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RELATIONSHIP BETWEEN THE MOLECULAR CONFORMATION OF CHOLINE ARYL ETHERS AND NICOTINE-LIKE STIMULANT ACTIVITY

BY

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The introduction of one or more methyl substituents into the structure of choline phenyl ether bromide (TM1) may result in large changes, both qualitative and quantitative, in pharmacological action. Thus, whereas choline *o*-tolyl ether bromide (*o*-Me-TM1), and α-methylcholine phenyl ether bromide (α-Me-TM1) are potent ganglion stimulants, being respectively one half and twice as active as choline phenyl ether (Hunt & Renshaw, 1936), choline 2,6-xylyl ether bromide (xylocholine) and β-methylcholine phenyl ether bromide (β-Me-TM1) are devoid of ganglion stimulant activity (Hey, 1952; Clark & Jana, 1966), and xylocholine possessès adrenergic neurone blocking activity.

An examination of molecular models of xylocholine and choline phenyl ether suggested that the difference in biological action might be steric in origin. Xylocholine is very sterically hindered, rotation about its phenyl-oxygen bond is virtually completely inhibited, and the plane containing the oxygen valencies is orthogonal to the plane of the benzene ring. In contrast choline phenyl ether is completely flexible and able to adopt different conformations in which the angle between the plane of the benzene ring and the plane containing the oxygen valencies ("angle-of-twist") varies between 0° and 90°. β-Me-TM1 lies between these two extremes; some degree of rotation about the phenyl-oxygen bond is possible but steric hindrance occurs between the *o*-hydrogens and the β-methyl group, and the "angle-of-twist" is always $>0°$. α-Me-TM1 and *o*-Me-TM1 on the other hand can both adopt conformations in which the "angle-of-twist" about the aryl-oxygen bond is 0°. It might be inferred from these observations on molecular models that for nicotine-like stimulant activity in choline aryl ethers it is necessary for the aryl ring, ether oxygen and β-methylene group of the choline moiety to lie in the same plane (that is, "angle-of-twist" $=0°$) when interacting with the nicotinic receptor and that for adrenergic neurone blocking activity the "angle-of-twist" needs to be 90°. The observation by Exley (1957) that *o*-Me-TM1 possesses adrenergic neurone blocking activity as well as ganglion stimulant activity could then be explained by assuming that the molecule adopts different conformations at the two different sites of action. In order

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to examine further the effects of molecular shape on the biological activity of choline aryl ethers, a number of heterocyclic analogues in which the " angle-of-twist " about the aryl-oxygen bond is fixed, were synthesized and a spectroscopic method developed for the determination of their conformations and for the minimum " angle-of-twist " of partially hindered molecules such as β-Me-TM1 (Clark & Williams, 1967).

The structural formulae of the heterocyclic compounds investigated, together with their code designations are shown in Fig. 1.

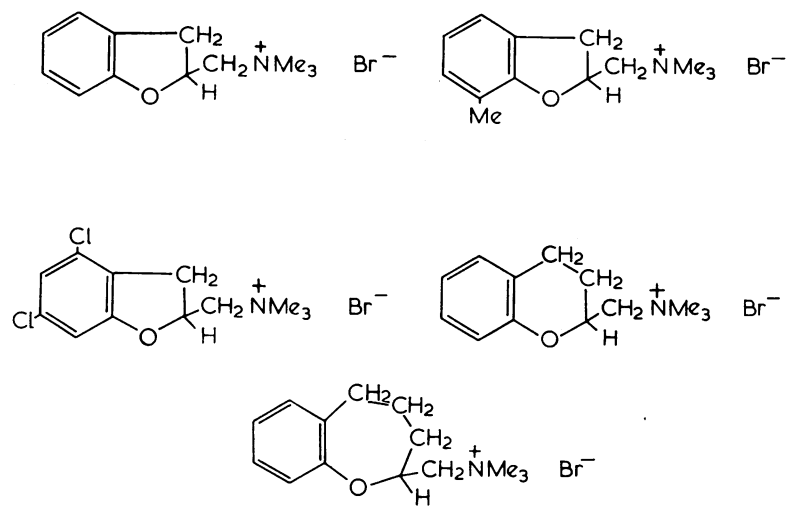

FIG. 1.

The ring systems of (2,3-dihydrobenzofuran-2-ylmethyl)trimethylammonium bromide (R5), (2,3-dihydro-7-methylbenzofuran-2-ylmethyl)trimethylammonium bromide (R5M) and (4,6-dichloro-2,3-dihydrobenzofuran-2-ylmethyl)trimethylammonium bromide (di-Cl-R5) are essentially planar ("angle-of-twist "$=0°$), whereas (chroman-2-ylmethyl)-trimethylammonium bromide (R6) and (2,3,4,5-tetrahydro-1-benzoxepin-2-ylmethyl) trimethylammonium bromide (R7) were found to have " angles-of-twist " of 34° and 64°, respectively. The spectroscopic method used to obtain these angles depended on the determination of the degree of interaction between the oxygen and the π electrons of the aromatic ring, and used the relationship $\frac{\varepsilon_{corr}}{\varepsilon_0} = \cos^2\theta$, where ε_{corr} is the molecular extinction coefficient of the compound under examination corrected for the effects of alkyl substituents, ε_0 is the molecular extinction when optimum interaction can occur between the oxygen and the π electrons of the benzene ring, and θ is the " angle-of-twist."

If this formula is applied to the spectroscopic data for choline phenyl ether bromide an " angle-of-twist " of 43.5° is obtained—consistent with the predicted possibility of free rotation about the phenyl-oxygen bond—that is, when combining with its receptor, choline phenyl ether may adopt a conformation in which the " angle-of-twist " has any value between 0° and 90°. This is in contrast to β-methylcholine phenyl ether in which

the derived " angle-of-twist " is 54.4° confirming the deductions made from molecular models that, though flexible, this molecule is unable to adopt a conformation in which the " angle-of-twist "=0°. Using the relationship $\frac{\varepsilon_{corr}}{\varepsilon_o} = \frac{1}{2}\left(\frac{1-\sin\theta'}{\pi - 2\theta'}\right)$ where θ' is the minimum " angle-of-twist " that such a partially restricted molecule can possess, Clark & Williams (1967) calculated that for β-methylcholine phenyl ether bromide the " angle-of-twist " has a minimum value of 24°.

