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A comparison of phenylalkyl- and phenoxyalkyl-trimethylammonium and triethylammonium salts; their apparent molal volumes at infinite dilution and effects on the frog rectus and guinea-pig ileum preparations

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Summary

1. The effects of phenoxyalkyltrimethylammonium and triethylammonium bromides on the frog rectus preparation and on the isolated guinea-pig ileum in the presence of hexamethonium have been compared with those of analogous phenylalkyltrimethylammonium and triethylammonium bromides. Affinity constants have been measured when possible.

2. The apparent molal volumes at infinite dilution of some of the compounds have been measured from estimates of density made with an Anton Paar precision density meter.

3. An ether oxygen occupies at infinite dilution in water only about one-third of the volume occupied by a methylene group.

4. The replacement of methylene by ether oxygen reduces nicotine-like activity and affinity for nicotine-sensitive receptors. The reduction in affinity may be partly due to the decrease in size.

5. The replacement of methylene by ether oxygen reduces activity and affinity at muscarine-sensitive receptors but in some compounds there is a bigger reduction in affinity than would be expected simply from the reduction in size.

6. It is suggested that the affinity of these compounds largely depends on hydrophobic bonding and that effects on water structure in the environment of the receptor may also be involved in the actions of agonists.

Introduction

Homologous series of phenylalkyl-trimethylammonium and triethylammonium salts were tested on the frog rectus preparation by Barlow, Thompson & Scott (1969) along with many other compounds related to nicotine. It was found that phenyl-propyl-trimethylammonium was appreciably more active than choline phenyl ether, which was surprising in view of the suggestions which have been made about the importance of the ether oxygen atom for nicotine-like activity (Hey, 1952; Clark, Dawes & Williams, 1968). The difference between the two compounds is also of interest because ether and methylene groups are often described as being isosteric (review, Burger, 1970).

We have, therefore, prepared analogous phenoxyalkl-trimethylammonium and triethylammonium salts, in order to observe the effects of replacing methylene by ether on activity at acetylcholine receptors in the frog rectus and guinea-pig ileum preparations. Most of the triethylammonium compounds were antagonists and with them it was possible actually to observe the effects of the change in structure on affinity for the receptors. The apparent molal volumes in aqueous solution of some of the compounds have also been measured in order to see to what extent the ether oxygen and methylene groups can really be regarded as isosteric. The apparent molal volume at infinite dilution, ϕ_v^o, is a measure of the volume which the solute appears to occupy in the solvent; reasons for applying it in attempts to interpret pharmacological activity have been discussed by Barlow, Lowe, Pearson, Rendall & Thompson (1971).

Methods

Compounds

Phenoxyalkylbromides were prepared by heating a large excess of the appropriate polymethylene dibromide with phenol and potassium hydroxide in methanol. The quaternary salts were obtained by heating the phenoxyalkyl bromide with a large excess of trimethylamine or triethylamine in ethanol, except for phenoxy-ethyl-triethylammonium bromide which could only be obtained satisfactorily by first preparing phenoxyethyldiethylamine and treating this with ethyl bromide.

Analyses and melting-points are shown in Table 1.

Frog rectus preparation

This was set up as described by Barlow, Scott & Stephenson (1967) but it was necessary to use *Rana temporaria* instead of *Rana pipiens*. The agonist was β-pyridyl-methyltrimethylammonium, which was allowed to act for 5 min and applied once every 30 minutes. The activities of agonists, expressed as equipotent molar ratios relative to β-pyridylmethyltrimethyl-ammonium, and the affinity constants of partial agonists and antagonists, were measured by the methods used by Barlow *et al.* (1969).

Guinea-pig ileum preparation

This was set up as described by Barlow, Scott & Stephenson (1963). The agonist was carbachol, allowed to act for 20 s and applied once every 90 s, and the Tyrode solution contained hexamethonium ($2{\cdot}76\times10^{-4}$M). The activities of agonists, expressed as equipotent molar ratios relative to β-pyridylmethyltrimethyl-ammonium, and the affinity constants of partial agonists and antagonists, were measured by the methods used by Abramson, Barlow, Mustafa & Stephenson (1969).

