Some effects of altering onium substituents on the internitrogen distance in ganglionic and neuromuscular blocking agents

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The conductances of butaethonium chloride and bromide, hexaethonium chloride and bromide, and hexadecaethonium iodide have been measured in water at 25°. The limiting equivalent cationic conductances have been interpreted to give a measure of the internitrogen distances, which are larger than in the corresponding methonium compounds, being 5·7–6·7 Å for butaethonium, 6·5–8·7 Å for hexaethonium, and 13·6–14·9 Å for hexadecaethonium. The changes of potency observed on changing the onium substituents are attributed to changes in internitrogen distance and of molecular geometry.

IN a previous paper (Elworthy, 1963b) the conductances of hexa-, deca-, and hexadecamethonium cations were interpreted in terms of internitrogen distance, showing that these molecules were considerably contracted in aqueous solution, due to the high interfacial energy present between the hydrocarbon chains and water. Measurements on deca-ethonium showed that the contraction was less pronounced than for decamethonium. As considerable changes in biological activity occur when the onium group substituents are altered, studies on additional ethonium compounds have been made in an attempt to relate changes of internitrogen distance and molecular geometry to biological activity.

Experimental

MATERIALS

The compounds were prepared by reacting the redistilled α–ω dibromo- or diiodo-alkanes with excess triethylamine in dry ethanol. The reaction mixture was kept for one week in the cold, refluxed for 4–5 hr, and the excess triethylamine and ethanol removed by distillation. The residue was recrystallised at least three times from a suitable solvent, and dried. Assays by releasing the free acid or free base on a strong cation (Zeocarb 225) or anion (Deacidite FF) exchange column, and titrating the effluent with standard alkali or acid, gave purities of 99·9% or better. Buta-ethonium and hexaethonium chlorides were prepared from the relevant bromides by passing their solutions down a strong anion-exchange column, which had been converted to the chloride form with Analar potassium chloride.

Water for preparing solutions was twice distilled and had specific conductance of 0·6–0·9 $\times$ 10^6 mho.

MEASUREMENTS

The conductance bridge used has been described (Elworthy, 1963b). The two cells previously used have been replaced by one flask type cell,

k = 0·6057, calibrated with solutions of recrystallised Analar potassium chloride using the Fuoss-Onsager equation to calculate the conductances of the solutions (Lind, Zwolenik & Fuoss, 1959). The cell had lightly platinised platinum electrodes, and included a polythene coated magnetic follower. Approximately 300 g water was weighed into the cell, which was then placed in thermostated oil bath (25° ± 0·003°), allowed to stand overnight, and the resistance of the water determined on the following day. The compounds were weighed into small pyrex tubes, which were then introduced one at a time into the cell, the stirrer started, and readings of the resistance taken until constant values were obtained for each tube. A run consisted of successively introducing 4–5 samples in this manner. 2–3 runs were made on each compound. The measuring resistance was calibrated from a Cambridge Precision Decade Bridge.

Results

The conductance results are shown in Figs 1 and 2 as plots of equivalent conductance (Λ) against (normality)$^{\frac{1}{2}}$; preliminary Λ_0 values were found by extrapolating these phoreograms to zero concentration. All the salts studied appeared to be slightly associated, approaching the Onsager limiting tangent from below, and the final fitting of the results to obtain Λ_0 was by the method of Fuoss & Shedlovsky (see Fuoss & Accascina, 1959). Decaethonium iodide was found to be associated in previous work. Using 78·1 for $\lambda_0(Br^-)$, 76·8 for $\lambda_0(I^-)$, and 76·4 for $\lambda_0(Cl^-)$ the λ_0 values found for the cations were:

