THE NEUROMUSCULAR BLOCKING ACTION OF TETRAETHYLAMMONIUM

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Received August 8, 1961

The abnormally low activity of tetraethylammonium at the neuromuscular junction of the rat-diaphragm has been investigated. It seems possible to explain this lack of action in terms of the charge delocalisation and the stereochemistry of tetraethylammonium. The neuromuscular blocking activities of tetraethylammonium, isomers of it and related quaternary ammonium compounds have been determined and the structure activity relationships are discussed.

JACOBJ and Hagenberg (1902) demonstrated that the pharmacological activity of tetraethylammonium differed from that of other simple mono-quaternary ammonium compounds. While toxic doses of tetramethylammonium given to frogs gave rise to both muscarinic and curariform responses, tetraethylammonium in similar doses produced neither.

The actions of a series of quaternary ammonium compounds on the anaesthetised mammal were investigated by Marshall (1913, 1914). The successive replacement of the methyl groups in tetramethylammonium by ethyl groups was shown to lead to a decline in neuromuscular blocking activity, tetraethylammonium being eighty times less active than tetramethylammonium as a neuromuscular blocking agent. These results were confirmed by Ing and Wright (1933), who used the frog rectus abdominis preparation.

The autonomic ganglionic blockade produced by tetraethylammonium was described by Acheson and Periera (1946), who stated that tetraethylammonium over a wide range of doses had no action other than a specific ganglionic effect. Atkinson (1952), and Jepson, Simeone and Lynn (1953), reported that tetraethylammonium injected intra-arterially in cats and dogs had no significant effect in doses below 10 mg./kg. With higher doses a transient neuromuscular depression occurred preceded by a small increase of muscle responses to both direct and indirect stimulation. Stovner (1957), investigated the action of tetraethylammonium bromide on the isolated phrenic nerve-diaphragm preparation of the rat. He showed that concentrations of tetraethylammonium up to 2 millimolar caused no neuromuscular block to either single or tetanic nerve stimulation. A neuromuscular block was produced with higher concentrations which could be antagonised with potassium but not with anticholinesterases.

In a review of the curariform action of onium salts, Ing (1936) stated that curariform activity appeared to depend primarily on the ionic character of onium ions and not on their detailed chemical structure but that it was difficult to account for the abnormally low activity of tetraethylammonium.

Holmes, Jenden and Taylor (1947), considered the charge delocalisation in onium ions in relation to their curariform activity and suggested that the charge density on the central atom was critical for curariform activity. The amount of charge delocalisation of onium ions is dependent on the electronegativity of the organic radicals linked to the central atom. Hence, it was suggested that the charge density of the nitrogen atom in the tetraethylammonium ion was unusually low because ethyl groups are more electronegative than other aliphatic groups, and this explained the lack of neuromuscular blocking action of tetraethylammonium. The evidence quoted as a basis for this postulate depended on the dissociation constants of aliphatic amines and carboxylic acids.

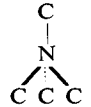
It would be expected, according to this postulate, that the replacement of a methyl group by an ethyl group in any quaternary ammonium compound should lead to a reduction in neuromuscular blocking activity. Ing and Wright (1933), however, demonstrated for a number of compounds that replacement of a methyl group by an ethyl group in quaternary ammonium compounds and quaternary arsonium compounds led to an increase in curariform activity.

The work of Thomas (1961b) on the anti-acetylcholinesterase activity of aliphatic quaternary ammonium compounds provides a possible explanation for the lack of curariform activity of tetraethylammonium. While the block of neuromuscular transmission is a more complex process than the anti-acetylcholinesterase action, involving a sequence of events and a number of different mechanisms (Van Rossum, Ariens, Linssen, 1958), the primary event in both actions is the adsorption of a compound onto a receptor and both receptors have the same natural substrate, acetylcholine. It appeared possible that the factors governing the adsorption of onium ions onto acetylcholinesterase would also have an influence on the adsorption of the ions onto the neuromuscular receptor and consequently on neuromuscular blocking action. The action of tetraethylammonium on the cholinesterases has been studied by a number of workers (Barlow and Ing, 1948; Bergman and Shimoni, 1951; Kensler and Elsner, 1951; Takagi, 1953; Thomas, 1961a) who all came to broadly the same conclusions, even though different sources of both acetylcholinesterase and cholinesterase were used. Tetraethylammonium is a very weak inhibitor of cholinesterase, particularly at low substrate concentrations, and under some conditions can even potentiate the enzyme.

