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THE GANGLION BLOCKING PROPERTIES OF HOMOLOGOUS COMPOUNDS IN THE METHONIUM SERIES

BY

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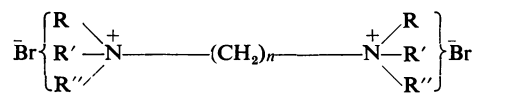
Speculation on the relationship of pharmacological activity to chemical structure is a fascinating occupation, though a complex one. An apparently small alteration in molecular configuration may profoundly change the actions of a compound ; a well-known example of this is the change in neuromuscular blocking activity brought about by the replacement of methyl by ethyl groups in quaternary ammonium ions (Ing, 1936). In a preceding paper (Wien and Mason, 1951) we found that the ability of hexamethonium to paralyse autonomic ganglia was influenced by the replacement of methyl by ethyl groups on the quaternary nitrogen atoms, and we have now extended our observations to a more systematic study of the tetra- to the hepta-methylene members in this alkamethonium series, as well as to several other quaternary ammonium compounds.

METHODS

The methods were the same as those described previously (Wien and Mason, 1951) for determining the paralysing effects of substances on transmission through the superior cervical ganglion of the cat ; on the peristaltic reflex of the isolated guinea-pig intestine ; and on transmission at the neuromuscular junction by the phrenic nerve-diaphragm preparation of the rabbit. Each result in Table I was the mean of several, at least two, experiments on each test object, permitting several comparisons, but no statistical analysis was attempted. In the experiments on the nictitating membrane care was taken to space the injections far enough apart (15 to 20 minutes) to prevent sub-threshold amounts of a previous injection affecting a later one ; the order of giving the compound to be tested and the standard for comparison was varied as a further safeguard. Toxicity estimations were determined intravenously in mice.

Compounds.—We examined sixteen polymethylene bis-trialkylammonium dibromides of general formula (I), where $n=4$, 5, 6, or 7, and $R=R'=R''=Me$, or $R=R'=Me$ and $R''=Et$, or $R=Me$ and $R'=R''=Et$, or $R=R'=R''=Et$. They were all prepared by our colleagues (Barber and Gaimster, 1952).



(I)

We also examined tetraethylammonium, triethylmethylammonium, diethyldimethylammonium, ethyltrimethylammonium, and tetramethylammonium bromides. The compound γ-phenoxypropyltriethylammonium iodide was kindly supplied by Professor

R. Paul, and some of its actions have been described by Depierre and Jacob (1950); it was also prepared by our colleague Dr. J. N. Ashley. The methyl homologue, γ-phenoxypropyltrimethylammonium bromide, has been described by Hey (1952) and it was kindly supplied by him.

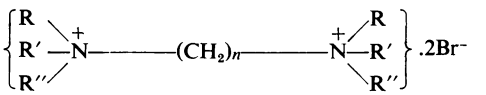
RESULTS

Ethyl homologues in the alkamethonium series

The parent bis-trimethylammonium compounds were described by Barlow and Ing (1948) and studied in some detail by Paton and Zaimis (1951), but the actions of the ethyl homologues have not hitherto been investigated. Barlow and Ing (1948) prepared the bis-triethylammonium series ($n=2$, 3, 4, 5, 7, 8, 9, 10, and 13), but did not study them in detail. Our results on this series of compounds are summarized in Table I.