Errors in estimates of log K

From repeated groups of estimates of log K on the guinea-pig ileum, Abramson *et al.* (1969) found that the variance of the estimates within a group was an underestimate of the real error. They concluded that on this preparation differences

TABLE 1. *Phenoxyalkyl bromides, trimethylammonium bromides and triethylammonium bromides: $PhO(CH_2)_nX$*

		Found			Theory		
$n=2$ X=		Br⁻	C	H	Br⁻	C	H
Br	b.p. 140°/37 mm m.p. 30·0–30·5°						
$\overset{+}{N}Me_3$ Br⁻	m.p. 168–9°	30·7			30·8		
$\overset{+}{N}Et_3$ Br⁻	m.p. 134–5°	26·3			26·4		
$n=3$ X=							
Br	b.p. 125°/15 mm N_D^{25} 1·5465						
$\overset{+}{N}Me_3$ Br⁻	m.p. 152–3°	29·0	52·5	7·27	29·2	52·5	7·37
$\overset{+}{N}Et_3$ Br⁻	m.p. 90–92°	25·3			25·3		
$n=4$ X=							
Br	b.p. 148°/14 mm m.p. 39·5–40·5°						
$\overset{+}{N}Me_3$ Br⁻	m.p. 176–7°	27·8	54·4	8·06	27·8	54·2	7·71
$\overset{+}{N}Et_3$ Br⁻	m.p. 150–151°	24·3			24·2		
$n=5$ X=							
Br	b.p. 167°/14 mm N_D^{19} 1·5363						
$\overset{+}{N}Me_3$ Br⁻	m.p. 186–7°	26·8	55·7	8·14	26·5	55·6	8·03
$\overset{+}{N}Et_3$ Br⁻	m.p. 91–2°	23·0			23·2		
$n=6$ X=							
Br	b.p. 179–182°/13 mm N_D^{22} 1·5319						
$\overset{+}{N}Me_3$ Br⁻	m.p. 199–200°	25·3	56·7	8·06	25·3	57·0	8·31
$\overset{+}{N}Et_3$ Br⁻	m.p. 113–4°	22·2			22·3		
$n=7$ X=							
Br	b.p. 123–8°/0·05 mm N_D^{20} 1·5247						
$\overset{+}{N}Me_3$ Br⁻	m.p. 192–3°	24·5	58·1	8·33	24·2	58·2	8·48
$n=8$ X=							
Br	b.p. 145–150°/0·2 mm N_D^{21} 1·5216						
$\overset{+}{N}Me_3$ Br⁻	m.p. 165–6°	23·3	59·2	8·50	23·2	59·3	8·81
$n=9$ X=							
Br	b.p. 153–160°/0·6 mm N_D^{21} 1·5183						
$\overset{+}{N}Me_3$ Br⁻	m.p. 184–5°	22·0	60·4	8·81	22·3	60·4	9·03

Melting-points were recorded with a Kofler hot-stage microscope and are uncorrected. Microanalyses for C and H were made by Dr. J. W. Minnis (Department Biochemistry); analyses for bromide ions were made gravimetrically with samples of 100–200 mg.

of up to 0·1 log units were unlikely to indicate real differences in affinity. This impression has been confirmed in subsequent work (Barlow, Franks & Pearson, 1972). Estimates with the frog rectus usually have a bigger variance (Barlow *et al.*, 1967; Barlow *et al.*, 1969) and it seems likely that differences of up to 0·2 log units do not indicate real differences in affinity on this preparation.

Apparent molal volumes

These were obtained from measurements of the density of dilute aqueous solutions of the compounds at 25° C, as described by Barlow *et al.* (1971), but with an Anton Paar Precision Density Meter, DMA 02C, instead of with a pycnometer. The density meter measures very accurately the period of oscillation of a glass tube, which is related to the density of the liquid with which the tube is filled. It must be calibrated by using solutions of known density; Lowe, MacGilp & Pritchard (1973) have described its use for measuring apparent molal volumes when calibrated with solutions of sodium chloride. In the present work the calibration involved measurements (at least in duplicate) with two concentrations of sodium chloride as well as with pure water (distilled and free from dissolved gases). Readings (at least in duplicate) were usually taken with two compounds, each in three concentrations between 0·01 M and 0·3 M, and arranged in increasing order of density. Further measurements with water were taken after the higher concentration of sodium chloride (whose density was greater than that of any of the other solutions). Because of the need to establish thermal equilibrium for each measurement, this procedure required about 6 hours.