	λ_0 from chloride	λ_0 from bromide	λ_0 from iodide
Butaethonium	42·8	42·7	
Hexaethonium	39·4	39·4	
Hexadecaethonium			31·6
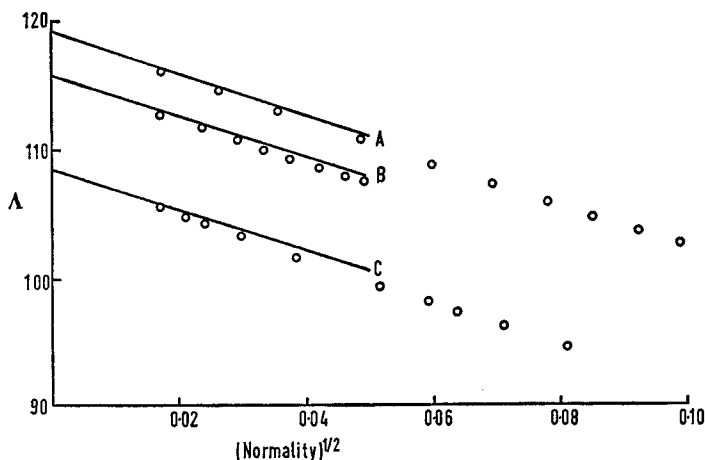
FIG. 1. Plots of equivalent conductance (Λ) against (normality)$^{\frac{1}{2}}$ for A. Butaethonium chloride. B. Hexaethonium chloride. C. Hexadecaethonium iodide.
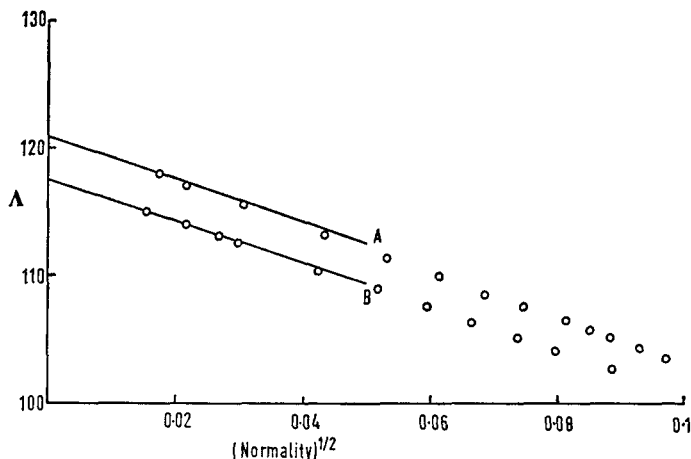
FIG. 2. Plots of equivalent conductance (Λ) against (normality)$^{\frac{1}{2}}$ for A. Buta-ethonium bromide. B. Hexaethonium bromide.

The limiting cationic conductances are interpreted in terms of molecular shape using the methods previously described (Elworthy, 1962, 1963a, 1963b) representing the molecules as prolate ellipsoids, and calculating the long (a) and short (b) semi-axes. From a, b, and the volume of each grouping in the molecule, values of the distance from the centre of the ellipsoid (distances, x, along the a axis) corresponding to the volume of the molecule inside and outside the nitrogen atoms were found (equation 6, Elworthy, 1963b).

The results on the ethonium compounds do not provide as clear an estimate of internitrogen distance as those on the methonium salts. This is due to the several possible arrangements of the ethyl groups on the nitrogen atoms. The three principal arrangements (see Elworthy, 1963b) are: (1). Ethyl groups lying in close contact with the main hydrocarbon chain which links the nitrogen atoms. (2). Two ethyl groups close to the main hydrocarbon chain, and one facing outwards in the x direction. (3). One ethyl group close to the chain, and two directed outwards. The internitrogen distances calculated for these alternative arrangements are given in Table 1.

TABLE 1. INTERNITROGEN DISTANCES FOR ETHONIUM COMPOUNDS ($\mathring{A}$)

$Et_3\overset{+}{N}[CH_2]_n\overset{+}{N}Et_3$

Position of ethyl groups	n = 4	n = 6	n = 10	n = 16
1	8·3	10·4	13·2	16·7
2	6·8	8·8	11·5	15·0
3	5·7	7·5	10·2	13·6
Nordic cross	6·0	7·9	10·6	14·0

The results of Wait & Powell (1958), on the crystal structure of tetra-ethylammonium iodide showed that the cation had the configuration of

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a nordic cross. Although in the case of the ethonium compounds, this configuration may not be maintained in solution, it falls between positions 2 and 3, which appear, from molecular models, to be the most strain free arrangements. In all it seems likely that the shaded area in Fig. 3 represents the most probable inter-onium distance for these compounds.

Discussion

Elworthy (1963b) has pointed out how an interfacial energy effect led to greater molecular extensions in the ethonium series, compared to the methonium series. This result is not predictable from Gill's (1959) calculations. At this stage it seems profitable to discuss some of the results in relation to biological activity.

The principal conclusions are:

(1)   The molecules are much contracted in solution.

(2)   Introduction of ethyl for methyl on the head groups increases the internitrogen distance.

It is usual to suggest a two point attachment theory for neuromuscular block, and in many cases for ganglionic block. It is also common to consider "holes" in receptor surfaces into which head groups drop neatly. The implication of conclusion (1) is that, due to contraction, the molecule resembles an ellipse from a side view; the diameter of the head group may thus be smaller than the diameter (2b) at the centre of the molecule in the methonium series. The implication of conclusion (2) is that the more extended configuration produced by substituting ethyl for methyl groups, which causes the chain to lengthen, also decreases the diameter at the centre of the molecule; the diameter at the position of the head groups is increased by the substitution. Some pharmacological results are examined in the light of these considerations.