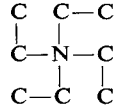
Thomas (1961b) postulated that the anti-acetylcholinesterase activity of simple quaternary ammonium compounds is related to the forces of adsorption between these ions and the active site of the enzyme and that the factors involved in the adsorption process are coulombic attraction and van der Waal's forces, with the distribution of the onium ion between the surface and the bulk of the solution also playing a part. It was further considered that the δ^+ charge on the α-carbon atoms of the quaternary ammonium group provided the major contribution to the coulombic attraction. The charge on the nitrogen atom was considered to make a relatively minor contribution to the total electrostatic binding

force. Since the configuration of a quaternary ammonium nitrogen atom is tetrahedral then the maximum number of α-carbon atoms which can be directed towards a surface is three, for example in tetramethylammonium (I). The abnormally low adsorption forces between tetraethylammonium and the enzyme, reflected by the lack of anti-acetylcholinesterase activity of the ion, may be explained only after a consideration of its stereochemistry.

Tetraethylammonium is an open chain molecule and consequently its configuration is not fixed because of possible rotation about carbon to nitrogen and carbon to carbon bonds. The configuration of tetraethylammonium in the crystalline state has been determined, however, by Waite and Powell (1958). It was shown that in projection the structure was that of a Nordic Cross (II).



(I)



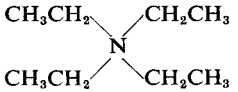
(II)

The inner carbon atoms were reported to be at the vertices of a regular tetrahedron with the nitrogen atom at the centroid. The N—C bond length was 1·49 Å and the N—C—C angle, 121° 44′, 12° greater than the normal regular tetrahedron angle. From studies in conformational analysis it is suggested that statistically the same arrangement of groups will predominate in aqueous solution where the restricting environment of a crystal lattice is absent. An examination of molecular models of such a molecule indicated that only two unhindered α-carbon atoms could be directed towards a surface or receptor whichever way the model was orientated. Consequently the coulombic forces of attraction between the tetraethylammonium ion and the anionic site of acetylcholinesterase are low. Since tetraethylammonium is the lowest homologue in which such a situation occurs then its anomalous behaviour as an anti-acetylcholinesterase agent may be explicable on this basis.

As methylene groups are added to tetramethylammonium in a symmetrical manner to produce tetraethylammonium, two factors are at work to modify the anti-acetylcholinesterase activity of the molecule; (*a*) the increase in methylene groups will tend to increase the activity as the van der Waal's forces and distribution factor are increased, and (*b*) the progressive decrease in the availability of the α-carbon atoms will decrease activity. Further addition of methylene groups to tetraethylammonium to produce n-alkyltriethylammonium compounds will increase activity by increasing van der Waal's forces and the distribution factor while coulombic forces remain constant.

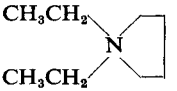
The theory is supported by the fact that 1,1′-spirobipyrrolidinium and *NN*-diethylpyrrolidinium have been found to be more powerful inhibitors of acetylcholinesterase than tetraethylammonium (Thomas, 1961a). The only difference between these compounds is that pairs of ethyl groups of tetraethylammonium are linked together to form the pyrrolidine ring.

The effect of this is to make the α-carbon atoms more available and this gave an increased activity. There is virtually no change in the van der Waal's forces or the distribution factor from one compound to the next.



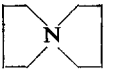
(III)

Tetraethylammonium



(IV)

NN-Diethylpyrrolidinium



(V)

1,1′-Spirobipyrrolidinium

The three compounds, III, IV, V, together with their isomers n-butyldimethylethylammonium bromide and n-pentyltrimethylammonium bromide, have now been examined for neuromuscular blocking activity.

