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OBSERVATIONS UPON ROLES OF ETHER OXYGEN, ALKYL GROUP SIZE, NUMBER OF ONIUM CENTRES AND INTER-ONIUM DISTANCE UPON NEUROMUSCULAR BLOCK IN BIS- AND POLYONIUM COMPOUNDS

BY

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Using the method of Van Rossum & Ariëns (1959), a number of bis-, tris- and tetra-onium neuromuscular blocking agents have been classified into depolarizing, non-depolarizing and intermediate-acting compounds. This investigation confirmed the results of experiments using conventional techniques. The role of the ether oxygen link, the effects of inter-onium distance, alkyl group size and the number and nature of the onium groups were also investigated, and the reasons underlying the observed quantitative and qualitative differences discussed.

Systems of classifying neuromuscular blocking agents which depend upon an attempted correlation of the type of block produced with molecular structural features have proved, in the main, to be unsatisfactory. Thus the stereochemical classification of neuromuscular blocking agents into pachycurares and leptocurares (Bovet, 1951) fails, for example, to account for the depolarizing activity of nicotine, the lack of depolarization by tridecamethonium, tridecamethylene-bis(trimethylammonium), in the chicken (Zaimis, 1952) and by many other linear polyonium muscle relaxants (see Edwards, Stenlake, Lewis & Stothers, 1961, for references). The equidistance concept of neuromuscular blocking activity by bis- and tris-onium compounds (for example, Barlow & Ing, 1948; Pfeiffer, 1948; Paton & Zaimis, 1949; Kimura & Unna, 1950) over-emphasized molecular linearity and a one-dimensional spatial arrangement and failed to consider the conformational isomerism possible about carbon bonds in non-rigid molecules. On the other hand, the value of using rigid molecules to interpret drug-receptor interaction appears to be limited, since the characteristics of the receptor may alter in response to the presence of the drug molecule (Koshland, 1958). Furthermore, a limited degree of molecular flexibility appears to be essential for activity at the ganglionic synapse (Gill, 1959) and at the neuromuscular junction (Wien & Mason, 1953).

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The method of classification proposed by Van Rossum, Ariëns & Linssen (1958) has the advantage that it is based only upon drug-receptor interaction and enables the effects of variation in chemical structure upon drug activity to be described without associating pharmacological properties with particular chemical features. Use of the dose/response curve obtained on the frog rectus abdominis muscle allows changes in pharmacological activity, brought about by changes in molecular structure, to be related to two basic constants ; the affinity and the intrinsic activity. The affinity is a measure of the ability of the drug to enter into complex formation with a receptor, and can be shown to be numerically equal to the reciprocal of the dissociation constant of the drug-receptor complex. The intrinsic activity is a measure of the power of the drug-receptor complex to evoke a positive biological response and is represented numerically by the proportionality constant relating the observed biological response to the proportion of receptors occupied when equilibrium has been achieved between the drug molecules and the receptors.

The Ariëns' (1954) approach assumes that the degree of biological response is a direct function of the proportion of the receptors occupied by the drug, that the concentration of the drug applied is large compared with the concentration of receptors but still sufficiently low to permit substitution of actual concentrations for thermodynamic activities at the dose levels employed, and that equilibrium conditions pertain. In its simplest form, the equation defining the dose/response curve is first order with respect to both drug and receptor, and the close agreement usually found between the shape of the theoretical curve and that obtained experimentally (for example, Ariëns, Van Rossum & Simonis, 1957) is strong circumstantial evidence for the validity of the approach. Differences between the theoretical and the experimental curves are readily explained by assuming deviation from second order kinetics (Cavanaugh & Hearon, 1954).

Further support for the validity of the approach is given by the identical form of the Ariëns' equation with that of the Langmuir isotherm and with that of the equation resulting from the kinetic approach of Michaelis and Menten (Ariëns, 1954), where it is assumed that a steady state exists and that the biological effect is proportional to the velocity of drug-receptor complex formation. Recently stress has been laid on the importance of the kinetic element in drug-receptor interaction (Paton, 1961). The Ariëns' intrinsic activity constant is, however, composite, itself incorporating the rate constant for the dissociation of the drug-receptor complex (Ariëns & Simonis, 1960). Challenges to the assumption that a biological response is a direct function of the number of receptors occupied have appeared (e.g., Stephenson, 1956 ; Nickerson, 1956), but these have, however, been rebutted (Rocha e Silva, 1957).