TABLE I

THE EFFECT OF SUCCESSIVE REPLACEMENT OF METHYL BY ETHYL GROUPS ON BOTH QUATERNARY NITROGEN ATOMS IN THE FORMULA



Compound				Toxicity (mice)		Relative potency paralysing ganglia (hexamethonium=100)		Neuromuscular paralysis (*d*-tubocurarine=100)
n	R	R′	R″	LD50 mg./kg. i.v.	% limits of error (P=0.05)	Sympathetic (Cat superior cervical)	Parasympathetic (Guinea-pig ileum)	Rabbit phrenic nerve-diaphragm
4	Me	Me	Me	122	(92–109)	1	5	Inactive
	Et	Me	Me	61	(89–113)	10	25	,,
	Et	Et	Me	35	(90–111)	100	25	,,
	Et	Et	Et	20	(91–110)	5	5	,,
5	Me	Me	Me	62	(81–119)	65	75	0.4
	Et	Me	Me	16	(84–120)	150	150	0.4
	Et	Et	Me	22	(91–110)	125	100	0.4
	Et	Et	Et	8	(85–118)	7	10	0.4
6	Me	Me	Me	50	(88–113)	100	100	0.5
	Et	Me	Me	22	(89–112)	150	200	0.2
	Et	Et	Me	12.5	(89–112)	75	100	0.4
	Et	Et	Et	2.5	(91–110)	<5	<2	4.0
7	Me	Me	Me	12	(90–112)	12	30	1.0
	Et	Me	Me	15	(94–107)	10	30	2.0
	Et	Et	Me	9	(91–111)	15	2	3.0
	Et	Et	Et	6	(91–111)	2	<2	5.0

These results showed several interesting facts. It was found that the substitution of ethyl for methyl groups profoundly affected the properties of the compounds. There was an increase in toxicity as more ethyl groups were introduced, reaching a maximum in the bis-triethylammonium compounds. This increase in toxicity was accompanied, in the hexa- and hepta-methylene members, by appreciable neuromuscular paralysis as well. In the whole animal, this neuromuscular paralysis may

be even more marked, as with decamethonium, which is several times more potent than *d*-tubocurarine in the sciatic-tibialis preparation of the cat, but less potent on the isolated phenic nerve-diaphragm preparation of the rabbit or kitten.

In the tetramethylene compounds (see Table I, where $n=4$), the presence of one ethyl group on each nitrogen caused a tenfold increase in activity on the superior

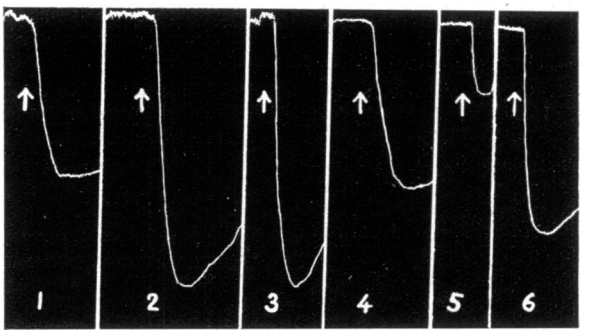


FIG. 1.—Cat, chloralose. Contractions of nictitating membrane on preganglionic stimulation of cervical sympathetic at 12 shocks/sec.; fall indicates relaxation. Compounds, as bromide salts, injected intravenously. (1) 0.25 mg., (3) 0.5 mg. hexamethonium; (2) 0.5 mg. tetramethylene-1:4-bis-diethylmethylammonium; (4) 10 mg. tetramethylene-1 : 4-bis-triethylammonium; (5) 50 mg. tetramethylene-1 : 4-bis-trimethylammonium; (6) 4 mg. tetramethylene-1 : 4-bis-ethyldimethylammonium. See text and Table I. Note that the effects in (2) and (3) are similar.