Experimental

Chemical

1,1′-Spirobipyrrolidinium bromide and NN-*diethylpyrrolidinium bromide* were prepared as described by Thomas (1961a) and *tetraethylammonium bromide* was obtained commercially. *n-Pentyltrimethylammonium bromide* was prepared by condensing n-pentyl bromide with trimethylamine, m.p. 194–195°. Found: C, 45·9; H, 9·5. Calc. for $C_8H_{20}BrN$. C, 45·7; H, 9·5.

n-Butyldimethylethylammonium bromide was prepared by standard reactions from dimethylamine, ethyl bromide and n-butyl bromide, m.p. 208–209·5°. Found: C, 45·8; H, 9·3. Calc. for $C_8H_{20}BrN$. C, 45·7; H, 9·5.

Pharmacological

The isolated phrenic nerve-diaphragm preparation of the rat was used.

Female albinos weighing 200 g. ± 20 g., were dissected as described by Bülbring (1946). The preparation was immersed in a modified Tyrode solution (Taugner and Fleckenstein, 1950) in a perspex organ bath of rectangular cross section, similar to that described by Raventos (1959). The solution, pH 7·2, at 29° ± 1°, was aerated with a mixture of oxygen 95 per cent and carbon dioxide 5 per cent.

The phrenic nerve was stimulated by square wave pulses of 10–20 volts and 0·1–1·0 msec. duration, delivered at a rate of 3/min., were used (Attree, 1950).

The molar concentration in the suspending medium which would produce a 50 per cent reduction of the response to indirect stimulation in 3 min. was determined for each compound. The assumption was made that, over the range of concentrations used, the response increased linearly with the dose. A statistical analysis of the results showed that this was valid.

An increase in the sensitivity of the preparation to the compounds under test was developed whatever the time interval between doses. Consequently, to obtain a reliable estimate of neuromuscular blocking activity, the results were calculated from observations of the effects of four different doses of each compound applied to four different rat phrenic nerve-diaphragm preparations.

Preliminary experiments were made with each compound and suitable doses chosen. Four doses, in constant ratio, which would be expected to produce blocks of between 20 per cent and 80 per cent in 3 min., were selected. The four doses were applied each to four rat phrenic nerve-diaphragm preparations in a randomised order by a Latin square design. Only four doses were applied to any one preparation. The experimental data from the four tissues was analysed statistically (Starmer, 1961). The results are given in Table I.

TABLE I

THE NEUROMUSCULAR BLOCKING ACTIVITIES OF TETRAETHYLAMMONIUM AND ITS ISOMERS ON THE RAT PHRENIC NERVE-DIAPHRAGM PREPARATION

Compound	Molecular weight	Concentration in m-moles/ml. to produce neuromuscular block*	Confidence limits (P = 0·95)	Relative activity T.E.A = 1
Tetraethylammonium ..	210	$3{\cdot}68 \times 10^{-2}$	$(3{\cdot}48–3{\cdot}88) \times 10^{-2}$	1
NN-Diethylpyrrolidinium ..	208	$2{\cdot}27 \times 10^{-2}$	$(2{\cdot}16–2{\cdot}38) \times 10^{-2}$	1·6
1,1 -Spirobipyrrolidinium ..	206	$1{\cdot}19 \times 10^{-2}$	$(1{\cdot}15–1{\cdot}23) \times 10^{-2}$	3·1
Dimethylethylbutylammonium	210	$1{\cdot}83 \times 10^{-3}$	$(1{\cdot}75–1{\cdot}91) \times 10^{-3}$	20
Trimethylpentylammonium	210	$4{\cdot}81 \times 10^{-4}$	$(4{\cdot}47–5{\cdot}17) \times 10^{-4}$	77

* Concentration per ml. of bath fluid required to produce a 50 per cent block in 3 min.

