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Cholinergic Blockade as Related to Chain Length of Monoquaternary Derivatives of Atropine and Benzoyl-tropine*

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Blockade of postganglionic parasympathetic effector sites by tropine esters as well as their methyl quaternary derivatives has long been recognized.[1,2] Recent studies of this aspect of anticholinergic activity include an extensive survey of the spasmolytic and mydriatic activities of a series of quaternized tropine esters[3] (the tropate, mandelate, benzoate, benzilate and xanthene 9-carboxylate esters of tropine have been included in that study) and a report on the spasmolytic activities of the benzilic acid ester derivatives of 6-methoxytropine.[4] According to these studies the actions at the postganglionic parasympathetic effector sites were generally increased by methyl quaternization.

In contrast to their effects on postganglionic sites, the effects of quaternary derivatives of tropines on autonomic ganglia have been elucidated only recently. Ganglionic blocking activity was established for the methyl derivatives of atropine,[5,6] of benzoyltropine,[7] of homatropine[5,8] and for many other quaternary tropane derivatives.[9]

It has long been known that quaternization of a wide range of tertiary alkaloids confers curare-like properties upon the molecule. Kimura *et al.*[10] described polymethylene-bis-quaternaries of atropine of which the decamethylene compound,[11] as had been predicted from atomic model studies, was found to have very potent curariform activity. The different anticholinergic properties of various quaternary tropane compounds have been recently reviewed.[9]

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While most of the studies of the aliphatic quaternary tropine derivatives have been limited to investigations of their atropine-like and spasmolytic potency, the present investigation was directed towards the delineation of the entire anticholinergic spectrum of action of compounds of two series of tropines: the methyl to dodecyl quaternary derivatives of atropine and benzoyl-tropine.

The availability of these two complete homologous series made it possible to define the changes in the spectrum of action resulting from stepwise lengthening of the aliphatic quaternary group. The atropine series was chosen because of its potent spasmolytic properties.[3] The benzoyl-tropine compounds were included in this study because their methyl to pentyl quaternaries were found to possess relatively pronounced ganglionic blocking potency associated with minimal spasmolytic activity.[12] Primary interest was focussed upon the 'within series' changes in potency, not upon the effect of quaternization as such.

The anticholinergic spectrum of action studied comprises the effects of the compounds at parasympathetic postganglionic effector sites, autonomic ganglia, and the neuromuscular junction of striated muscle.

Materials

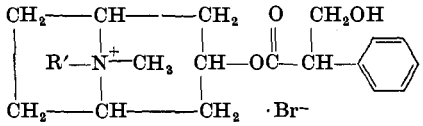
Compounds tested were the *n*-alkyl monoquaternary, methyl to dodecyl, derivatives of DL-tropyl-tropine and of benzoyl-tropine; their structural formulae are depicted in Table I. They were synthesized by Dr. F. Adickes and kindly supplied by Professor K. Zeile of C. H. Boehringer Sohn, Ingelheim, Germany. All compounds were obtained in the form of their bromide salts as colourless crystalline substances.

Water solubility at 20°C decreases markedly and in direct relation to the increase in length of the alkyl chain of the compounds in both series. The methyl derivative of either series is approximately ten thousand times more soluble than the dodecyl compound of the same series. The reverse is true of chloroform solubility, e.g. the dodecyl derivatives of both series are approximately one thousand times more soluble than the methyl compounds. The distribution of the compounds between water and

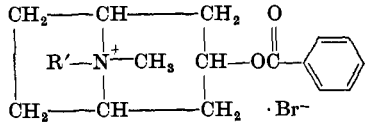
chloroform has been reported for the atropine series.[3] Members of the atropine series were considerably more soluble in water than were their benzoyl-tropine homologues; chloroform solubilities

Table I. Potency of blocking action at postganglionic parasympathetic effector sites

Atropine Series



Benzoyl-tropine Series



R′	Anti-acetylcholine action on isolated rabbit ileum				Mydriasis in mice	
	Atropine series		Benzoyl-tropine series		Atropine series	Benzoyl-tropine series
	Mean[a]	S.E.[b]	Mean	S.E.	Mean	Mean
CH_3	1·0		0·01		1·0	0·007
C_2H_5	0·33	0·04	0·003		0·4	0·003
C_3H_7	0·11	0·03	0·003		0·03	0·003
C_4H_9	0·04		0·003		0·04	<0·0025
C_5H_{11}	0·04	0·008	0·0015		0·03	<0·005
C_6H_{13}	0·16		0·0025		0·04	<0·0012
C_7H_{15}	0·2	0·06	0·0015		0·05	<0·0012
C_8H_{17}	0·11	0·015	0·0035		0·016	0·0012
C_9H_{19}	0·15	0·033	0·01	0·001	0·025	0·001
$C_{10}H_{21}$	0·06	0·006	0·014	0·004	0·01	0·0015
$C_{11}H_{23}$	0·05	0·001	0·04	0·003	0·007	
$C_{12}H_{25}$	0·17		0·025		0·0025	

[a] Mean relative potencies (methylatropinium bromide = 1·0).