In addition to affecting the three-dimensional shape of the molecule, the " angle-of-twist " about the phenyl oxyben bond, by influencing the degree of interaction between the oxygen and the π orbitals of the benzene ring, will influence the basicity of the ether oxygen. Thus xylocholine, with minimum interaction, will be the most basic and R5 will be the least basic of the heterocyclic compounds possessing no ring substituents. Clark & Williams (1967) showed that the presence of the methyl substituent in the aromatic ring of R5M produces an inhibition of resonance between the oxygen and π electrons of the benzene ring which results in an increase in basicity of the ether oxygen (equivalent to that of a structure with an " angle-of-twist " about the aryl oxygen bond of 29°), but they believe that the actual " angle-of-twist " is 0°, as it is in R5. The spectroscopic method described by Clark & Williams (1967) is not capable of handling the halogen substituted di-Cl-R5. By analogy with R5, the ring structure of this compound is also believed to be essentially planar, but the presence of the chlorine substituents is expected to produce a lower electron density on the ether oxygen than that found in R5.

It was hoped that an examination of the pharmacological actions of these compounds, which possess differences in conformation and basicity of the ether oxygen, might throw some light on the structural requirements for nicotine-like ganglion stimulant activity and adrenergic neurone blocking activity.

METHODS

Blood pressure recording. Anaesthesia of cats (1.5–3 kg) of either sex was induced with ether after intraperitoneal injection of atropine 1 mg/kg. The trachea was cannulated. Spinal preparations were made according to Dale's method (Burn, 1952) or anaesthesia was maintained with chloralose, 80 mg/kg injected intravenously. Arterial blood pressure was recorded with a mercury manometer through a siliconized cannula containing 4% sodium citrate solution, in the left common carotid artery. Drugs were dissolved in 0.9% sodium chloride solution, injected into the right femoral vein through a polythene cannula and washed in with 1 ml. of saline.

Nictitating membrane preparation. The right postganglionic and the left preganglionic cervical sympathetic nerves were exposed and stimulated electrically for 15 sec every minute using rectangular pulses of 0.5 msec duration and supramaximal voltage delivered at the rate of 50 pulses/sec through shielded platinum electrodes. The exposed nerves were covered with cotton-wool soaked in warm liquid paraffin. Movements of the nictitating membranes were recorded on smoked paper with isotonic levers fitted with frontal writing points. Drugs were administered as described above.

Isolated superior cervical ganglion preparation. Rabbits of either sex and weighing 2–4.5 kg were killed by an injection of air into a marginal ear vein. The right superior cervical ganglion, together with short lengths of preganglionic and postganglionic nerves, was quickly excised and placed in a dish of cold Krebs solution containing (g/l.): sodium chloride, 6.92 ; potassium chloride, 0.345 ; calcium chloride (anhydrous), 0.285 ; magnesium sulphate heptahydrate, 0.294 ; sodium bicarbonate, 2.10 ; potassium dihydrogen phosphate, 0.102 ; and dextrose, 2.0 ; and gassed with 95%

oxygen and 5% carbon dioxide. The ganglion was desheathed under a dissecting microscope, connective tissue removed and the preparation transferred to seven platinum electrodes which were incorporated in the lid of a moist chamber (Eccles, 1952). A mixture of 95% oxygen and 5% carbon dioxide, previously warmed and moistened by passage through water at 30° C, flowed continuously through the chamber. The first two electrodes were used to stimulate the preganglionic nerve, the next electrode served to earth the preparation, the next pair, placed on the preganglionic nerve, were used to record the preganglionic action potential and the final pair, one electrode of which was placed on the ganglion and the other on the postganglionic nerve, served to record the ganglionic action potential. The preparation, which was tied to the first and last electrodes, was bathed in Krebs solution at 30° C except during recording periods when the chamber was drained for not more than 2 min. The moist chamber assembly was partially immersed in a bath containing water maintained at 30° C. The preganglionic nerve was stimulated with single supramaximal rectangular pulses of 0.05 msec duration delivered through an isolating transformer. Action potentials were recorded on a Solartron oscilloscope after amplification by a condenser-coupled Tektronix 122 preamplifier.

The experimental procedure was similar to that described by Elliott & Quilliam (1964). After allowing the tissue to equilibrate with the Krebs solution for 1 hr, control action potentials were recorded and the tissue was then immersed in Krebs solution containing the drug. Recordings were again made after allowing 15 min for the drug to act. Successive applications of the drug in increasing concentrations were then made without intervening washing to regain control potentials. This procedure is believed to be justified because successive additions, without intervening washing of the tissue, of a concentration of hexamethonium sufficient to produce 50% reduction of the ganglionic action potential were found to produce a constant degree of inhibition. This might be taken to indicate that accumulation of the drug in the tissue is negligible. Dose response curves were constructed for the test compounds, from which the concentration of drug necessary to reduce the ganglionic action potential by 50% was obtained. Each determination of the 50% blocking concentration of a given drug was performed using a different isolated ganglion preparation. Only the effects of the drugs on the S_a spike (Eccles, 1952) of the ganglionic action potential complex have been used in the assessment of ganglionic blocking activity.

Rabbit isolated intestine-sympathetic nerve preparation. Segments of rabbit isolated ileum or duodenum with attached periarterial nerves were prepared according to the method of Finkleman (1930), suspended in "double glucose" Tyrode solution of the following composition (g/l.): NaCl, 8.0; KCl, 0.2; $CaCl_2$, 0.2; NaH_2PO_4, 0.05; $MgCl_2$, 0.2; $NaHCO_3$, 1.0; dextrose, 2.0; and the solution, maintained at 37° C, bubbled with a mixture of 95% oxygen and 5% carbon dioxide.