RESULTS AND DISCUSSION

The factors which constitute the total binding force appear to be those suggested by Thomas (1961b) to be important for the adsorption of quaternary ammonium compounds onto acetylcholinesterase.

The explanation for the anomalously low activity of tetraethylammonium at the neuromuscular junction can therefore be explained in terms of stereochemistry and charge delocalisation.

Trimethylpentylammonium bromide. The structure of this compound is such that the conditions for its adsorption onto an anionic receptor are the best possible, three α-carbon atoms being completely available for electrostatic binding whichever way the molecule approaches the surface. Consequently, the coulombic forces would be at a maximum for an aliphatic onium ion. Since the pentyl chain is normal once the onium ion had become associated with the anionic area, it could orientate itself to come into close contact with the surface of the receptor. Thus potentially a van der Waal's bond could be formed between all the carbon atoms of the chain and the atoms of the enzyme surface. Finally, because of the polar–nonpolar asymmetry of the molecule it would be expected that it would concentrate at the surface of a solution. These three factors lead to the conclusion that *a priori* trimethylpentylammonium would be adsorbed onto an anionic receptor more avidly than any other of the compounds examined and, therefore, should be the most active neuromuscular blocking compound of the series.

Dimethylethylbutylammonium bromide. Statistically, the availability of the α-carbon atoms is less with this structure because of the substitution of one of the methyl groups, but the van der Waal's forces and the

concentration of the ion at the surface of the solution would be similar to trimethylpentylammonium. Consequently, dimethylethylbutylammonium should be a weaker neuromuscular blocking agent than trimethylpentylammonium.

Tetraethylammonium bromide. We have already shown that the binding forces between tetraethylammonium and the anionic site on acetylcholinesterase are unusually low, with the result that tetraethylammonium is a very weak inhibitor of acetylcholinesterase. It can be seen from Table I that it is also the weakest neuromuscular blocking agent of the series.

Diethylpyrrolidinium bromide. The structure of this compound is similar to that of tetraethylammonium but two of the ethyl groups are linked through the β-carbon atoms to form a pyrrolidine ring. From a stereochemical point of view the α-carbon atoms are made more available for binding to the anionic site of acetylcholinesterase than with tetraethylammonium. The other factors, such as potential van der Waal's forces and distribution between bulk and interface in solution, should be similar in both tetraethylammonium and *NN*-diethylpyrrolidinium. The effect of the change in structure from tetraethylammonium would be to increase the binding forces between an anionic receptor and *NN*-diethylpyrrolidinium. *NN*-Diethylpyrrolidinium is a more powerful inhibitor of acetylcholinesterase than tetraethylammonium (Thomas, 1961a) and from Table I it may be seen that it has also a more powerful neuromuscular blocking action.

1,1′-*Spirobipyrrolidinium bromide.* In this compound the rotation of all the ethyl groups is restricted with the result that, theoretically, the α-carbon atoms are more available for binding than in the previous compound. Since the other factors are virtually constant it would be expected that 1,1′-spirobipyrrolidinium would be more active than *NN*-diethylpyrrolidinium. However, to offset the advantage conferred by making the molecule more rigid, it may be that the potential van der Waal's forces would be less because the ethyl groups would not be able to orientate towards a surface under the influence of the free energy of the surface atoms of the receptors. On balance, it is difficult to assess which factors would have the greater influence, but the spiran compound should be more active than tetraethylammonium in all tests. It may be seen from Table I that 1,1′-spirobipyrrolidinium is a more powerful neuromuscular blocking agent than tetraethylammonium and it has also been shown (Thomas, 1961a) that the spiran compound is more active as an inhibitor of acetylcholinesterase than tetraethylammonium. However, when *NN*-diethylpyrrolidinium and 1,1′-spirobipyrrolidinium are compared it is seen that the spiran is a more powerful neuromuscular blocking agent (Table I) but less active as an inhibitor of acetylcholinesterase (Thomas, 1961a). This may be taken to reflect the opposition of the two factors.

If the compounds listed in the table are analysed from the point of view of charge delocalisation and stereochemistry the order of activities becomes explicable.

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