction of the ileum was determined. All drug solutions were made up on a molecular basis.

TABLE I

ANTI-ACETYLCHOLINE ACTIVITY OF SOME ATROPINE ESTERS ON THE GUINEA PIG ILEUM

Drug	No. of experiments averaged	Molar concentration	Inhibition Time (minutes)
Hyoscine hydrobromide	13	$0{\cdot}22 \times 10^{-8}$	12
Atropine hydrobromide	13	$0{\cdot}5 \times 10^{-8}$	12
Atropine methylbromide ..	5	$0{\cdot}6 \times 10^{-8}$	15
Acetylatropine hydrobromide ..	5	$0{\cdot}7 \times 10^{-8}$	12
Acetylatropine methylbromide	3	$0{\cdot}5 \times 10^{-7}$	16
Propionylatropine hydrobromide	5	$0{\cdot}75 \times 10^{-8}$	13
Propionylatropine methylbromide	4	$0{\cdot}2 \times 10^{-7}$	13
Valerylatropine methylbromide	1	$0{\cdot}2 \times 10^{-8}$	15

Using this method each preparation acted as its own control against atropine, and so eliminated errors due to the variation in sensitivity of different preparations.

TABLE II

GANGLIONIC BLOCKING ACTIVITY OF SOME DRUGS ON THE CAT SUPERIOR CERVICAL GANGLION

Cat. No.	Drug	Dose mg./kg.	Time in minutes	
			total block	partial block
1	PAMN	1	4	11
	Atropine sulphate	1	No block	10
	AMB	1	5	6
3	PAMN	1	No block	9
	Atropine sulphate	1	,,	No block
	AMB	1	,,	8
	VAMB	1	,,	4
	Buscopan	1	,,	5
	Hyoscine MeBr	1·7	,,	No block
4	PAMN	1·7	4	8
	Atropine sulphate	1·7	No block	4
	Hyoscine MeBr	0·7	,,	No block
		1·3	,,	6
		3·3	,,	8
	AMB	0·7	,,	6
		1·7	,,	13
	PAMN	0·7	,,	9
		1·7	,,	15
	VAMB	0·7	,,	11
		1·7	,,	15
	Oxyphenonium	1·7	,,	No block
		3·3	,,	4
	Pipenzolate	3·3	,,	5
	PAMN	1·7	,,	8
		3·3	,,	13
	C 5	3·3	,,	30
6	Hyoscine MeBr	1·0	,,	8
	PAMN	1·7	2	11
	AMB	1·7	1	10
	VAMB	1·0	No block	6
	Pipenzolate	1·0	,,	7
	Oxyphenonium	1·0	,,	7
	PAMN	1·0	,,	11
	AMB	1·0	,,	14
	C 5	1·0	,,	37

Total block means the time during which there is no contraction of the nictitating membrane, partial block means the time from the first signs of recovery until this is complete.

Cat Superior Cervical Ganglion

The ganglion was dissected with its accompanying pre- and post-ganglionic fibres (the sympathetic were carefully separated from the parasympathetic fibres). The drugs were injected intravenously and the contraction of the nictitating membrane was recorded after appropriate stimulation of the pre-, and post ganglionic fibres.

Shay Rat

The operative technique of Shay and others[3,4] was followed. The rats were anaesthetised with 20 mg./kg. of pentobarbitone i.p. As gastric secretion does not take place whilst the animal is unconscious, 20 mg./kg.

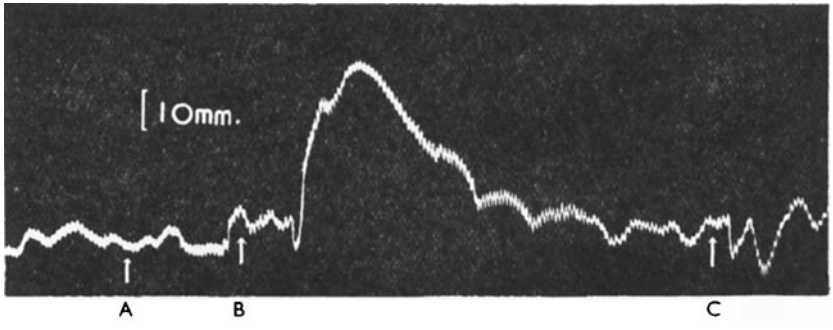

FIG. 1. A, 12.30 p.m. PAMN 0·17 mg./kg; B, 1.10 p.m. nicotine 0·2 mg./kg.; C, 1.33 p.m. PAMN × nicotine 0·17 and 0·2 mg./kg.

of bemegride was injected i.p. at the end of the operation and caused an almost immediate arousal of the animals. The drugs used were injected subcutaneously, immediately at the completion of the operation.