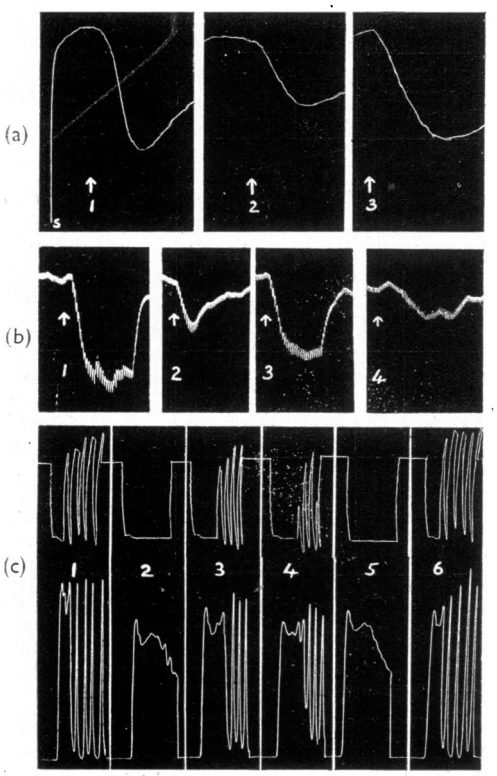


FIG. 2.—(*a*) Nictitating membrane contractions of cat (chloralose) on sustained preganglionic stimulation at S. (1) 0.2 mg., (2) 0.1 mg. intravenously of tetramethylene-1 : 4-bis-diethylmethylammonium, and (3) 0.2 mg. of hexamethonium bromides. (*b*) Blood pressure record of cat. (1) and (3) control effects on stimulation of peripheral end of severed right vagus; (2) and (4) after 0.5 mg./kg. intravenously of tetramethylene-1 : 4-bis-diethylmethylammonium and 0.25 mg./kg. of hexamethonium respectively. (*c*) Peristaltic responses of guinea-pig ileum; upper circular, and lower longitudinal, movements. (1) and (6) control effects; (2) 0.5 mg. and (3) 0.2 mg. of pentamethonium; (4) 0.5 mg. and (5) 1.0 mg. of tetramethylene-1 : 4-bis-diethylmethylammonium bromides.

cervical ganglion. The symmetrical replacement of two more methyl groups by ethyl caused a further tenfold increase, making it 100 times as active as the parent compound. This compound (tetramethylene-1 : 4-bis-diethylmethylammonium dibromide) was consequently as active as hexamethonium (Fig. 1). This compound, too, showed some selectivity in its action, since it had a relatively greater effect on the superior cervical ganglion than on the intestine and the vagus (Fig. 2). From Fig. 2 it will be seen that it was (*a*) as active as C6 on the superior cervical ganglion—the onset was quicker, but the duration was similar ; (*b*) about one-half as effective in preventing the fall of blood pressure produced by stimulation of the cardiac vagus ; and (*c*) about one-half as effective as C5 (equal to 37.5 per cent of C6) on the guinea-pig ileum.

There was a marked decrease in ganglion blocking potency in all the bis-triethylammonium compounds, but there was an increase in potency in the bis-ethyldimethylammonium homologues of penta- and hexa-methonium, in which only one methyl group on each nitrogen atom was replaced by ethyl (Wien and Mason, 1951).

Homologues of tetraethylammonium

Though the neuromuscular paralysing actions of several ethyl homologues of tetramethylammonium (Me_4N^+) have been studied by Marshall (1914), Burn and Dale (1914), Ing (1936), and Raventós (1937), and Dallemagne and Philippot (1951) have investigated a series Me_3RN^+, where $R = C_n H_{2n+1}$ and $n = 2$ to 18, we are unaware of any comparative data on the ganglion paralysing activities of these compounds, studied in the manner described in this paper. We have examined the effects on autonomic ganglia, and an illustration of the results obtained is shown in Fig. 3. In these experiments we found that tetraethylammonium (Et_4N^+) was