The periarterial nerves were stimulated supramaximally for 30 sec in every 10 min, through platinum electrodes, with square wave pulses of 0.5 msec duration at 50 pulses/sec. Longitudinal contractions of the preparation were recorded by means of an isotonic lever with a frontal writing point on smoked paper.

Electrical stimulation was discontinued when the effects of added adrenaline were being observed.

Chemistry. All the compounds used have been described previously except di-C1-R5.

2-Allyl-3,5-dichlorophenol (b.p. 154° C/21 mm) obtained by Fries rearrangement of Allyl 3,5-dichlorophenyl ether, was ring closed and brominated by the method described by Mills & Adams (1923) to yield 4,6-dichloro-2,3-dihydrobenzofuran-2-ylmethyl bromide via the corresponding mercurichloride. Heating (150° C) the bromide with excess dimethylamine in benzene in a sealed tube for 15 hr gave N-(4,6-dichloro-2,3-dihydrobenzofuran-2-ylmethyl)dimethylamine, b.p. 115° C/0.3 mm (Found: C, 53.8; H, 5.5; Cl, 28.7; N, 6.05. $C_{11}H_{13}Cl_2NO$ requires C, 53.65; H, 5.3; Cl, 28.8; N, 5.7%), which with methyl bromide gave the required (4,6-dichloro-2,3-dihydrobenzofuran-2-ylmethyl)trimethylammonium bromide, m.p. 235–237° C (Found C, 41.5; H, 4.7; Br+Cl, 43.8; N, 4.15. $C_{12}H_{16}BrCl_2NO$ requires C, 42.25; H, 4.7; Br+Cl 44.2; N, 4.1%).

RESULTS

Intravenous injection of di-C1-R5 into atropinized spinal cats or cats under chloralose anaesthesia raised the carotid arterial blood pressure; a dose of 0.3 μ-mole/kg in the

spinal animal caused a rise of approximately 60 mm of mercury. The response was abolished by hexamethonium (27.6 μ-mole/kg) (Fig. 2). By comparison of log dose-response curves, it was found that di-C1-R5 had approximately one-third of the potency of choline phenyl ether on a molar basis. R5 and R5M also increased the arterial blood pressure, the responses being abolished by hexamethonium (1.4 μ-mole/kg). They had

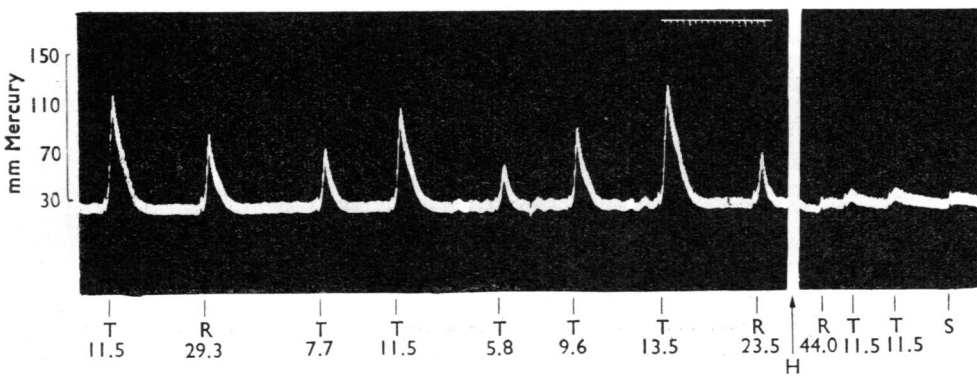

Fig. 2. Cat (1.5 kg), spinal, atropine (1 mg/kg). Recording of carotid arterial blood pressure. The first part shows the effects of TM1 (administered at T) and di-C1-R5 (administered at R), the numerals indicating the dose in m-moles ($\times 10^{-5}$)/kg injected intravenously. The second part shows the effect of hexamethonium (27.6 μ-moles/kg intravenously at H) on the response to injected TM1 and di-C1-R5. First and second part of record are separated by a 5 min interval. Time signal: 30 sec.

respectively approximately one thirtieth and one hundredth of the potency of choline phenyl ether on a molar basis. The response to R5M showed marked tachyphylaxis. Intravenous administration of R5 (2.75 μ-mole/kg) and R5M (10.5 μ-mole/kg) to anaesthetized cats caused the nictitating membranes to contract, the response being abolished by tetraethylammonium.

In contrast to the dihydrobenzofuran derivatives, R6 and R7 (7–17 μ-mole/kg) produced a fall in the blood pressure of the anaesthetized cat (Fig. 3). This hypotension was associated with a reduced response of the blood pressure to ganglionic stimulants and to injected adrenaline (Fig. 4). The duration of the antagonism to ganglionic stimulation by choline phenyl ether and 1,1-dimethyl-4-phenyl piperazinium iodide appeared to be more prolonged than did the antiadrenaline action. Intravenous injection of R6 and R7 (7–17 μ-mole/kg) had little effect on the nictitating membrane or caused slight relaxation, and the contractions elicited by electrical stimulation of the preganglionic and postganglionic cervical sympathetic nerves were reduced. The responses of the nictitating membranes to adrenaline injected intravenously were depressed by R6 and R7 for a slightly longer period than was the response to preganglionic electrical stimulation of the cervical sympathetic nerve (Fig. 5).

The relative ganglion-stimulant potencies of the compounds examined, as determined from their effects on blood pressure, are given in table 1.