[b] Numbers without standard error refer to the mean of less than 4 (usually 3) experiments.

were approximately the same in both series. Compounds of both series were insoluble in olive oil and in benzene. Solutions of undecyl and dodecyl benzoyl-tropine could not be prepared in concentrations sufficiently great for testing mydriatic activity in mice.

Methods

Blockade at parasympathetic postganglionic effector sites was determined (a) by measuring the decrease in the acetylcholine (ACh)-induced contraction of the isolated rabbit ileum, and (b) by measuring mydriasis in mice following intraperitoneal injection of the compounds.

Isolated Rabbit Ileum. Segments of ileum were suspended in a tissue bath containing aerated Tyrode solution at 37°C. A constant dose of ACh was chosen to elicit submaximal contractions, then the blocking compounds were given in dose ratios of 1:4, two minutes prior to addition of ACh. The doses of the compounds required to produce 50 per cent diminution of the ACh contractions (ED_{50}) were determined in four point assays and were related to the potency of methylatropinium bromide. The mean equipotent dose ratios and their standard errors were calculated.

Mydriasis in Mice. The method of Pulewka[13] was used on albino mice weighing 20–30 g. Three or four logarithmically spaced doses of the blocking agents were injected intraperitoneally into groups of 6–12 mice. Each compound was tested on 18–36 mice. Pupil size was measured before and 15, 30, and, in some cases, 60 min after injection, using a binocular dissecting microscope at 20 × magnification. Calculations were based upon the maximum mydriatic responses, usually obtained within 30 min after injection. The activities of the compounds were expressed as relative to methylatropinium bromide.

Blockade of autonomic ganglia was measured by two methods, '*in vitro*' and '*in vivo*'.

Isolated Rabbit Ileum. The method was used as described above, except that 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP) was used to elicit contractions by stimulating the parasympathetic ganglia of the intestine. For determining blocking action of the compounds, four point assays were made on at least three different ileum strips, the ED_{50}'s against DMPP were calculated, and converted to potencies relative to methylatropinium bromide.

Dog Pelvic Nerve-Bladder Preparation. Under pentobarbital anaesthesia, the bladders of small (8–12 kg) female dogs were

exposed; the urethra was cannulated and connected to a water manometer. The pelvic nerves were stimulated by condenser discharge pulses (frequency 40–200/sec, time constant 1–2 msec, strength 1–5 V) through a Sherrington electrode each 60 sec for 1 sec. Changes in the tone of the bladder were measured by a strain gauge transducer and recorded on a multichannel polygraph. Doses in a ratio of 1 : 2 or 1 : 4 were injected intravenously, through a cannula in the femoral vein. The degree of diminution of bladder contractions was determined and four point assays were made with methylatropinium bromide as standard.

In some animals the left cervical sympathetic trunk was exposed, cut, placed on a Sherrington electrode, and stimulated by condenser discharge pulses (frequency 50/sec, time constant 2 msec, strength 2–10 V) each 60 sec for 1 sec; the pelvic and cervical nerves were stimulated simultaneously and pressure changes of the bladder and the contractions of the nictitating membrane were recorded with mechano-transducers on a polygraph. Evaluation was made in a manner comparable to that used in the bladder experiments.

Blockade of the neuromuscular junction was tested by two methods for the atropine series. Since roughly similar values were obtained by both methods only the frog method was used for the benzoyl-tropine series.

Curare-like Action on Frogs. The compounds were injected into the ventral lymph sacs of large frogs (*Rana pipiens*, 40–70 g). Five frogs were used for each of the three dose levels, which differed by ratios of 1:2:4. Flaccid paralysis, as shown by complete loss of the righting reflex, was considered a positive response. The percentage of positive reactors was converted into probit values and the ED_{50} and its standard error calculated according to the graphic method of Miller and Tainter (1944). The results are presented in Table IV. In some animals, the sciatic nerve was stimulated electrically, and the response was compared with that obtained from frogs treated with tubocurarine.