For neuromuscular block, decamethonium had peak activity, having an internitrogen distance of 9·5 Å. Decaethonium, however, has an internitrogen distance of 10·8 Å (mean value from shaded area in Fig. 3), and its potency is 10 to 25% that of decamethonium (Barlow, Roberts & Reid, 1953; Thesleff & Unna, 1954). Wein, Mason, Edge & Langston (1952) showed that converting the methonium into an ethonium head group for compounds with n = 6 and 7 enhanced their neuromuscular blocking power. For n = 6 the change in internitrogen distance is from 6·3 to 8·0 Å, for n = 7 from 7·2 to 8·8 Å; the ethonium compounds come closer to the 9·5 Å distance of decamethonium, which is presumably necessary for peak activity. Thesleff & Unna give results showing the same general trend. However, the potency (or type of action) of a molecule expanded by introduction of ethyl groups cannot be expected to be the same as that of a longer chain methonium compound, because the geometry of the molecules is different, as is the fit on the receptor site, and the distances from the centre of the onium group to the receptor atoms. Nevertheless, the change of internitrogen distance on altering
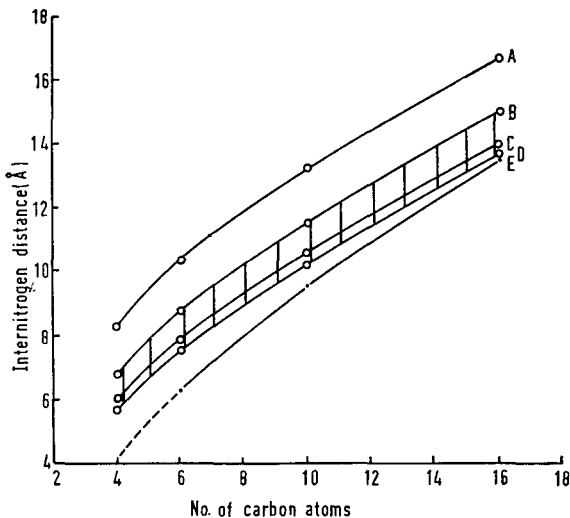
FIG. 3. Internitrogen distance against number of carbon atoms separating the nitrogen atoms. Ethonium Compounds: A. Position 1. B. Position 2. C. Nordic cross. D. Position 3. E. Methonium Compounds.

the head groups does give a guide to explaining some of the potency changes.

It is interesting to speculate on the implications of a geometrical arrangement of receptor points for neuromuscular block. Using 9·5 Å as the critical distance between receptor points, and letting them be present on the corners of a square, we have a hypotenuse of 13·6 Å.
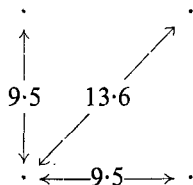
This distance should provide a second set of receptor points for attaching molecules to the surface. Molecules having the required internitrogen distance are n = 16–17 for methonium and n = 14–15 for ethonium derivatives. Barlow & Ing (1948) showed that the most potent member of the ethonium series tested was n = 13, no tests of longer chain compounds being made; n = 13 has an internitrogen distance of 12·6 Å. Examination of Paton & Zaimis's (1949) results shows that in most of the species of animal used, the n = 18 methonium derivatives was more potent than n = 11 or n = 12 methonium derivatives. The n = 18 compounds give an approximate fit on the second site. It was realised that the n = 16 methonium compound should also fit the longer site; a preliminary test by courtesy of Mr. J. J. Lewis indicated a comparable

potency of the n = 16 and n = 18 methonium compounds (comparison of n = 16 methonium and tubocurarine, and using Paton & Zaimis' n = 18 methonium and tubocurarine figures).

Turning now to ganglionic block, the n = 4 methonium compound shows an increase of potency on introducing ethyl groups, the increase in internitrogen distance being from 4·2 Å to 6·4 Å. This second distance is almost exactly that of hexamethonium, but the ganglionic blocking potency of butaethonium is 6% that of hexamethonium. The indication is that other geometrical factors in the molecule are as important as internitrogen distance. The shorter chain molecules, like hexamethonium, show a smaller percentage contraction in solution than the longer ones; their equatorial diameter (2b) is not so great. Thus the bulkiness of head groups should have a more significant effect on the fit of short chain molecules to receptor sites than on that of long chain ones. Of particular interest are the potencies of n = 5 and n = 6 compounds; with Me_2EtN^+ and $BuMe_2N^+$ head groups these compounds have greater potencies than the corresponding methonium compounds. Although the hydrocarbon chains will be more expanded in these derivatives, some small groups are still present, suitable orientation of which could still bring the onium nitrogens atoms close to the receptor surface.

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