The ganglionic action potentials of the isolated superior cervical ganglion of the rabbit were reduced by all the compounds investigated. The mean 50% blocking concentrations

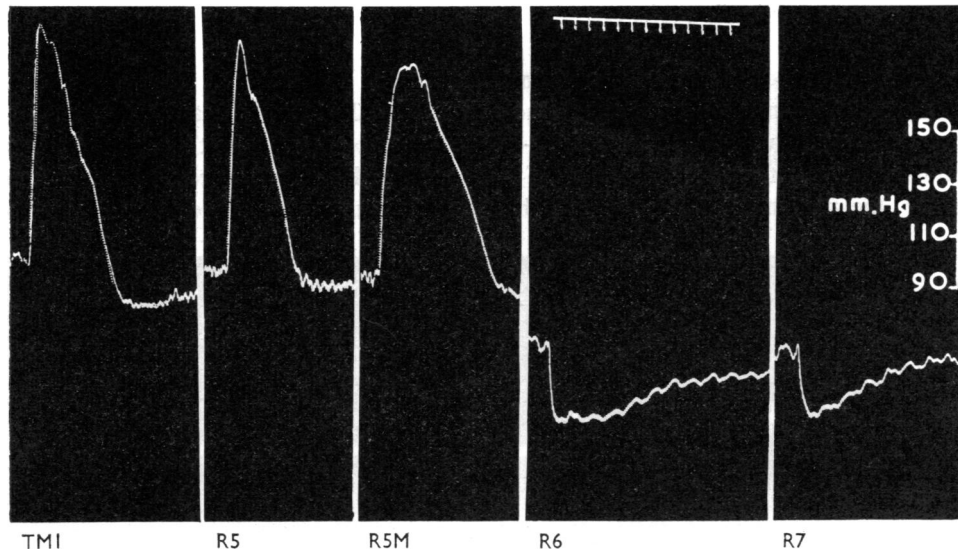

Fig. 3. Cat (2 kg), chloralose anaesthesia, atropine (1 mg/kg). Effects of intravenous administration of TM1 (6.35×10^{-2} μ-moles/kg), R5 (1.37 μ-moles/kg), R5M (5.25 μ-moles/kg), R6 (7 μ-moles/kg) and R7 (6.7 μ-moles/kg) on the carotid arterial blood pressure. Time signal: 30 sec.

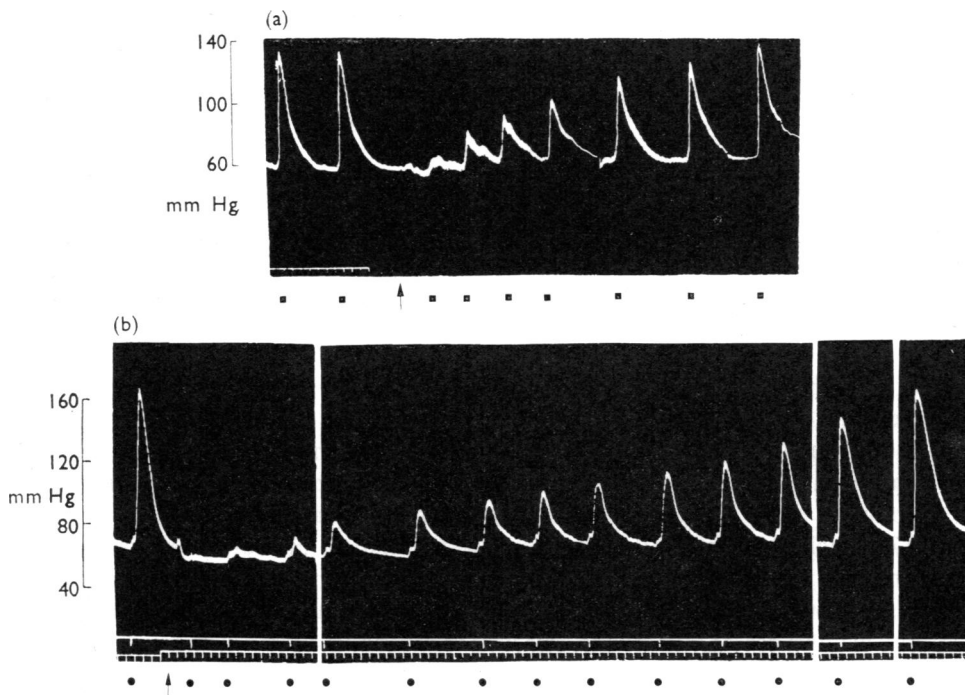

Fig. 4. Cat (2 kg), chloralose anaesthesia, atropine (1 mg/kg). Showing the effect of R7 (16.6 μ-mole/kg intravenously) at the arrows, on the response of the blood pressure to (a) adrenaline (1.4×10^{-2} μ-mole/kg intravenously) at ■ and (b) dimethylphenylpiperazinium bromide (1.5×10^{-1} μ-mole/kg intravenously) at ●. Interval between first and second parts of trace (b) was 10 min, and between second and third and third and fourth parts was 5 min. Time signal: 30 sec.