In the present work the method of Van Rossum & Ariëns (1959) has been applied to the study of a number of polymethylene bis-, tris- and tetra-onium salts showing neuromuscular blocking activity, in order to secure both a convenient classification and also a theoretical interpretation of the results.

METHODS

The method employed was that of Van Rossum & Ariëns (1959), but using *Rana temporaria* instead of *R. esculenta*.

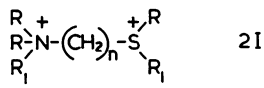
In all experiments the standard agonist was decamethonium iodide (2 μmole/ml.) and cumulative dose/response curves were constructed by step-wise addition of this drug in the sequence: a, a, 2a, 4a, 8a and so on (a=0.01 ml. added by an automatic constriction pipette). When the maximum effect had been recorded the muscle was washed thoroughly until complete relaxation was attained (1 to 1.5 hr). Compounds possessing a similar agonistic effect were compared with decamethonium by noting the height of the dose/response curve obtained by adding them to the bath as described above. Compounds having other types of muscle relaxant activity were investigated by adding them to the bath 15 min before the addition of decamethonium, and by recording their effect on the subsequent dose/response curve.

RESULTS

The chemical structures of the compounds investigated, together with their affinity and/or intrinsic activity values, where measured, are shown in Table 1.

TABLE 1

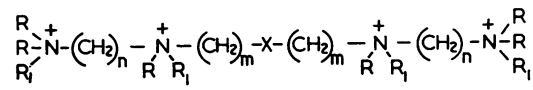
THE CHEMICAL STRUCTURES, CODE NUMBERS, AFFINITIES AND INTRINSIC ACTIVITIES OF COMPOUNDS IN GROUPS 1, 2a AND 2b



$2I^-$

Group 1

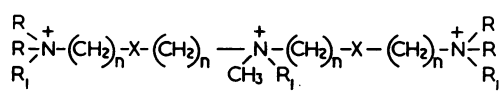
Code no.	R	R_1	n	Intrinsic activity	Affinity	Relative affinity (decamethonium=1)
I	CH_3	C_2H_5	8	0·32	0·025	0·13
II	CH_3	CH_3	10	1·0	0·13	0·68
III	CH_3	CH_3	8	0·62	0·062	0·33
Oxydipentonium				1·0	0·16	0·84
Decamethonium				1·0	0·19	1·0



$4I^-$

Group 2a

Code no.	R	R_1	n	m	X	Inhibition index	Affinity	Relative affinity (tubocurarine=1)
E_{77}	C_2H_5	C_2H_5	6	2	O	0·0163	61·35	2·95
E_{79}	C_2H_5	C_2H_5	6	2	CH_2	0·034	29·41	1·41
E_{78}	C_2H_5	CH_3	6	2	O	0·243	4·12	0·20
E_{80}	C_2H_5	CH_3	6	2	CH_2	0·307	3·26	0·16



$3I^-$

Group 2b

Code no.	R	R_1	n	X	Inhibition index	Affinity	Relative affinity (tubocurarine=1)
E_{98}	C_2H_5	C_2H_5	2	O	2·10	0·48	0·023
E_{99}	C_2H_5	CH_3	2	O	2·60	0·39	0·019
E_{100}	CH_3	CH_3	2	O	7·15	0·14	0·007
E_{101}	CH_3	C_2H_5	2	O	6·33	0·16	0·008
E_{102}	CH_3	n-C_3H_7	2	O	5·23	0·19	0·009
E_{103}	C_2H_5	n-C_3H_7	2	O	1·96	0·51	0·025
E_{151}	CH_3	CH_3	6	Nil	2·43	0·41	0·02
Tubocurarine dimethyl ether					0·168	5·95	0·27

The interaction of a substance A with a receptor system R containing r_o receptors, is represented by the equation $E_A = r_o \alpha / \frac{K_A}{[A]} + 1$ (Ariëns & De Groot, 1954) where E_A is the effect of substance A, K_A the dissociation constant of the complex RA; α the intrinsic activity of A, the concentration of which is represented by [A].

The maximum contractural effect of a compound is proportional to its intrinsic activity (Ariëns & De Groot, 1954). The dose of a compound producing a constant fraction of the maximum response, for example 50%, is proportional to the value of the dissociation constant of the drug-receptor complex and inversely proportional to its affinity (Ariëns & De Groot, 1954).