Neuromuscular Junction

The neuromuscular blocking action of PAMN was tested on the rat phrenic nerve-diaphragm preparation[5].

Toxicity

The acute toxicity was estimated on mice by the intraperitoneal route and the LD50 was calculated by the standard probit technique. Semi-acute toxicity tests were carried out on mice and rats by injecting the animals intraperitoneally twice a day for five days at a dose ratio of 0·56 of the LD50 to mice.

RESULTS

Isolated Intestine

The anticholinergic activity of the atropine derivatives is given in Table I. The results indicate that the degree of activity of hyoscine is about twice that of atropine and that esterification does not affect the activity quantitatively or qualitatively. However quaternisation of the esters does result in a decrease in spasmolytic activity.

Superior Cervical Ganglion

The ganglionic blocking activity of the atropine derivatives is shown in Table II. The order is hyoscine methyl bromide < *N*-butyryl hyoscine bromide, oxyphenonium, pipenzolate < AMB, PAMN < VAMB, < C5. Thus PAMN has marked ganglionic blocking activity but is not as

TABLE III

THE EFFECT OF DRUGS ON BLOOD PRESSURE OF THE ANAESTHESTISED CAT

Drug	Dose mg./kg.	Fall mm.Hg	Duration minutes
Hyoscine MeBr ..	1	33	4
PAMN	1·7	48	9
AMB	1·7	41	11
VAMB	1	35	10
Pipenzolate.. ..	1	23	5
Oxyphenonium ..	1	15	5
PAMN	1	35	10
AMB	1	37	8
C 5	1	36	45
Hyoscine MeBr ..	1	33	4

powerful as pentamethonium. The site of action of PAMN is at the ganglion because stimulation of the post ganglionic fibres, when the ganglion is blocked, still results in contraction of the nictitating membrane.

PAMN and Nicotine. As is well known nicotine stimulates all sympathetic ganglia, producing contraction of the nictitating membrane and a rise in the blood pressure.

TABLE IV

SHAY RAT EXPERIMENT

Drug dose, 350 mg./kg.	Number experiments averaged	Collection time hours	Analysis of Stomach Content		
			total volume	pH	m.eq. of acid per kg.
Atropine hydrobromide	17	22	1·2	3·9	0·329
Atropine methyl bromide	4	20	1·7	3·8	0·420
Methyl atropine methyl bromide ..	1	22	?	?	0·139
Acetyl atropine hydrobromide	4	21	0·95	4·0	0·480
Acetyl atropine methyl bromide ..	4	20	0·48	5·2	0·277
Propionyl atropine methyl bromide ..	7	22	1·54	5·8	0·227
Propionyl atropine methyl nitrate ..	8	22	1·4	4·4	0·206
Valeryl atropine methyl nitrate	9	21	1·2	3·3	0·471
Hyoscine hydrobromide	7	21	2·2	2·9	1·201
Acetyl hyoscine hydrobromide	3	20	1·95	3·6	0·368
Hyoscine methyl bromide	6	21	1·50	4·6	0·508
Propionyl hyoscine hydrobromide ..	7	21	1·84	3·1	0·981
Butyryl hyoscine hydrobromide ..	7	22	2·3	3·5	0·662
Valeryl hyoscine hydrobromide	3	21	1·17	4·1	0·398
*iso*Valeryl hyoscine hydrobromide ..	9	21	2·0	3·5	0·844
Benzoyl hyoscine hydrobromide (250 mg./kg.)	4	21	1·6	5·6	0·507
Benzoyl hyoscine hydrobromide (220 mg./kg.)	1	21	4·2	3·0	2·579
Benzoyl hyoscine hydrobromide (157 mg./kg.)	1	21	1·7	3·5	0·686
Acetyl hyoscine amino oxide hydrobromide	2	21	2·7	2·5	0·620
Acetyl hydrocyamine hydrobromide ..	2	21	3·3	2·5	0·852
Acetyl homatropine hydrobromide ..	2	22	2·6	2·3	0·950
Benzoyl homatropine hydrobromide (200 mg./kg.)	2	22	0·7	5·8	0·084
No Drug	3	22	9·7	<2·0	2·541