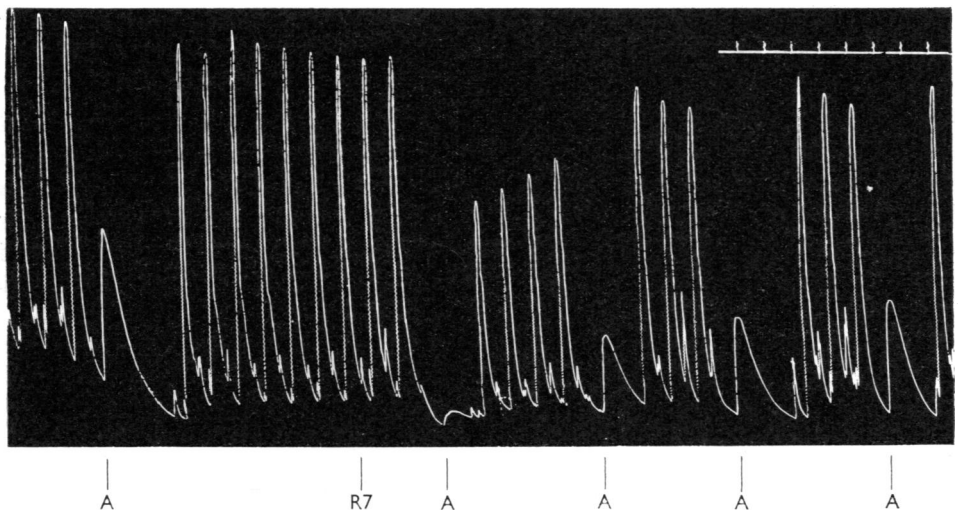

Fig. 5. Cat (2.3 kg), chloralose anaesthesia, atropine (1 mg/kg). The record shows contractions of the left nictitating membrane in response to supramaximal preganglionic stimulation of the left cervical sympathetic nerve for 15 sec in every minute. Electrical stimulation was stopped and adrenaline (2.4×10^{-2} μ-mole/kg) injected intravenously at A. R7 (14.4 μ-mole/kg) was injected intravenously as indicated. Time signal: 30 sec.

and standard errors are shown in Table 1, in which the known ganglion blocking agents hexamethonium and tetraethylammonium and the ganglion stimulant tetramethylammonium bromide have been included for comparison. No correlation was noted between the amplitude of the control ganglionic spike and the 50% blocking concentration.

Compounds capable of stimulating autonomic ganglia *in vivo* reduced the ganglionic action potential in the isolated preparation as did the "non-stimulants," β-Me-TM1,

TABLE 1

* May adopt any conformation in which "angle-of-twist" varies between 0 and 90°; † actual "angle-of-twist" =0° (see text); ‡ may adopt any conformation in which "angle-of-twist" varies between 24° and 90°; § Hunt & Renshaw, 1936; || Hey, 1952; ¶ Clark & Jana, 1966.

Compound	Spectroscopically determined "angle-of-twist"	Relative molar ganglion stimulant potency (TM1 = 100) as determined on the blood pressure of the cat	Mean 50% blocking conc. for isolated superior cervical ganglion ($\times10^{-5}$ M)
TM1	43·5*	100	1·275±0·24
o-Me-TM1	45*	50§	1·68±0·13
di-Cl-R5	—	27·1±2·1	0·56±0·06
R5	0	3·0±0·56	2·045±0·44
R5M	27·5†	0·92±0·13	5·35±1·63
β-Me-TM1	54·5‡	0‖	13·22±4·49
R6	34	0	17·89±3·11
R7	63·5	0	21·55±0·80
Xylocholine	90	0¶	18·65±3·56
Hexamethonium	—		7·90±0·89
TEA	—		37·75±5·22
TMA	—		3·40±0·17

xylocholine, R6 and R7. The transient blockade of transmission in the superior cervical ganglion of the cat by xylocholine, given intravenously, has previously been reported by Exley (1957).

The preganglionic action potential was relatively unaffected during ganglionic blockade produced by the test compounds. In only three experiments, each with a different drug, was the preganglionic action potential reduced by more than 15% when the ganglionic spike was essentially abolished, and the results from these experiments were not used in calculating the mean 50% blocking concentrations. After incubation with procaine hydrochloride (7.35×10^{-3}–4.4×10^{-1} m-mole/l.), preganglionic and ganglionic action potentials were reduced in parallel.

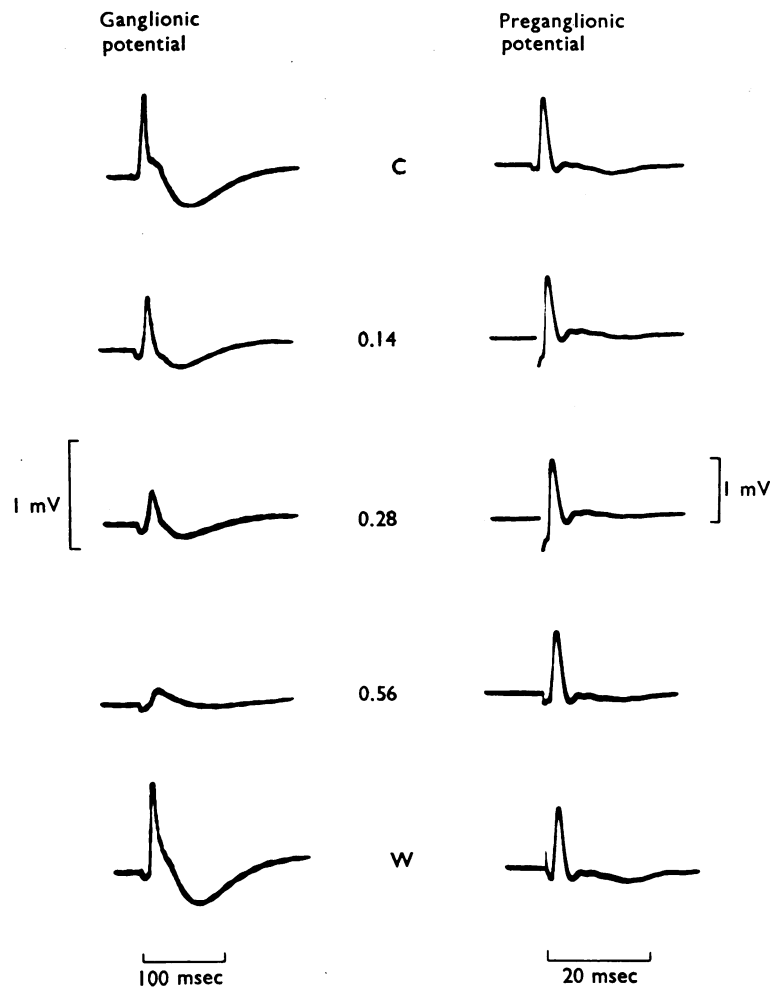

Fig. 6. Effect of β-Me-TM1 on the ganglionic and preganglionic action potentials of the isolated superior cervical ganglion of the rabbit. C indicates the control response. The numerals between the records indicate concentrations of the drug in m-mole/l. present in the bath for 15 min before recording. The responses at W were obtained after repeated washing of the preparation.