The agonistic, contracture-inducing properties of decamethonium (with intrinsic activity arbitrarily assigned the value of unity), oxydipentonium (5,5'-bis(trimethylammonium)dipentyl ether dichloride) and compounds I, II and III (Table 1) are compared in Fig. 1. The order of addition of the drug and of decamethonium to the bath was determined from a table of random numbers, which assigned equal chances of coming first or second to either decamethonium or the drug with which it was being compared. Oxydipentonium and compound II were each compared

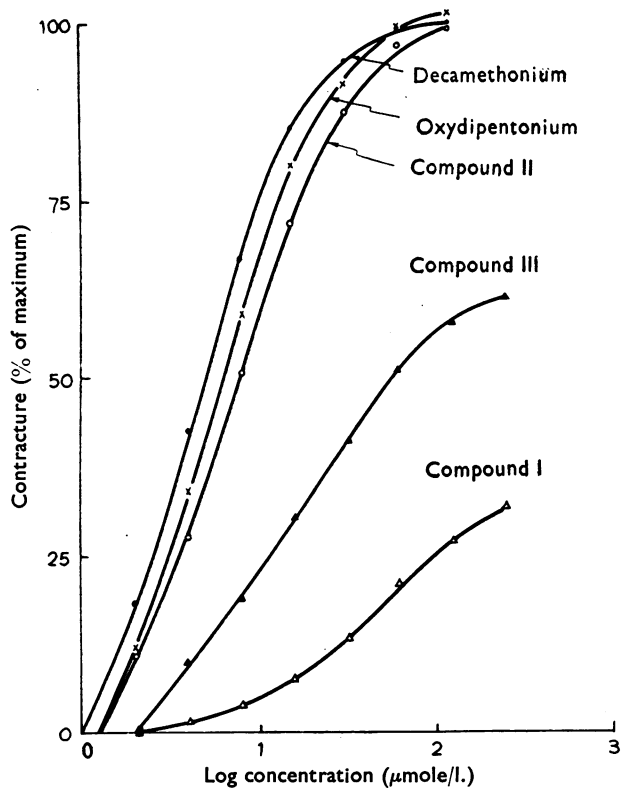


Fig. 1. Comparison of the cumulative log dose/response curves for decamethonium, oxydipentonium and compounds I, II and III. The differences in the maximum heights and the horizontal displacements of the curves along the abscissa indicate the differences in the intrinsic activities and the affinity values of these compounds. See Table 1 for chemical formulae.

with decamethonium in twenty experiments, while compounds I and III were each investigated ten times. Using Student's *t*-test (Snedecor, 1959) no significant differences in the intrinsic activity values of decamethonium, oxydipentonium and compound II ($0.2>P>0.1$) were revealed. There were, however, significant differences between the intrinsic activity values of decamethonium and compound I ($P<0.001$) and between compound III and decamethonium ($P<0.001$) as indicated by the reduction in the maximum height of contracture produced.

The values of the dissociation constant (K_A) and the affinity for each drug in Group 1 were also calculated (Table 1) (Ariëns & De Groot, 1954).

The comparatively low intrinsic activity of compound I, confirmed by observations with the conventional frog rectus assay procedure (Muir & Lewis, 1959), prompted a further investigation of the mode of action of this drug. Accordingly, a series of log dose/response curves was constructed for decamethonium, alone, and in the presence of constant concentrations of compound I. The shape of the curves obtained (Fig. 2) indicated that this drug possessed both agonistic and non-competitive antagonistic blocking properties (Van Rossum *et al.*, 1958). At higher dose levels the auto-antagonistic action (Ariëns *et al.*, 1957) of the drug could also be demonstrated.