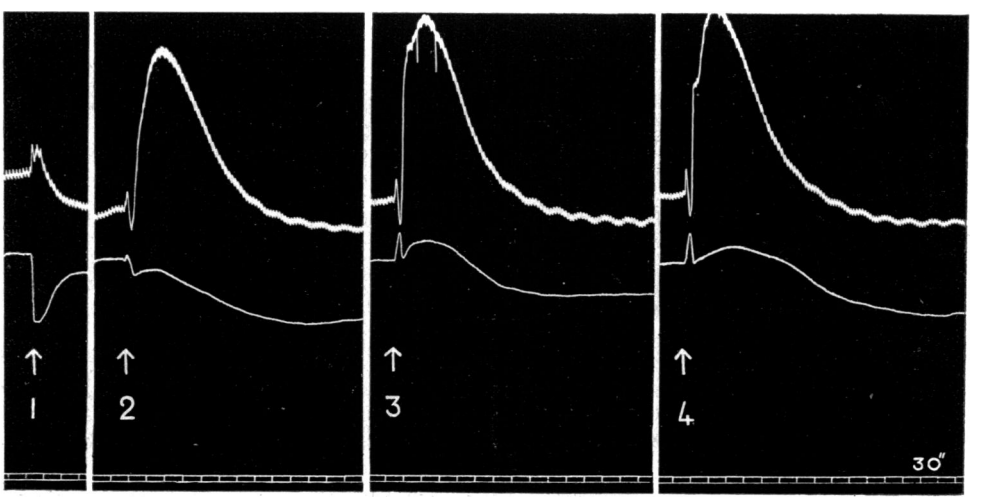


FIG. 3.—Cat, chloralose. Upper record, blood pressure. Lower record, contractions of nictitating membrane on sustained preganglionic stimulation; upstroke is contraction, downstroke is relaxation. Intravenously, (1) 5 mg. tetraethylammonium bromide; (2) 20 mg. diethyldimethylammonium bromide; (3) 5 mg. ethyltrimethylammonium bromide; (4) 2 mg. tetramethylammonium bromide.

between five and seven times less potent than hexamethonium. Ethyltrimethylammonium ($EtMe_3N^+$) had a marked nicotine-like pressor effect (comparable with tetramethylammonium) in doses which partially blocked ganglionic transmission to the nictitating membrane ; its effect was different from that of tetraethylammonium, and similar to that of tetramethylammonium. A stimulant action was observed also on the longitudinal muscle of the guinea-pig ileum, at all concentrations which caused an inhibition of the peristaltic movements. Triethylmethylammonium (Et_3MeN^+) had about the same ganglion blocking activity (one-quarter of Et_4N^+) as diethyldimethylammonium ($Et_2Me_2N^+$), but it was devoid of the stimulant actions of the latter compound, and its depressor effect was even more transient than that of tetraethylammonium.

γ-Phenoxypropyltriethylammonium and its trimethyl homologue

The triethylammonium compound was found by Depierre and Jacob (1950) to be about three times as potent as tetraethylammonium in paralysing the effects of nicotine on the blood pressure in dogs. We have found in the cat (chloralose) that it was one-half as potent as hexamethonium in paralysing transmission through the superior cervical ganglion ; its action, however, was much more evanescent. On the guinea-pig ileum preparation it was equally as active as hexamethonium in reducing the peristaltic reflex. This compound, like hexamethonium, affected only pre-ganglionic excitation, leaving post-ganglionic stimulation unmodified. Its trimethyl homologue, γ-phenoxypropyltrimethylammonium bromide, was almost devoid of any ganglionic paralysing effect (Fig. 4) ; consequently in these two monoquaternary salts the complete replacement of methyl by ethyl groups increased ganglionic paralysing activity, whereas the reverse was true in the bis-quaternary ammonium compounds examined.

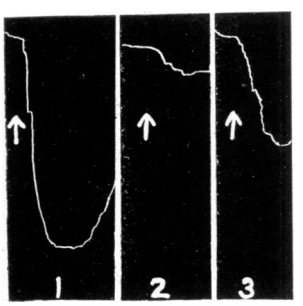


FIG. 4.—Cat, chloralose. Contractions of nictitating membrane on preganglionic stimulation. (1) 0.2 mg., (3) 0.1 mg. γ-phenoxypropyltriethylammonium iodide; (2) 1.0 mg. γ-phenoxypropyltrimethylammonium bromide.