The results from a typical experiment using the isolated ganglion are shown in Fig. 6 and the log dose-response curves derived from this experiment in Fig. 7.

The log dose-response curves for the ganglion blocking activity of some representative "stimulant" and "non-stimulant" choline analogues are shown in Fig. 8. There is a significant difference ($P<0.01$) between the summed slopes of the "stimulants," di-C1-R5, TM1, R5, and the "non-stimulants," β-Me-TM1, R7 and xylocholine. The compound R5M, which possesses only weak pressor activity in the anaesthetized cat, seems to have a slope intermediate between those of the "stimulants" and "non stimulants."

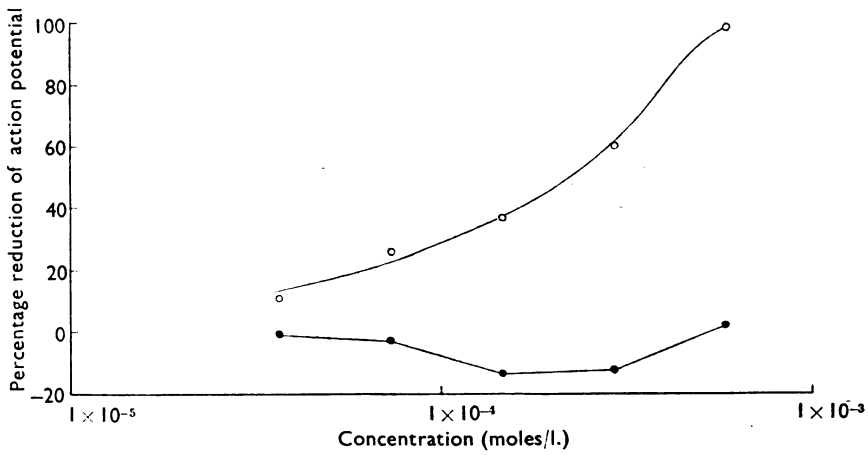

Fig. 7. Showing the relationship between the amplitudes of ganglionic (○) and preganglionic (●) action potentials (shown in Fig. 6) of the isolated superior cervical ganglion of the rabbit and the molar concentration of β-Me-TMI present in the bath fluid 15 min before recording.

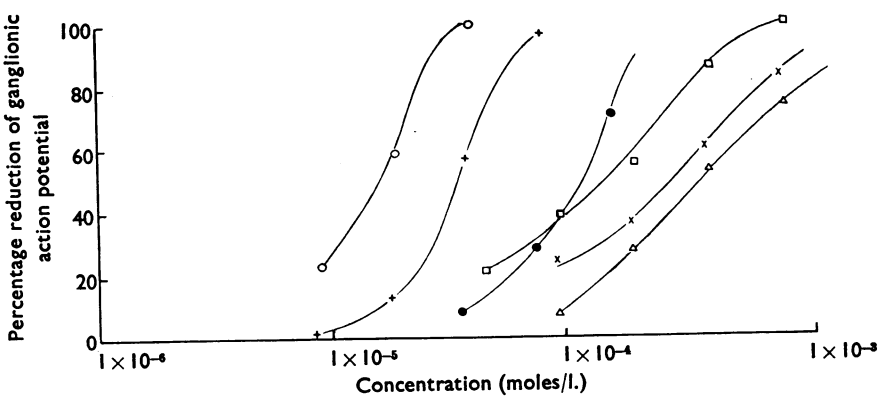

Fig. 8. A comparison of the graphs of reduction of the ganglionic action potential of the isolated superior cervical ganglion of the rabbit plotted against molar concentration of the following compounds: ○, TM1; +, R5; ●, R5M; □, R6; ×, R7; △, xylocholine. A different ganglion was used for each compound, and each curve is the result of a single experiment.

R5 at a concentration of 1.84×10^{-2} m-mole/l. and R5M at 9.4×10^{-2} m-mole/l. caused a considerable increase in the tone of the preparation of rabbit isolated small intestine. R6 was somewhat less potent in its stimulant action and R7 had no effect at a concentration of 1.67×10^{-1} m-mole/l. Both R6 (7×10^{-2} m-mole/l.) and R7 (6.7×10^{-2} m-mole/l.) abolished the response to nerve stimulation (see Fig. 9), and a similar effect could be demonstrated with R5 (7.4×10^{-2} m-mole/l.) and with R5M (3.5×10^{-1} m-mole/l.) when hexamethonium (2.76×10^{-1} m-mole/l.) was present in the bath to abolish the increased tone.

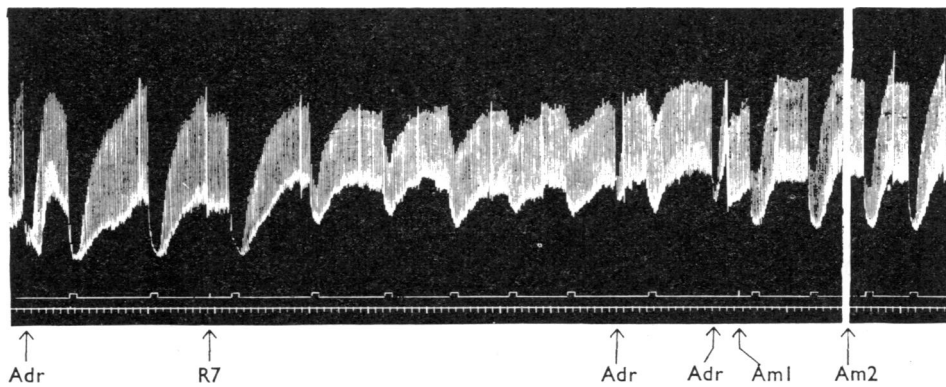

Fig. 9. Rabbit duodenum, Finkleman preparation. The periarterial nerves were stimulated at supramaximal voltage for 30 sec every 10 min at the rectangular signal. Adrenaline (Adr ; 2.7×10^{-1} μ-mole/l.) was added before and after the addition of R7 (6.7×10^{-2} m-mole/l.). The bath fluid was changed after each response to adrenaline. Dexamphetamine (27 μ-mole/l.) was added to the bath at Am1 and a further 54 μ-mole/l. in the interval between the two parts of the trace (Am2). Time signal: 30 sec. The drum was stopped between each response. Interval between the two parts of the record was 20 min.