The density of solutions of sodium chloride can be expressed as $d=d_w+A_1m+A_2m^{3/2}+A_3m^2$ and from published results, Lowe, MacGilp & Pritchard (1973) calculated that the best fit by the method of least squares, was obtained when $A_1=0{\cdot}04166$, $A_2=-0{\cdot}0014972$ and $A_3=-0{\cdot}0009876$; d_w at 25° C is 0·997046. The relationship between the density of the solution and the period of the oscillator, t, is $d=At^2+B$, so the measurements with pure water and with the solutions of sodium chloride can be used to calculate values of A and B which best fit the results (usually at least 8 in number, 4 with pure water and 2 with each of the concentrations of sodium chloride).

The values of t for the other solutions can therefore be used to calculate their density, which is related to the apparent molal volume ϕ_v by the relation:

$$\phi_v=\frac{1}{m}\left(\frac{1000+mM}{d}-\frac{1000}{d_w}\right)$$

where m is the molality and M is the molecular weght. The relation between ϕ_v and concentration is

$$\phi_v=\phi_v^{\circ}+S_vc^{1/2}+jc$$

where ϕ_v° is the apparent molal volume at infinite dilution, c is the molarity, S_v is a constant determined by the physical properties of the solvent (for water $S_v=1{\cdot}868$; Barlow *et al.*, 1971), and j is a constant determined by the solute. The computer programme used by Lowe *et al.* (1973), which was also used in this work, calculates the densities of the solutions from the values of t, the corresponding values of ϕ_v (from the weights of solvent and solute and the molecular weight), and gives estimates of ϕ_v° and j together with their standard errors.

Measurements with the density meter require much less material than is needed for pycnometry. Satisfactory results can be obtained with as little as 200 mg, compared with at least 5 grams. The method is also potentially more accurate, with the standard error of an estimate usually less than 0·1 cm^3/mole (about 0·05%). The accuracy is limited, however, by the accuracy of the calibrations

with the solutions of sodium chloride, which must be performed whenever the instrument is used. It is also limited by the accuracy with which the temperature can be controlled. In this work a Haake Model FT circulating thermostat was used with a temperature constancy of $\pm 0{\cdot}005°$ C.

Results

Frog rectus preparation

The results obtained with the phenoxyalkyl compounds on preparations from *Rana temporaria* are compared with the results obtained by Barlow *et al.* (1969) with the phenylalkyl compounds on preparations from *Rana pipiens* in Table 2A. The value of log equipotent molar ratio for choline phenyl ether on *Rana temporaria* (1·25) is even larger relative to the standard (corresponding to lower activity) than the value obtained on *Rana pipiens* (0·89; Barlow *et al.*, 1969) and gives some idea of the differences which may be expected from the use of

TABLE 2. *Values of log K and log equipotent molar ratios*

A. Frog rectus abdominis

	Rana pipiens	*Rana temporaria*
	$Ph(CH_2)_n\overset{+}{N}Me_3$	$PhO(CH_2)_n\overset{+}{N}Me_3$
$n=1$	1·97*±0·04 (6)	
2	1·05*±0·01 (4)	
3	0·58*±0·03 (4)	1·25*±0·02 (7)**
4	1·61*±0·03 (4)	1·98*±0·02 (4)
5	1·23*±0·02 (8)	3·10† (4) A
6	6·14†±0·06 (6) C	3·90† (6) A
7	6·03 ±0·05 (4)	3·74†±0·14 (7) A
	$Ph(CH_2)_n\overset{+}{N}Et_3$	$PhO(CH_2)_n\overset{+}{N}Et_3$
1	4·03 ±0·05 (7)	
2	3·39†±0·16 (4) A	
3	4·03†±0·10 (3) A, B	4·37±0·10 (3)
4	5·08 ±0·04 (4)	5·14±0·05 (4)
5	5·10 ±0·05 (5)	5·48±0·12 (3)
6	6·30 ±0·05 (6)	noncompetitive
7	6·52 ±0·05 (6)	