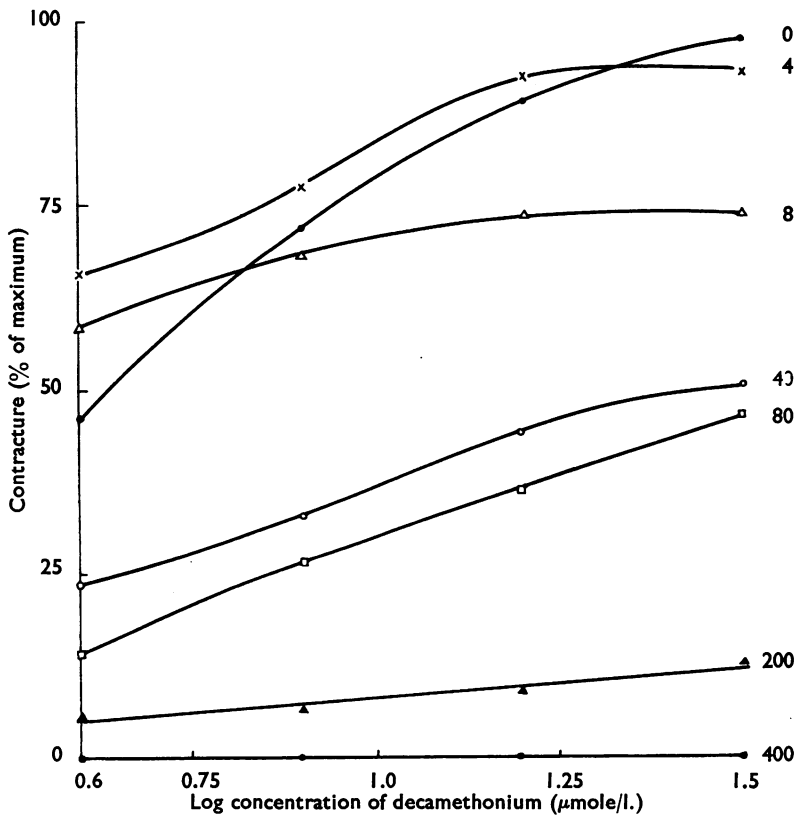


Fig. 2. The agonistic and non-competitively antagonistic properties of compound I (concentrations on right) are observed as a contracture and a decline of the maximum height of the dose/response curves for decamethonium.

All the remaining compounds lack depolarizing properties. The initial dose chosen for investigation was obtained from experiments using the conventional frog rectus assay method. The dose of tubocurarine initially used was 1 μmole/l. Subsequently, integral multiples of this dose of tubocurarine and of the other compounds were used.

Of the compounds in Group 2 (Table 1) none produced contractures of the frog rectus abdominis muscle but, when placed in the bath before the addition of decamethonium, each displaced the log dose/response curve along the abscissa without affecting either the slope or the maximum observed contractural response. Thus, in the presence of constant doses of these compounds, the decamethonium log dose/response curve suffered a parallel displacement along the abscissa indicating the presence of affinity for the receptors but the absence of measurable intrinsic activity in the compounds of this group (Van Rossum *et al.*, 1958).

The interaction of depolarizing and non-depolarizing compounds has been discussed by Ariëns & De Groot (1954). The mathematical relationship derived to represent this interaction has been expressed as

$$\frac{E_{max}}{E_{AB}} = \frac{K_A}{K_B} \times \frac{[B]}{[A]} + 1$$

where E_{max} is the maximum effect, and E_{AB} is the combined effect of the respective depolarizing and non-depolarizing substances A and B. The ratio $\frac{[B]}{[A]}$, for doses