The blockade by either R7 or R5 of the response of the small intestine to sympathetic nerve stimulation was readily reversed, following change of the bath fluid, by dexamphetamine (3.7×10^{-2} m-mole/l.), but only with difficulty by washing alone.

The anti-adrenaline activity of R6 and R7 observed on the blood pressure and nictitating membrane preparation of the cat was not so apparent on the isolated small intestine of the rabbit. Only a small percentage of inhibition of the effects of exogenous adrenaline (2.74×10^{-1} μ-mole/l.) occurred when almost complete abolition of the effects of nerve stimulation had been achieved.

DISCUSSION

As far as could be detected from observing the preganglionic action potentials, depression of conduction along the preganglionic nerve fibres did not contribute significantly to the ganglionic blockade produced by the experimental compounds. In contrast, the local anaesthetic effect of procaine was readily detectable by the reduction of the preganglionic spike. Nevertheless, depression of conduction in the non-myelinated nerve terminals, and consequent depression of transmitter release, cannot be entirely excluded as a possible

cause of the reduction in the ganglionic spike potential by the test compounds. The related quaternary compound, N-*n*-octyl-N,N-dimethyl-2-(2,6-xylyloxy)ethylammonium bromide (8 DM 10 ; Clark & Hughes 1966) has been shown to reduce the preganglionic action potential and the ganglionic spike simultaneously. The introduction of the *n*-octyl group into the quaternary head of xylocholine was shown by Clark & Hughes (1966) to affect the surface properties and the difference between the nerve blockade exhibited by 8 DM 10 and xylocholine may be solely the result of their differing lipophilic properties. Certainly, at the *p*H of Krebs solution, procaine is only partially ionized, unionized molecules being available for penetration of the lipid barrier surrounding the preganglionic nerve trunk. It is probable that an obvious local anaesthetic effect on the nerve trunk would be detected by the effect on the preganglionic action potential but local anaesthesia at the nerve terminals cannot be excluded.

Other possible mechanisms for the suppression of the ganglionic spike potential which cannot be excluded by the present experiments are a selective effect on the mechanism of release or synthesis of transmitter, or a non-specific effect on the post-synaptic membrane.

Blockade at an accessory ganglion (Douglas & Ritchie, 1956) situated between the preganglionic and ganglionic recording electrodes would reduce the recorded ganglionic spike but would be undetected in the preganglionic recordings. No evidence of an accessory ganglion in this situation has been found microscopically in the preparations used and if the accessory ganglion were situated between the stimulating and preganglionic recording electrodes, a later negative wave originating in fibres which had synapsed would be expected to arise in the ganglionic recordings. No evidence of this has been seen regularly. As concluded by Elliott (1963) from his experiments, the preganglionic nerve was probably sectioned cranial to the accessory ganglion which was reported by Douglas & Ritchie (1956) to occur approximately 4 cm central to the main ganglion.

Of the heterocyclic analogues of choline phenyl ether, only di-C1-R5, R5 and R5M showed any ganglionic (nicotine-like) stimulant activity on the blood pressure and nictitating membrane of the cat, and the stimulant effect was readily abolished by hexamethonium. R5M was only weakly active as a ganglionic stimulating agent and the onset of tachyphylaxis suggests that it is a partial agonist. R6 and R7 exhibited no ganglion stimulating activity in the cat, and the weak stimulant activity of R6 on the rabbit small intestine was only partially abolished by hexamethonium.

The compounds which are most potent in reducing ganglionic transmission in the isolated ganglion preparation are also capable of stimulating ganglia *in vivo*. The blockade produced by these compounds might therefore be caused by persistent depolarization of the ganglion cells, a mechanism postulated for the initial phase of the blockade caused by nicotine (Paton & Perry, 1953) and tetramethylammonium (Gebber & Volle, 1966), and for the equilibrium blockade produced by nicotine (Eccles, 1956). In contrast, the " non-stimulants " may be acting either as pure antagonists or by affecting, directly or indirectly, the release of transmitter. Different mechanisms of action for the two groups of drugs are suggested by the different slopes of the graphs of reduction of the ganglionic action potential against $\log_{10}$ concentration of the drug (Fig. 8). Moreover, in

a few experiments using d.c. recording through non-polarizable silver-silver chloride electrodes, TM1 was shown to depolarize the ganglion whereas R7 did not.

The importance of the ring system is demonstrated by the fact that, although R5 has approximately half the ganglionic stimulating potency of tetramethylammonium, it is more potent than the simple onium ion in reducing the ganglionic action potential. This finding is possibly an indication that depolarization alone is not the sole factor influencing the blocking action of R5. The finding that TM1, *o*-Me-TM1, di-Cl-R5 and R5 are more potent than tetramethylammonium in reducing the ganglionic action potential suggests that the moiety linked to the trimethylammonium grouping contributes to the blocking potency.

Thus ability to stimulate ganglion-cells and the greatest ability to blockade ganglionic transmission in the isolated ganglion preparation is found in those molecules, such as R5 and di-Cl-R5, which have planar ring systems and in those molecules which are sufficiently flexible to adopt a conformation in which the aryl ring, ether oxygen and C-atom located β to the quaternary nitrogen are in the same plane. Structures such as R6 and R7 which are fixed in conformations in which the ring systems are non-planar, and partially hindered molecules such as β-Me-TM1 which cannot adopt a conformation in which the benzene ring, ether oxygen and methynyl group are in one plane, completely lack ganglion stimulating activity and are only weakly active as ganglion blocking agents. We may conclude that planarity of the aromatic ring, ether oxygen and β-carbon is optimal for linkage with the ganglionic receptor.