Dog Sciatic Nerve—Gastrocnemius Muscle Preparation. Female dogs weighing 8–10 kg were anaesthetized with pentobarbital (30 mg/kg). The sciatic nerve was exposed, sectioned and placed on a Sherrington electrode. The Achilles tendon was sectioned and connected to a tension transducer. The hind leg was rigidly

fixed by a drill bored through the distal end of the femur. The peripheral end of the sciatic nerve was stimulated by short tetanic condenser discharges (frequency 100/sec, time constant of 2 msec with supramaximal intensity of 2–4 V) every 10 sec for $\frac{1}{5}$ sec. The contractions of the gastrocnemius muscle, blood pressure, and respiration were recorded on a multi-channel polygraph. Injections were made into the abdominal aorta via a cannula inserted through the contralateral femoral artery. The drugs were given in 2–3 dose levels and four point assays performed, using *d*-tubocurarine chloride as a standard. The relative potency of each compound was determined graphically on the basis of two to three experiments. Antagonism by edrophonium chloride (Tensilon) was studied with some of the compounds to determine the nature of the blockade.

Results

Postganglionic Parasympathetic Blockade

Results obtained from isolated ileum experiments (Table I) indicate that potency in the atropine series drops sharply as the side chain increases in length from methyl to propyl; propyl to dodecyl derivatives are all of relatively low potency and no consistent relationship to chain length is evident.

Members of the benzoyl-tropine series possess only about one-hundredth of the potency of the analogous atropine compounds using rabbit ileum. However, changes related to length of the side chain are similar; there is a decrease in potency with the change from methyl to ethyl and the successive compounds of the series are of lower potency. In contrast to the atropine series, the nonyl to dodecyl compounds are equal to or more potent than the methyl derivative.

Results obtained with mouse pupil roughly paralleled those obtained with the isolated ileum preparation with a few important exceptions (Table I). Only the methyl and ethyl atropinium derivatives had pronounced activity; potency decreased tenfold with the change from propyl to undecyl substitution; the dodecyl compound was practically devoid of mydriatic activity. The benzoyl-tropine series again had less activity than the atropine series; only the methyl quaternary salt showed any mydriatic activity; other members of this series were inactive up to toxic

dose levels. Nonyl and decyl benzoyl-tropinium salts were no more potent than the methyl and ethyl derivatives in the isolated tissue. The low solubilities and toxicities of the undecyl and dodecyl compounds precluded determination of their potencies.

Ganglionic Blockade

Table II indicates that ganglionic blocking potency, in contrast to postganglionic blocking potency, is either unaffected or strik-

Table II. Potency of blocking action at parasympathetic ganglia

R′	Isolated rabbit ileum (anti-DMPP action)		Dog pelvic nerve-bladder preparation			
	Atropine series	Benzoyl-tropine series	Atropine series		Benzoyl-tropine series	
	Mean[a]	Mean	Mean	S.E. or range[b]	Mean	S.E. or range
CH_3	1·0	0·75	1·0		0·8	0·35
C_2H_5	1·5	0·65	1·0	(0·8–1·4)	1·2	(1–1·7)
C_3H_7	1·5	2·1	1·8	0·43	1·0	0·16
C_4H_9	1·5	0·55	2·0	0·45	0·9	0·1
C_5H_{11}	3·7	0·65	3·3	0·40	0·85	0·1
C_6H_{13}	4·3	3·7	6·8	1·6	2·3	(1·7–3·3)
C_7H_{15}	1·0	2·5	6·8	1·3	1·5	0·27
C_8H_{17}	7·0	2·6	9·9	1·1	1·2	(0·8–1·7)
C_9H_{19}	2·7	4·3	2·4	(1·2–4)	0·8	(0·6–1·0)
$C_{10}H_{21}$	4·0	7·5	1·3	0·24	<0·4	
$C_{11}H_{23}$	7·0	7·5	<1·0		<0·4	
$C_{12}H_{25}$	8·3	6·9	<0·2		<0·4	

[a] Mean relative potencies (methylatropinium bromide=1·0).
[b] S.E. values refer to four or more experiments. The range is given on the basis of three experiments.

ingly increased by lengthening of the alkyl chain. On the isolated ileum, both series were about equally potent; however, the changes in potency were not related to changes in chain length.