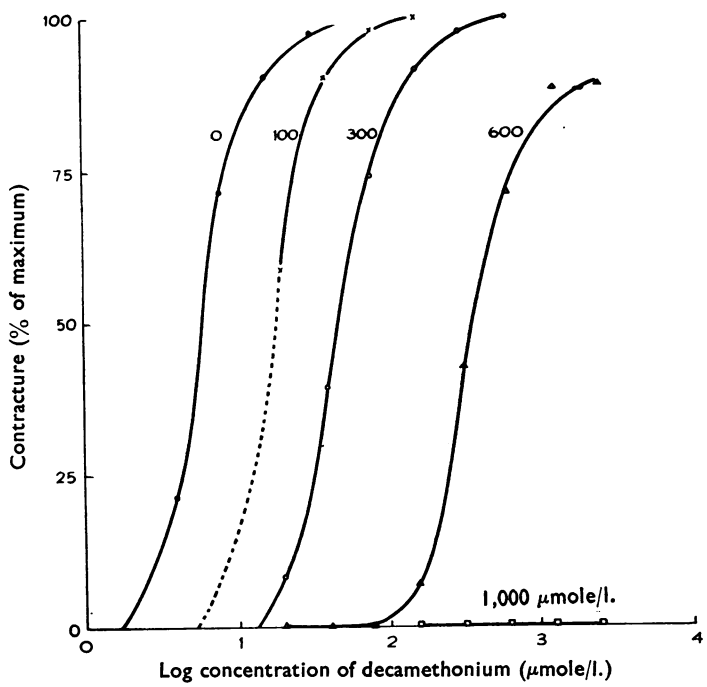


Fig. 3. The competitively antagonistic properties of compound E_{101} (100 and 300 μmole/l.) in the presence of decamethonium (abscissa) gives way to apparent non-competitively antagonistic effects at higher concentrations (600 and 1,000 μmole/l.) as indicated by the decline in the maximum height of the log dose/response curves.

producing 50% of the maximum response, has been termed the " inhibition index " (Ariëns & De Groot, 1954) and is constant for a truly competitive antagonism between two drugs. Thus, for the interaction of such compounds, the " inhibition indices " may be used as measures of the dissociation constants, or the affinities, of the compounds concerned. When several non-depolarizing compounds are investigated against the same depolarizing compound (for example, decamethonium) then, since K_A for the latter is constant, the " inhibition indices " are inversely proportional to the affinities between the receptor and non-depolarizing compounds (Ariëns & De Groot, 1954). " Inhibition indices " for the compounds in Groups 2a and 2b were accordingly calculated as means of at least three individual results (Table 1). When the doses of the drugs used were greater than those usually required to inhibit completely acetylcholine-induced contractures, differences in the shapes and heights of the decamethonium log dose/response curves were observed. A non-parallel displacement of the curves along the abscissa, and a decline in the maximum height of the contractures, were also observed. These effects are characteristic of a non-competitive antagonism (Van Rossum *et al.*, 1958 ; Fig. 3).

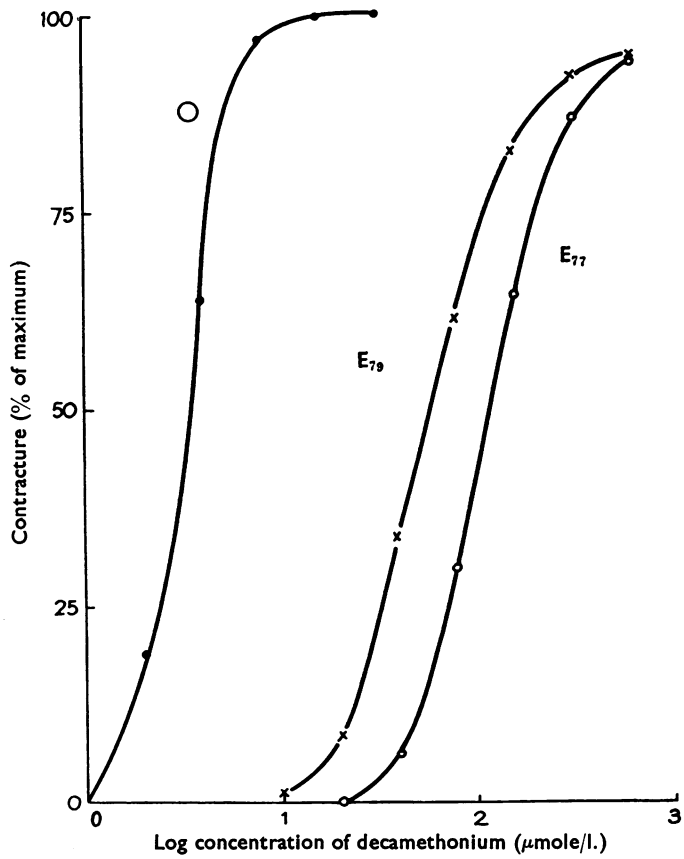


Fig. 4. The difference in the horizontal displacement of the log dose/response curves of decamethonium in the absence (O) and in the presence of E_{77} and E_{79} (2 μmole/l.) represents a difference in the affinity of the two compounds (see Table 1). Both E_{77} and E_{79} show competitively antagonistic properties.