DISCUSSION

We have found that in the alkamethonium series the type of terminal groupings on the two quaternary nitrogen atoms, as well as the intervening chain length, modifies ganglion blocking activity. This is illustrated in Fig. 5. Whereas increase in chain length from four to six carbon atoms caused a marked increase in potency, the same increase could be achieved by partial replacement of methyl by ethyl groups on the quaternary nitrogen atoms. It may well be that the most important factor is the preservation of the integrity of the terminal, basic polar groups ; any change in the quaternary nitrogens, for example, to tertiary nitrogens, results in an almost complete (95 per cent) reduction in activity. The characteristic feature, then, would

appear to be two cationic groups connected by an inert polymethylene chain. The association of biological activity with a quaternary nitrogen atom has been frequently observed; besides the paralysis of ganglia, such properties as neuromuscular paralysis and even activity against certain bacteria and trypanosomes have been observed. This might be due to some special property of the quaternary ammonium ion (for example, Lorente de Nó (1949) has shown its ability to replace sodium ions for the maintenance of the excitability of frog nerve), and indeed it is known that an enormous increase in basicity occurs on passing from a tertiary to a quaternary ammonium hydroxide, so that the positive charge associated with the quaternary ion might be responsible for an electrostatic attraction between it and the anion of the receptor grouping, particularly if the latter is regarded as of a weakly acidic nature.

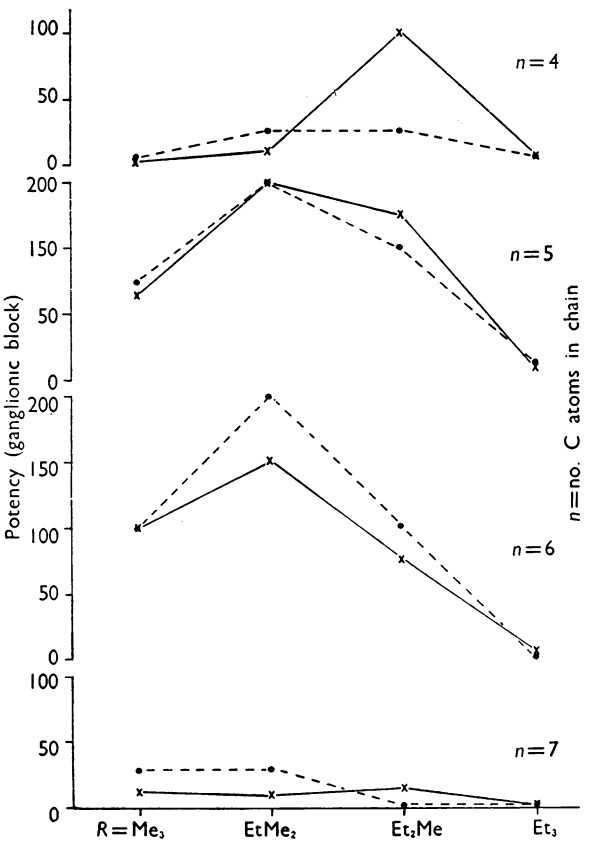


FIG. 5.—Relationship between ganglion blocking potency, chain length (*n*), and terminal groups (R) in the series $R.N^+(CH_2)_nN^+.R$; the maximal potencies vary with both *n* and R. × ——— × relative potencies on cat superior cervical ganglion. • - - - - • relative potencies on guinea-pig ileum.

We have not attempted to determine the exact mode of action of each compound, and it is conceivable that some of these compounds may indeed have mixed actions. Nevertheless, the substitution of one ethyl group for a methyl group on both nitrogen atoms was most effective where the intervening chain length was five or six carbon atoms, whereas the substitution of two ethyl groups was most effective where the chain

length was four carbon atoms. Tetramethylene-1 : 4-bis-diethylmethylammonium had a greater effect on the superior cervical ganglion relative to its effect on the intestine, showing that a ganglion blocking compound of high potency can be obtained with an affinity for a particular ganglionic system. But one of us (N.D.E.), in a quantitative study of drugs producing mydriasis in the mouse by paralysis of the ciliary ganglion, found this compound to be not less but 2.9 times more potent than hexamethonium, which may indicate a true differential action within the parasympathetic system or merely a difference due to species variation. With a chain length of seven carbon atoms substitution on the terminal nitrogens made little difference except to increase the neuromuscular blocking properties.