B. Guinea-pig ileum

	$Ph(CH_2)_n\overset{+}{N}Me_3$	$PhO(CH_2)_n\overset{+}{N}Me_3$
$n=1$	0·330*±0·022 (4)	
2	0·788*±0·063 (5)	
3	1·274*±0·026 (6)	1·827*±0·128 (8)
4	4·771 ±0·060 (4)	4·438 ±0·044 (7)
5	5·134 ±0·050 (8)	3·954 ±0·098 (9)
6	5·393 ±0·020 (10)	4·398 ±0·042 (7)
7	5·265 ±0·027 (9)	5·053 ±0·018 (4)
	$Ph(CH_2)_n\overset{+}{N}Et_3$	$PhO(CH_2)_n\overset{+}{N}Et_3$
1	4·831 ±0·014 (4)	
2	4·947 ±0·046 (5)	
3	5·185 ±0·050 (8)	4·938 ±0·023 (6)
4	5·480 ±0·031 (4)	5·050 ±0·040 (5)
5	5·779 ±0·030 (8)	4·925 ±0·021 (7)
6	5·970 ±0·020 (10)	5·172 ±0·031 (7)
7	6·168 ±0·020 (5)	5·651 ±0·020 (7)

All equipotent molar ratios* are relative to β-pyridylmethyltrimethylammonium; values of log K for partial agonists† were obtained by the reciprocal plot method (A), by the addition method (B), or by treating the compound as an antagonist (C) as described by Barlow *et al.* (1969). Carbachol was the agonist in affinity constant measurements on the ileum and hexamethonium was present ($2{\cdot}76\times10^{-4}$M). β-Pyridylmethyltrimethylammonium was the agonist in affinity constant measurements on the rectus. Values of log epmr are affected by the species, the double asterisk (**) indicates that the corresponding value for *Rana pipiens* was 0·89.

a different species of frog. The phenoxyalkyltrimethylammonium compounds have been tested on *Rana pipiens* elsewhere (Hamilton, personal communication; Barlow, 1965; Hersey, 1968) and appear to have higher efficacy on this than on *Rana temporaria*; even phenoxyhexyl-trimethylammonium was a partial agonist whereas in this work even the phenoxybutyl compound was an antagonist. In both species there was a general decline in activity with chain length for both phenylalkyl- and phenoxyalkl- trimethylammonium salts and the longer compounds were antagonists. With the phenoxalkyl compounds, however, there was a maximum in activity at the phenylpropyl homologue and with *Rana pipiens* there was a secondary maximum at the phenylpentyl homologue. Affinity appears generally to increase with chain length.

It is clear that the phenoxyalkyltrimethylammonium compounds have lower activity and lower affinity than their phenylalkyl analogues of comparable chain length. The phenoxyalkyltriethyl-ammonium compounds, however, have affinity very similar to that of their phenylalkyltriethylammonium analogues.

Guinea-pig ileum preparation

The results are shown in Table 2B. As with frog rectus, an increase in chain length or in the size of the onium group generally leads to a decrease in activity and an increase in affinity. On this preparation, however, the activity of the phenylalkyltrimethylammonium compounds declined steadily from the benzyl homologue with no suggestion of any secondary maximum at either the phenylpropyl or phenylpentyl homologues. Nevertheless, as on the rectus, the phenoxy compounds clearly had lower activity and lower affinity than their phenylalkyl analogues of comparable chain length.

Apparant molal volume

Estimates of $\emptyset_v^\circ$, the apparent molal volume at infinite dilution, are shown in Table 3 and illustrated in Figure 1. The average increment for a methylene group is 16·0 cm³/mole, which is in good agreement with 16·3 (cm³/mole)/methylene

TABLE 3. *Apparent molal volumes at infinite dilution ($\emptyset v^o$) in cm³/mole at 25° C*