The role of the ether oxygen function was investigated by comparing the degree of displacement of the decamethonium log dose/response curve produced by equimolar doses of the ethers and the related non-ethers. In addition to oxydipentonium and decamethonium, three pairs of compounds were investigated, namely compounds E_{77} and E_{79}, E_{78} and E_{80} and tubocurarine dimethyl ether and tubocurarine (Table 1). The non-isosteric compounds E_{100} and E_{151} were similarly tested. All the compounds were used in doses at which they antagonized decamethonium competitively. A qualitatively similar effect was produced by each of the eight compounds, but quantitative variations in the degree of displacement of the decamethonium log dose/response curve were observed between the ether and non-ether derivatives. The tetra-onium ether derivatives E_{77} and E_{78} had respectively a greater affinity for the decamethonium receptor site than had the corresponding non-ether derivatives E_{79} and E_{80} (Fig. 4). On the other hand, the insertion of a second ether oxygen function which had previously been observed to exert a markedly deleterious effect on potency (Edwards, Lewis, McPhail, Muir & Stenlake, 1960) might also be a contributing factor, together with differences in chain length, to the reduction in affinity of compound E_{100} below that of E_{151}.

The insertion of an additional ether oxygen function into the molecule of decamethonium produced a slight and statistically insignificant reduction in the affinity value of the compound (oxydipentonium) (Fig. 1).

DISCUSSION

In the bis-onium compounds, simple replacement of a quaternary nitrogen atom by a tertiary sulphur atom (compound II) does not significantly alter potency but, when the chain length is also reduced, both the affinity and the intrinsic activity values fall. This may be due to a reduction in the Van der Waals' forces associated with the tri-substituted sulphur atom, as against the tetra-substituted nitrogen atom, together with the effects of shortening the inter-onium chain. Charge density effects, influencing the strength of the electrostatic bond, generally accepted to be responsible for muscle relaxant activity, would also be operative.

The effect of alteration of the size of the alkyl onium substituents depended upon whether or not depolarizing activity was involved. While it has been suggested that the receptors for depolarizing compounds could differ from those occupied by non-depolarizing agents and that the molecular properties required for a high affinity may be different in each instance (Van Rossum & Ariëns, 1959), no particular dimension or configuration of the acetylcholine receptor is implied by the Ariëns' approach. Indeed, it could equally well be that the same receptor is involved for both depolarizing and non-depolarizing compounds. Consequently, alteration of a particular chemical feature in the molecule of a depolarizing compound might modify either the high affinity or the high intrinsic activity possessed by compounds of this class, or both of these factors. A similar chemical change in the non-depolarizing compounds which possess a very low intrinsic activity might be expected to affect mainly the general fields of force uniting drug and receptor, that is, the affinity of the compound concerned.

In the depolarizing bis-onium compounds of Group 1, the molecular conformation adopted at the receptor may not be the maximally extended form, due to the formation of an ion-pair complex involving a single anion (Cavallito & Gray, 1960). If this is so, the bulkier ethyl groups could reduce the concentration of the twin cationic onium charges by sterically hindering their approach to each other as well as their combined interaction with a single anionic site.

On the other hand, the molecular conformation adopted by the non-depolarizing polyonium compounds of Group 2 could be the extended one, in which case the increased Van der Waals' bonding, possible with the ethyl groups, would favour a higher affinity. It is further suggested that since these compounds, by definition, have a low intrinsic activity (Van Rossum *et al.*, 1958) such effects will be confined to alterations in the affinity of the compounds concerned. Since no structural alterations other than the size of the alkyl onium groups are involved the observed differences in affinities between E_{98}, E_{99} and E_{103}; E_{100}, E_{101} and E_{102}; E_{77} and E_{78} and between E_{79} and E_{80} can be related to this effect. These non-depolarizing compounds act purely by preventing the access of acetylcholine to the receptor or to non-ionic satellite receptors (Meier, Tripod & Bruni, 1955; Carey, Edwards, Lewis & Stenlake, 1959).

The influence of the ether-oxygen function in the compounds studied is therefore related to changes in the amount of the drug-receptor complex formed (that is, to changes in affinity).