If the PAMN and nicotine were injected simultaneously the results were variable; the usual pattern was a rise in blood pressure and contraction of the nictitating membrane less than that given by the control dose of

nicotine. The control doses of PAMN had little effect on the blood pressure and no effect on the nictitating membrane. One interpretation of these results would be that the PAMN was antagonising the effect of nicotine at the ganglia (see Fig. 1). When PAMN itself produced a fall

TABLE V

SUMMARY—SHAY-RAT EXPERIMENT

Drugs which produced a pH of 6 or more in rat stomach.

Drug	Number of experiments	pH 6 or more
Atropine hydrobromide	17	2 (pH 7·3, pH 8·0)
Acetyl atropine methyl bromide ..	4	1 (pH 8·5)
Propionyl atropine methyl bromide ..	7	3 (pH 8·5, pH 7·0, pH 7·5)
Propionyl atropine methyl nitrate ..	8	1 (pH 8·8)
Hyoscine methyl bromide	6	1 (pH 7·9)
*iso*Valeryl hyoscine hydrobromide ..	9	1 (pH 6·4)
No Drug (Control)	3	0 (pH < 2 in all cases)

in blood pressure of any magnitude it could be argued that the anullment of the blood pressure rise due to the nicotine plus PAMN was the result of the summing of the effects peripherally.

Effect on Blood Pressure

All the compounds tested gave a fall in blood pressure (Table III). In general the results on the blood pressure paralleled the ganglionic blocking activity of the compounds.

TABLE VI

THE EFFECT OF SOME ATROPINE ESTERS ON THE RAT PHRENIC NERVE DIAPHRAGM

Drug	Molar concentration in bath	Time for complete inhibition (minutes)
Tubocurarine	1×10^{-6}	5
,,	1×10^{-5}	1·5
Atropine ..	1×10^{-3}	No effect
AMB ..	1×10^{-3}	3
,, ..	5×10^{-4}	5
PAMN ..	1×10^{-3}	2
,, ..	5×10^{-4}	5
,, ..	2×10^{-4}	No effect
VAMB ..	5×10^{-4}	1
,, ..	5×10^{-5}	Slight effect

Shay Rat

As the compounds so far tested had both anti-muscarinic and ganglionic blocking activity it appeared as though they might be useful for the symptomatic treatment of peptic ulcers. The results are shown in Tables IV and V.

This test illustrated that a single subcutaneous dose of the drugs decreased the total volume and the acidity of gastric secretion (for a period of 18 to 22 hours). It has been shown (Shay and others[3,4]), that if unbuffered gastric juice is allowed to accumulate in the stomach of the rat for this period, ulcerations of the stomach are produced and the stomach is distended with highly acid gastric fluid, pH below 2. Both

these symptoms were reduced by atropine, hyoscine, or any of the derivatives tested. No ulceration occurred in any medicated animal, and the volume of secretion was also greatly reduced. Because of wide individual variation between animals, 121 rat experiments were performed. The results indicate that the atropine compounds exhibit a stronger antisecretory activity than the hyoscine compounds. In each case where

TABLE VII

TOXICITY (LD 50 MICE)*

Substance	mg./kg. intraperitoneal	Limits 0·95	mg./kg. Oral
Propionyl atropine methyl nitrate	114	93–141	2000
Benzoyl hyoscine hydrobromide..	158	144–172	
Atropine sulphate	355	329–384	1000
Hyoscine hydrobromide	757	732–783	

* The LD 50 for the substances by the intraperitoneal route is calculated by means of probit analysis. The figures for the oral route are only an approximation because it was evident that the compounds were non-toxic and to have completed a statistical series would have required more material than was available.