$Ph(CH_2)_n\overset{+}{N}Me_3\ Br^-$		Δ_1	$PhO(CH_2)_n\overset{+}{N}Me_3\ Br^-$		Δ_1	Δ_2
$n=1$	176·0					
2	191·9	15·9				
3	207·5	15·6	$n=2$	196·8		10·7
4	224·7	17·2	3	212·9	16·1	11·8
5	240·1	15·4	4	229·9	17·0	10·2
6	256·5	16·4				
7	272·2	15·7				
$Ph(CH_2)_n\overset{+}{N}Et_3\ Br^-$			$PhO(CH_2)_n\overset{+}{N}Et_3\ Br^-$			
$n=1$	219·2					
2	236·8	17·6				
3	251·7	14·9	$n=2$	241·9		9·8
4	268·4*	16·7	3	257·6	15·7	10·8
			4	274·4	16·8	

Δ_1 is the increment in $\emptyset^\circ_v$ for the additional methylene group; Δ_2 is the increment for the replacement of –O– by $-CH_2-$. The values of $\emptyset^\circ_v$ have been obtained by extrapolation of the values of $\emptyset_v$ for three concentrations in the range 0·01 to 0·3M; the standard error of $\emptyset^\circ_v$, estimated from the variance of the values of $\emptyset_v$, is usually less than 0·1 cm³/mole, but there may be systematic errors greater than this.

* The value for the corresponding iodide was 279·9 cm³/mole and the difference between bromide and iodide, 11·5 cm³/mole, is identical with the expected value (Barlow *et al.*, 1971).

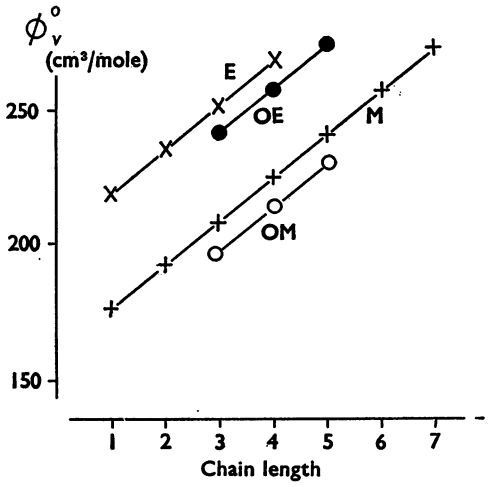


FIG. 1. Apparent molal volumes at infinite dilution (ϕ_v°) plotted against the total number of methylene and ether groups ('chain length'). M indicates the series $Ph(CH_2)_n{}^+NMe_3\ Br^-$, E indicates the corresponding triethylammonium bromides. OM indicates the series $PhO(CH_2)_n{}^+NMe_3\ Br^-$, OE indicates the corresponding triethylammonium bromides. Note that a compound containing n methylene groups has an appreciably bigger volume than its analogue containing $(n-1)$ methylene groups and an ether oxygen. Temperature, 25° C.

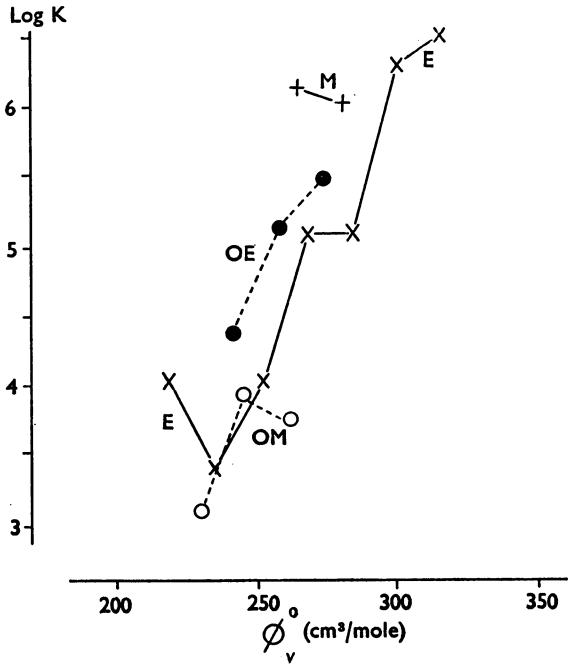
group for alkyltrimethylammonium iodides (Barlow, *et al.*, 1971). The actual values of the increment, however, differ by small amounts which should be significant and are worth further investigation.