In contrast, the blockade of the response of dog bladder by

members of the atropine series gradually increased with increasing chain length to C_8 and then fell markedly with further increase in chain length (Fig. 1). Octylatropinium bromide was about ten times more potent than methylatropinium bromide; this

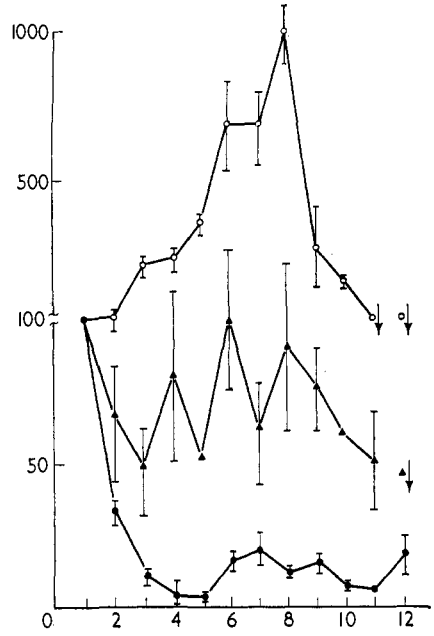


Fig. 1. Relation of the chain length of alkylatropinium compounds to their anticholinergic potencies

Abscissa: number of carbon atoms in the alkyl chain.
Ordinate: potency expressed percentage, related to methylatropinium bromide = 100 per cent.
○——○ Autonomic ganglia (dog bladder)
▲——▲ Neuromuscular junctions (frog)
●——● Postganglionic parasympathetic effector sites (rabbit ileum)
The brackets represent standard deviations of the mean.

corresponds to thirty to forty times the activity of tetraethylammonium bromide. The benzoyl-tropine series showed no such stepwise increase in potency with lengthening of the chain. In general, the members were less potent than the corresponding members of the atropine series. The C_{10}–C_{12} compounds of both series showed remarkable differences in potency on the two test organs.

Simultaneous recording of the contraction of the nictitating membrane and contractions of the bladder in the same dog enabled the investigation of the effects of selected compounds simultaneously on ganglia of both the parasympathetic and sympathetic systems. The values of the blocking potencies obtained on the two (sympathetic and parasympathetic) ganglion preparations were similar (Table III). This is indicated by the

Table III. Ganglionic blocking activities investigated simultaneously on sympathetic and parasympathetic ganglia of the dog

	Experiment No.	Nictitating membrane	Bladder	Ratio membrane: bladder
Atropine series				
R′ C_8H_{17}	1	13·6	8·0	1·7
	2	10·0	8·0	1·2
	3	11·2	13·6	0·8
Benzoyl-tropine series				
R′ CH_3	1	<3·0	2·0	<1·5
	2	1·2	0·6	2·0
C_7H_{15}	1	2·0	2·7	0·7
	2	2·5	2·0	1·2
C_9H_{19}	1	0·5	1·0	0·5
	2	0·4	0·7	0·6

Potencies related to methylatropinium bromide=1.

values in the last column of Table III expressing ratios of the potencies of the two tests. All of these values were close to 1·0.

Members of the atropine series, except for the C_{10}–C_{12} compounds, have a rather uniform potency without relation to chain length when tested by the sciatic nerve–gastrocnemius method (Table IV). The action of the methyl and ethyl compounds was reversed by edrophonium chloride. The compounds were generally about one-tenth as potent as tubocurarine chloride. Flaccid paralysis in frogs was produced by approximately equal doses of compounds of either series. Electrical stimulation of the sciatic nerve of the treated frogs showed complete block; however, direct stimulation

of the gastrocnemius muscle also failed to evoke a maximum contraction.

Table IV. Potency of blocking action at neuromuscular junctions

R′	Frog experiments				Dog sciatic nerve-gastrocnemius preparation
	Atropine series		Benzoyl-tropine series		Atropine series
	Mean	S.E.	Mean	S.E.	Mean
CH_3	1·0		0·8	0·25	1·0
C_2H_5	0·64	0·2	2·0	0·6	1·0
C_3H_7	0·47	0·15	1·0	0·4	1·0
C_4H_9	0·81	0·3	1·0	0·3	0·8
C_5H_{11}	~0·5		1·1	0·4	1·1
C_6H_{13}	1·0	0·25	~1·4		0·8
C_7H_{15}	0·6	0·18	~1·4		0·5
C_8H_{17}	0·9	0·3	~0·45		0·6
C_9H_{19}	0·75	0·15	0·62	0·2	0·7
$C_{10}H_{21}$	~0·6		0·81	0·3	<0·25
$C_{11}H_{23}$	0·5	0·18	0·6	0·2	<0·17
$C_{12}C_{25}$	<0·45		<0·23		<0·7

Mean relative potencies (methylatropinium bromide=1·0).