Tubocurarine dimethyl ether has been reported to have a greater affinity than tubocurarine for the acetylcholine receptor site in frog skeletal muscle (Kalow, 1954), although the opposite effect has been demonstrated with other species including man (Unna & Pelikan, 1951; Foldes, Machaj, Hunt, McNall & Carberry, 1952). In the present work, however, tubocurarine had a greater affinity than its dimethyl ether. The methoxyl groups of the latter which replace the two hydroxyl groups of tubocurarine render it incapable of carrying any anionic charge; the two phenolic hydroxyl groups of tubocurarine are, however, capable of undergoing dissociation (Swann, 1951; Kalow, 1953). The pK_A value of each hydroxyl group is different, that in the ortho position to the methoxyl groups being the less acidic. Consequently it has been suggested that tubocurarine can exist in more than one ionized form (Kalow, 1954). Maximum pharmacological activity is at *p*H 6.7 when the hydroxyl groups are virtually un-ionized and, at higher *p*H values, the existence of a partial zwitterion would reduce the net cationic charge of the molecule. In consequence, tubocurarine may, at certain *p*H values, be equiactive with (Swanson, Henderson & Chen, 1949; Swanson, Gibson & Powell, 1952) or less active than the dimethyl ether (Kalow, 1954). This explanation without additional consideration of steric effects fails, however, to account for the very low potency of the di-*n*-butyl and the dibenzyl ethers of tubocurarine (Wintersteiner, 1959). Studies on the binding of muscle relaxants with the acetylcholine receptor protein of the electric eel (Ehrenpreis, 1960) have shown that, at *p*H 7.5, the dimethyl ether has less affinity than tubocurarine but at *p*H 9.5 the opposite applies. This can be explained by the ionization of the phenolic hydroxyl groups in tubocurarine at the higher *p*H and supports the view of Kalow (1954) and the present results.

The importance of the number and position of the methoxy substituents in molecules of this type has been emphasized by the marked increase in potency in a series of *N,N*-dimethyl-1,10-decamethylene-bis-tetrahydroquinolinium and tetrahydro-isoquinolinium derivatives, with increasing substitution by methoxyl groups. The increased activity of tubocurarine compared with its dimethyl ether could, however, be attributed to steric factors. Alternatively, the hydrogen atom of the hydroxyl group in the former may hydrogen bond with the receptor, a situation not possible with tubocurarine dimethyl ether.

Differences in the distribution of tubocurarine and its dimethyl ether *in vivo* (Collier, Paris & Woolf, 1948; Marsh, 1952) have indicated the influence of permeability differences, the biophase concentration attained and the availability of each drug at the receptor site. Thus, while the dimethyl ether has a slightly higher solubility in lipids than tubocurarine, it is no more effective in blocking conduction at the node of Ranvier in a single nerve fibre preparation.

Insertion of an ether oxygen function into decamethonium (as in oxydipentonium) increases chain length slightly and there is a slight though insignificant reduction in the affinity of the resulting compound (Van Rossum, 1960). This effect may be explained by the increase in chain length (Paton & Zaimis, 1949) and/or the physicochemical characteristics of the ether oxygen atom which could increase bonding at sites of loss and disturb the lipophilic-hydrophilic balance of the molecule. The reduced affinity of the ether-containing derivative compared to decamethonium confirms the anti-bonding effect of the ether oxygen link, although the reverse was observed among chemically related ganglion-blocking compounds (Fakstorp & Pedersen, 1957). Furthermore, the presence of two ether links in the bis-onium compounds was also associated with a reduction in affinity and intrinsic activity when compared to decamethonium (Van Rossum & Ariëns, 1959).

The role of the ether oxygen function in the mono-ether tetra-onium compounds E_{77} and E_{78} can be more precisely defined. In each compound the affinity is increased when compared to that of E_{79} and E_{80} where the ether group is replaced by a methylene unit. Here alterations in chain length and the change in bond angles produced by the ether function are small and seem unlikely to cause the observed differences in potency. It is more likely that the physicochemical properties of the ether oxygen itself, not the alteration in chain length, are responsible. The increased affinity of the ether-containing compound may be due to an intensification of the secondary bonding characteristics of the drug receptor complex associated with an increased degree of electrical field interaction.

The lack of a dramatic alteration in muscle relaxant activity produced by the ether oxygen link confirms the findings of Bovet (1951) with some phenolic ethers, but not those of Pradhan, Ray, Varadan & De (1954) with aliphatic derivatives.

In the examination of drugs as potential neuromuscular blocking agents, the technique of Ariëns and his co-workers is particularly useful. Depolarizing agents and compounds with mixed actions can be eliminated without the sacrifice of an undue number of animals, and non-depolarizing drugs can then be more rigorously tested by routine methods.

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