The average effect of replacing ether by methylene is to increase ϕ_v° by 10·7 cm³/mole, which agrees well with results obtained with alkyl- and alkoxyalkyl-onium iodides (10·6 cm³/mole ; Barlow *et al.*, 1971).

Discussion

Ether and methylene groups cannot reasonably be regarded as 'isosteric'. Although the carbon-oxygen and carbon-carbon bond lengths are not very different (143 and 154 pm, respectively) and because of their geometry lead to roughly equal increases in overall chain length, the increment in ϕ_v° for an ether link is only about one-third of that for a methylene group. The relatively low affinity of the ethers might, therefore, be due at least in part to the smaller amount of material available for binding to the receptor, rather than to a poorer ability of the oxygen atom to become bound. When the affinities of the compounds were plotted against ϕ_v° the results for the frog rectus (Fig. 2A) showed a marked rise in binding with increasing size. In view of the errors attached to the estimates and the steepness of the rise, it is doubtful whether the results show any detectable difference between the binding of phenoxyalkyl- and phenylalkyl-compounds of equal apparent molal volume at infinite dilution. There may be differences, the high value for the partial agonist phenylhexyltrimethylammonium for example, but these are small compared with the large overall increase in affinity with chain length, for which the increase in size could be mainly responsible.

The results for the muscarine-sensitive receptors of the guinea-pig ileum (Fig. 2B) showed a less steep rise and in view of their greater accuracy and regularity, it



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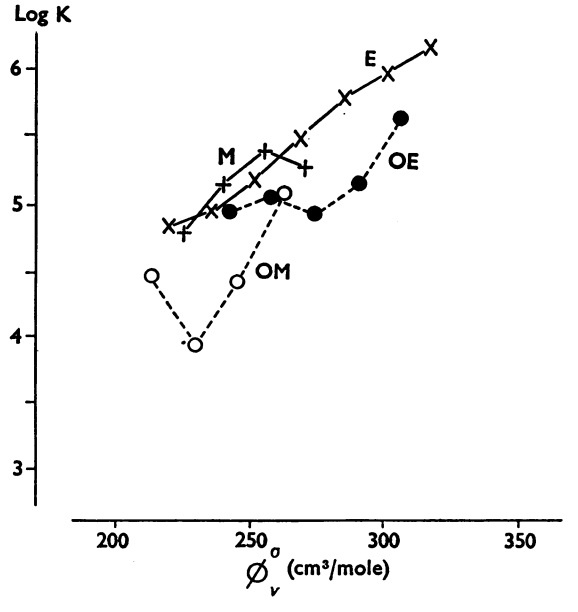

B

FIG. 2. Affinity for acetylcholine receptors plotted (as log K) against apparent molal volumes at infinite dilution ($\emptyset_v^\circ$). Where necessary these have been estimated by extrapolation of the results in Table 3. A, results for the frog rectus ; B, results for postganglionic receptors of the guinea-pig ileum.

appears that in certain positions of some molecules an ether group makes a much smaller contribution to binding than does a methylene group. Because this adverse effect depends on position in the molecule, it may indicate differences in water structure in various regions of the receptor. Alternatively it must be supposed that the receptor contains groups at some points which repel the ether oxygen.

The lower activity of the ethers compared with their methylene analogues is the reverse of what would be expected from the suggestion made by Hey (1952) that nicotine-like activity might be 'associated with a reduction of the electron density of the ether oxygen atom of choline ethers and esters'. It is, moreover, difficult to interpret the activity of these simple (but quite active) molecules in terms of the binding of groups in the receptor with groups in the molecule because the molecules lack features other than methylene and phenyl groups. The regular increase of affinity with chain length, for instance, suggests that it is largely dependent on hydrophobic bonding. If there are differences in water structure in various regions of the receptor, between for example the regions where the onium and phenyl groups are bound, the interaction between drug and receptor will include effects on water. Simple molecules, such as those tested in this work, might then have relatively complex structure-activity relationships, depending on the regional differences in water structure. It is even possible that effects on water structure may be involved in the actions of agonists, perhaps by leading to changes in the conformation of the receptor protein. It is certainly remarkable that the substituents which enhance the nicotine-like activity of phenylalkyltrimethylammonium salts are groups like hydroxyl and amino (Barlow *et al.*, 1969).

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