Two homologues of tetraethylammonium, namely diethyldimethyl- and ethyltrimethyl-ammonium bromides, displayed marked nicotine-like properties ; these compounds exemplify the well-recognized transition in properties between tetraethyl- and tetramethyl-ammonium, originally described by Burn and Dale (1914). The active ganglion blocking compound, γ-phenoxypropyltriethylammonium iodide, and its corresponding inactive trimethyl homologue, may be compared with the difference between tetraethylammonium and tetramethylammonium, all mono-quaternary compounds. In the bis-quaternary series the reverse was true, the complete substitution of methyl by ethyl groups rendering the compounds less effective. One explanation for this might be the existence of different receptors for the mono- and bis-quaternary ammonium compounds. Though ganglion blocking activity in the bis-quaternary compounds was very sensitive not only to the length of the alkyl chain, but also to the types of groups on the quaternary nitrogen atoms, it does not necessarily follow—since there is no direct evidence—that there is a corresponding optimal distance between the receptors. Moreover, the molecules are flexible and may assume various configurations within limitations. We would suggest, nevertheless, that an analysis of the electron configuration in the compounds we have examined might help to explain our findings ; obviously more information is wanted. An attractive hypothesis put forward by Taylor (1951) for the difference in curariform activity of Me_4N^+ and Et_4N^+ rests on differences in electron densities, and we think that similar deductions in the alkamethonium series might reveal values associated with changes in ganglion blocking activity.

Summary

1. The ganglion blocking properties have been studied of (*a*) a series of sixteen homologous compounds in the alkamethonium series in which methyl groups were successively replaced by ethyl groups on the two quaternary nitrogen atoms ; (*b*) three homologues of tetraethylammonium ; (*c*) γ-phenoxypropyltriethylammonium and its trimethyl homologue.

2. The potency of all these compounds was considerably influenced by the relatively minor change of ethyl for methyl groups on the quaternary nitrogen atoms.

3. In the alkamethonium series complete replacement of all the methyl by ethyl groups invariably led to a reduction in activity. Partial replacement caused an increase in potency, maximum at a chain length of four carbon atoms with the replacement by two ethyl groups on each nitrogen atom ; and at a chain length of five or six carbon atoms with the replacement by one ethyl group on each nitrogen

atom. Ganglion blocking activity was related not only to chain length but also to the type of terminal grouping. This was remarkably illustrated in the tetramethylene compounds, where the substitution of two ethyl groups produced as sharp and as great an increase in potency as alteration in chain length. The bearing of this on structure-action relationships in this series has been discussed, the observations not readily falling into line with any current theories. The alteration in potency resulting from alteration in structure usually occurred *pari passu* on both sympathetic and parasympathetic ganglia.

REFERENCES

Barber, H. J., and Gaimster, K. (1952). *J. appl. Chem.* (in press).
Barlow, R. B., and Ing, H. R. (1948). *Brit. J. Pharmacol.*, **3**, 298.
Burn, J. H., and Dale, H. H. (1914). *J. Pharmacol.*, **6**, 417.
Dallemagne, M. J., and Philippot, E. (1951). *Arch. int. Pharmacodyn.*, **87**, 127.
Depierre, F., and Jacob, J. (1950). *C. R. Acad. Sci., Paris*, **231**, 554.
Hey, P. (1952). *Brit. J. Pharmacol.*, **7**, 117.
Ing, H. R. (1936). *Physiol. Rev.*, **16**, 527.
Lorente de Nó, R. (1949). *J. cell. comp. Physiol.*, **33**, 3.
Marshall, C. R. (1914). *Trans. roy. Soc. Edinb.*, **50**, 379.
Paton, W. D. M., and Zaimis, E. J. (1951). *Brit. J. Pharmacol.*, **6**, 155.
Raventós, J. (1937). *Quart. J. exp. Physiol.*, **26**, 361.
Taylor, D. B. (1951). *Pharmacol. Rev.*, **3**, 412.
Wien, R., and Mason, D. F. J. (1951). *Brit. J. Pharmacol.*, **6**, 611.