Incorporation of the ether oxygen into the dihydrobenzofuran ring system, however, in order to ensure planarity of this part of the molecule forces the methylenic carbon, situated α to the quaternary nitrogen, out of the plane of the benzene ring, and limits the position relative to the aryl ring which can be taken up by the quaternary nitrogen. No such restriction is present in the flexible molecules of TM1 and *o*-Me-TM1. It is widely accepted that the onium group is responsible for the stimulant activity of many biologically active quaternary ammonium compounds (Ing, 1949 ; Barlow & Hamilton, 1962 ; Burgen, 1965), and the marked lowering of stimulant activity found in R5 when compared with that of TM1 or *o*-Me-TM1 may be the result of a sub-optimum steric relationship between the quaternary nitrogen and the planar ring system, which prevents the charged nitrogen from approaching the receptor site as closely as it is able to do in TM1. The strain free conformations of R6 and R7 result in even greater limitations on the relative arrangements in space of the aryl ring and the quaternary nitrogen, presumably completely preventing interaction between the nitrogen and its receptor site.

From a consideration of ring substituted analogues of TM1, Hey (1952) suggested that nicotine-like stimulant activity increased as the electron density on the ether oxygen decreased. The values found for the relative nicotinic activities of an extended series of such analogues by Coleman, Hume & Holland (1965), however, though not interpreted in these terms by the authors, suggest that there may be an optimum electron density requirement after which a further decrease in the basicity of the ether oxygen leads to reduced potency.

A corollary of the inhibition of resonance between the oxygen and π orbitals of the benzene ring when the " angle-of-twist " about the aryl-oxygen bond is 90° is that the

oxygen is more basic in character. This has been demonstrated elegantly by Brouwer, Mackor & Maclean (1966) who showed by nuclear magnetic resonance studies that whereas 2,3- and 2,5-dimethylanisole are protonated on the 4-carbon, 2,6-dimethylanisole is protonated exclusively on the ether oxygen.

On the assumption that basicity of the ether oxygen varies inversely with the degree of interaction between the oxygen and the π orbitals of the aromatic ring, the order of increasing basicity will be R5<R5M<R6<R7<TM10, and the finding that only the "planar" structures possess appreciable nicotine-like stimulant activity may be merely the result of the decreased electron density on the ether oxygen. In order to fit the flexible molecules of TM1 and *o*-Me-TM1 into this picture it must be assumed that they preferentially adopt a conformation in which the "angle-of-twist" about the aryl-oxygen bond is 0°, thus ensuring a maximum degree of interaction between the ether oxygen and benzene ring and hence a minimum basicity for the ether oxygen atom. Support for the view that a low basicity for the ether oxygen is advantageous for nicotine-like stimulant activity comes from the observation that di-Cl-R5, in which the basicity of the oxygen is expected to be less than that in R5 because of the electron attraction of the halogen groups, is more active than R5, whereas R5M, in which there is a steric inhibition of resonance resulting in an increased oxygen basicity without loss of planarity of the ring system, is less active than R5.

The need for "planarity" as an essential requirement for nicotinic activity in aryl choline ethers is demonstrated by the inactivity of β-Me-TM1. This molecule is capable of adopting a conformation possessing a minimum value for the "angle-of-twist" about the aryl-oxygen bond of 24°, and in this conformation the basicity of the ether oxygen is expected to be less than that of the oxygen in the planar R5M, the basicity of which is equivalent to that of a molecule having an "angle-of-twist" of 29°. The lack of ganglion stimulant activity in β-Me-TM1 and its presence in R5M emphasizes the part played by conformation as distinct from its secondary effects on the basicity of the ether oxygen.

From the evidence available no such specific conformational requirements can be laid down for adrenergic neurone blocking activity. In the anaesthetized cat none of the heterocyclic compounds exhibited the slowly developing blockade of the nictitating membrane characteristic of xylocholine, guanethidine and bretylium, and it might be concluded that whereas for nicotine-like stimulant activity an "angle-of-twist" about the aryl–oxygen bond of 0° is required, an angle of 90° is required for adrenergic neurone blockade. This simple picture is, however, complicated by the experiments *in vitro* on the Finkleman preparation, where it was found that, provided its ganglion stimulant activity was masked with hexamethonium, R5 blocked the effects of stimulation of the adrenergic nerves as efficiently as R7. It seems unlikely that a single receptor is involved in the sympathetic blockade in the Finkleman preparation, because this result would mean that the receptor has ill-defined structural requirments. It is more likely that different mechanisms are involved in the blockade of the sympathetic nerves achieved by these structurally very different compounds.

SUMMARY

1. The pharmacological actions of a series of heterocyclic analogues of choline phenyl ether in which the conformations vary in a known way have been examined.

2. Ability to stimulate sympathetic ganglia, as evidenced by effects on the blood pressure of the cat, was found only in those compounds in which the aromatic ring, ether oxygen, and carbon atom positioned β to the quaternary nitrogen were in the same plane.

3. The same " planar " compounds were also most active in blocking ganglionic transmission in the isolated superior cervical ganglion of the rabbit, probably as a result of depolarization of the ganglion-cells.

4. It was concluded that flexible molecules such as TM1 and *o*-Me-TM1 adopt a virtually " planar " conformation when interacting with the nicotinic receptor.

5. It is suggested that the reduction in electron density on the ether oxygen which accompanies " planarity " is a further factor which contributes to ganglion stimulant activity.

6. The lack of ganglion stimulant activity found in β-Me-TM1 is attributed to its inability to adopt a " planar " conformation.

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