Discussion

In general, the two series of monoalkyltropinium ions behave similarly to the monoaralkyltropinium salts previously investigated. The results support the postulate[9, 15] that the anticholinergic spectrum of a compound can be markedly altered by changing only one group attached to the quaternary nitrogen atom. Fig. 1 graphically illustrates that marked changes in potency occur with changes in chain length. The relative potency at autonomic ganglia is of a different order of magnitude to that at neuromuscular junctions and postganglionic parasympathetic effector sites throughout the series. In fact, the characteristic change produced by quaternization of tropine esters is

the introduction of notable ganglionic blocking activity. Although not depicted, a similar figure of the benzoyl-tropine series presents an analogous appearance. Thus, from the present two series, compounds can be selected which show fairly dominant blocking properties with respect to one of the cholinergic sites. For example, methylatropinium bromide is a potent postganglionic blocking agent, with moderate ganglionic blocking activity.[5] Octylatropinium bromide has considerable potency at autonomic ganglia while its effect at postganglionic parasympathetic effector sites and neuromuscular junctions is moderate and is of the same order of magnitude as the other members of the atropine series (except the methyl compound at postganglionic parasympathetic effector sites). Furthermore, *N*-ethylbenzoyl-tropinium bromide produces primarily neuromuscular blockade with only slight activity at the other two sites.

Differences have been found between the ganglionic blocking potencies of the compounds obtained with the two methods employed. One possible explanation of such a phenomenon is that the ileum preparation is also detecting a property of the drug other than ganglionic blockade. Although DMPP is known as a selective ganglionic stimulant[17] and stimulates the postganglionic parasympathetic effector sites only through the ganglia, its blockade in these experiments might be construed as being due to the postganglionic blocking effect of the compounds. However, this is probably not the case because the results of the DMPP-test paralleled the results of the dog ganglion preparation rather than those obtained on the ACh-stimulated ileum preparation. From Tables I and II it may be noted that long-chain compounds, C_9–C_{12}, especially benzoyl-tropine derivatives, showed appreciable spasmolytic potency on the isolated ileum against ACh and also DMPP-induced contraction. In contrast, they produced only very slight mydriatic activity in mice. Furthermore, they were the least active compounds of either series on the dog-bladder preparation. It was also observed that these compounds had a pronounced tendency to foam and appeared to have detergent properties. Supporting this observation are the data[3] which show that the higher alkylatropinium salts reduce the surface tension of water. Thus, the potency of these higher chain compounds in the isolated tissue bath may be partly due to a musculotropic spasmolytic

property of surface active agents. This and other properties of the compounds are currently under study.

Results obtained in the experiments on the bladder and on the superior cervical ganglia of dogs agreed with each other, as might be expected since they measure a similar reaction, i.e. response of smooth muscle organs to preganglionic electric stimulation.

There are certain discrepancies (C_3–C_6, C_8 and C_{11}) between the values obtained for *in vitro* spasmolytic activity of the atropine series[3] and our results. This may be partly due to a difference in the assay technique but different sensitivities of the intestines of the guinea-pig and the rabbit to some of the compounds is probably the most important factor. The mouse mydriasis experiments differed from those of Engelhardt and Wick[3] only in the route of administration; the results are in close agreement.

Many of the newer, potent anticholinergic drugs possess some ganglionic blocking activity. This action is pronounced in two recently introduced atropine derivatives: *n*-octylatropinium bromide[3] and *p*-phenylbenzylatropinium bromide.[16] Ganglionic blocking activity supposedly plays an important role in their therapeutic action and may offer advantages over the Belladonna alkaloids and the older anticholinergic drugs.

Summary. 1. Investigation of the anticholinergic spectra of C_1–C_{12} alkyl quaternary derivatives of tropyl-tropine and benzoyl-tropine has demonstrated that, (a) the length of the aliphatic chain influences the potency of each compound at the different cholinergic sites, (b) both potency and selectivity vary independently of one another, and (c) ganglionic blockade is the most characteristic change introduced by quaternization.

2. In the atropine series, postganglionic blockade decreases sharply in compounds with groups larger than methyl; ganglionic blockade increases directly, reaches a peak at octyl, and then drops off sharply; neuromuscular blockade remains weak throughout.

3. The benzoyl-tropine series follows a pattern similar to the atropine series except that postganglionic and ganglionic blockade are weaker, and neuromuscular blockade is slightly stronger.

4. Anticholinergic agents of the type investigated may, depending on their structure, have predominant action at any of the following sites; (a) methylatropinium at parasympathetic postganglionic effector sites; (b) octylatropinium at autonomic ganglia, and (c) *N*-ethyl benzoyl-tropinium at the neuromuscular junction.

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