tertiary and quaternary compounds were investigated, the quaternary derivatives were considerably more active. Several of the compounds caused an alkaline gastric content in some of the test animals. (See Table IV.) In these instances the total volume of secretion although small, was not so small as to be entirely attributable to swallowed saliva, particularly as these drugs tend to decrease salivary secretion. Normal gastric secretion is a mixture obtained from three types of secretory cells, which may not be equally affected by these drugs. Probably a greater inhibition of the acid-secreting cells was obtained than of the mucin-secreting cells. The secretion of the latter is alkaline in nature and also because of its high protein content acts as a buffer for any small amount of acid that may be present.

TABLE VIII

TOXICITY (SEMI-ACUTE)

Substance	Dose mg./kg. i.p.	Injection frequency	No. of animals	Period to death
Propionyl atropine methyl nitrate ..	64·25	2 per day	10 rats	did not die within 5 days
,, ,, ,, ,, ...	,,	,,	10 mice	3 died 4–5 days
Atropine sulphate ,, .. ,, ..	200	,,	6 rats	2 died 3–4 days
	,,	,,	6 mice	3 died 3–5 days

Neuromuscular Junction

PAMN has a curariform action at the neuromuscular junction. This action is only one fiftieth that of tubocurarine. Other results are given in Tabel VI.

Toxicity

Table VII gives the intraperitoneal LD 50 to mice of several of these compounds. Table VIII gives the semi-acute toxicity when the drugs were administered at a ratio of 0·56 of their LD 50 to mice. During the Shay Rat experiments it was noted that benzoyl hyoscine hydrobromide

was very toxic. The dose used for other compounds 350 mg./kg.,resulted in the death of all animals. As has been found elsewhere the toxicity of quaternary compounds is much less by mouth than by parenteral administration[2,6].

Discussion

In an earlier paper Shaw and Rosenblum[1] postulated that the esters (acetyl, butyryl, propionyl etc.) of hyoscine might owe their lesser degree of activity but more prolonged action to the fact that they were hydrolysed in the body before they exerted their pharmacological actions. Subsequent unpublished work has not confirmed this. In the present series the acetyl and propionyl esters of atropine had the same qualitative and quantitative spasmolytic action on the guinea pig ileum. Quaternisation reduced the anti-muscarinic activity to one tenth that of the tertiary amines.

As was to be expected the quaternary derivatives showed ganglionic blocking activity. The activity of PAMN was about the same as that of atropine methyl bromide but greater than that of the other therapeutically employed gastric "sedatives".

PAMN also caused a fall in blood pressure in the anaesthetised cat, presumably due to its ganglionic blocking activity. A fall in blood pressure was not noted in 6 paraplegic patients receiving 24 mg. of PAMN per day. Peptic ulcer patients receiving the drug for a period of 6 months, at about the same dose level had no complaints of orthostatic hypotension.

It was not surprising that several of the atropine and hyoscine derivatives reduced the volume and acidity of the gastric content of the Shay rat preparation. It is interesting that the quaternary derivatives were as active, or more active, than atropine. Yet these compounds had only one tenth the antiacetylcholine activity on the guinea pig ileum. It would thus appear that ganglionic block is more important than anti-muscarinic activity in the prevention of gastric secretion.

The results of toxicity tests of PAMN and related compounds at first appear to be paradoxical. By the intraperitoneal route PAMN was three times as toxic as atropine, and benzoyl hyoscine hydrobromide was six times as toxic as hyoscine. Orally PAMN was only half as toxic as atropine. These findings are in agreement with work of other authors[2,6], who have shown that quarternary are more toxic than tertiary compounds when given parenterally and the reverse holds orally. We have shown the same concept to be true for quinine (Shaw and others unpublished) When a fixed ratio of the LD 50 was chosen for a semi-acute toxicity the quaternary compound appeared to be relatively less toxic.

PAMN had slight neuromuscular junction blocking activity but no muscular weakness was noticed in man consuming this drug over long periods.

PAMN thus shows possibilities as a therapeutic substance for symptomatic treatment of peptic ulcer. This matter will be considered further in another publication (Herman and Shaw[7]).

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