MOLECULAR PHARMACOLOGY, 7, 357-366

Ion Size and Activity at Acetylcholine Receptors

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(Received November 2, 1970)

SUMMARY

The apparent molal volumes at infinite dilution, ϕ_v^0, of a number of quaternary ammonium salts, tested on the isolated frog rectus and guinea pig ileum preparations, have been determined from precise pycnometric density measurements with dilute aqueous solutions at 25°. In these salts the differences in ϕ_v^0 for related compounds arise largely from differences in intrinsic volume.

The apparent molal volumes have been compared with the activity and affinity of the compounds at the acetylcholine receptors of the frog rectus and guinea pig ileum. Increases in size are associated with increases in affinity at both types of receptor, but efficacy (ability to activate receptors) is decreased. It is suggested that efficacy is limited by the ability of the ions to penetrate to sites within the receptor at which they can act to produce a change in the conformation of the receptor.

INTRODUCTION

The biological activity of drugs depends on molecular size, among other things. Attempts to assess this are handicapped, however, by the difficulty of obtaining a satisfactory measure of the size of drugs in solution, particularly if they are ionized. Molecular size is not necessarily the same as in the crystal lattice, especially if the ions interact with solvent molecules, and estimates of molecular size based on X-ray crystallographic data may not be valid. The estimation of solute volumes from conductance measurements (1) may also be criticized, since the theoretical relationship between conductance and ion size is known with certainty only for solutions in which the ions are very much larger than the solvent molecules, and in which the solute has a negligible effect on the solvent structure. Since there are few, if any, ions which do not exert some influence on water structure, even the use of empirical correlations between ion size and conductance, such as that described by Robinson and Stokes (1), may be questioned (2). For similar reasons, the determination of solute volumes from viscosity measurements (3) is also open to criticism.

Another, possibly more reliable measure of ion size in solution is given by the apparent molal volume, ϕ_v. This may be determined from density measurements on aqueous solutions and is defined by the relationship

$$\phi_v = \frac{(V - n_1 V_1^0)}{n_2}$$

in which V is the volume of solution that contains n_1 moles of solvent and n_2 moles of

We thank the Medical Research Council for a grant for equipment, and the Science Research Council and Tobacco Research Council for support (to H.M.R. and G.M.T., respectively).

solute; V_1^0 is the volume occupied by a mole of pure solvent, and hence $n_1V_1^0$ is the volume the solvent molecules would occupy in the solution if they were unaffected by the solute. The apparent molal volume is that which the solute *appears* to occupy; e.g., if the solute fitted exactly into a hole in the solvent structure, V would equal $n_1V_1^0$ and the apparent molal volume would be zero. This is, of course, an extreme case which merely serves to illustrate why, even for a large drug molecule, the apparent molal volume may be less than the intrinsic volume, i.e., the absolute amount of space occupied by the drug. However, there is no reason to believe that for the interpretation of pharmacological activity the intrinsic size of the solute is operationally significant; it seems to us at least equally likely that it is the apparent molal volume which is important because this contains, in addition to the intrinsic volume, a contribution representing the ability of the drug to fit into the solvent structure.

This paper attempts to assess the possible importance of size in relation to biological activity. To reduce complications, we have restricted our observations to closely related compounds, tested on simple isolated preparations. We studied compounds related to acetylcholine, tested on the isolated guinea pig ileum by Abramson, Barlow, Mustafa, and Stephenson (4), and compounds related to nicotine, tested on the frog rectus preparation by Barlow and Thompson (5). We also include in our discussion compounds related to tetramethylammonium, whose limiting conductances and viscosity B-coefficients, as well as apparent molal volumes, were measured by Lowe and Rendall (6), and which were tested on the frog rectus by Barlow, Scott, and Stephenson (7). The affinity constants of many of the compounds for the acetylcholine receptors in these tissues are known, and we have attempted to correlate them with estimates of ion size based on apparent molal volumes. Because the affinity constants were measured under conditions approaching equilibrium, we should be able to assess the importance of size in relation to binding at these receptors, uncomplicated by factors such as rates of diffusion. We have also attempted to correlate apparent molal volumes of those of the compounds which were agonists with their activity relative to a standard agonist. From the observed effects of size on the affinity of the antagonists and partial agonists it may then be possible to assess effects of size on ability to activate receptors.

EXPERIMENTAL PROCEDURE

Compounds. Acetylcholine iodide, chromatographically pure, was obtained from British Drug Houses, Ltd. Acetyl-β-methylcholine bromide was obtained from Koch-Light, Ltd., and was recrystallized before use. Acetyl-α-methylcholine iodide was prepared from L(+)-alanine as described by Beckett, Harper, and Clitherow (8). The sample had a melting point of 108.6–109.2°, $[\alpha]_D^{20}$ $-7.15°$ (0.2 M in water); Beckett, Harper, and Clitherow recorded a melting point of 108–109°, $[\alpha]_D^{27}$ $-9.07°$ (in 90% ethanol, v/v). The spiro compounds were prepared as described by Thomas (9), who recorded melting points of 309° (with decomposition), 260–261°, and 261–262° for 1,1-spirobipiperidinium bromide, spiropiperidine-1,1-pyrrolidinium bromide, and 1,1-spirobipyrrolidinium bromide, respectively. We obtained the following values for these compounds: m.p. 300°, found, Br^- 34.19, theory 34.12%; m.p. 266.0–266.5°, found, Br^- 36.35, theory 36.29%; m.p. 261.5–262.0°, found, Br^- 38.66, theory 38.76%. Details of the preparation of the other compounds used in this work are given by Barlow and Hamilton (10), Barlow, Scott, and Stephenson (7, 11), and Barlow and Thompson (5). The samples used had melting points and analyses which indicated that they were at least as pure as, and usually purer than, the samples described in these papers.

Apparent molal volumes. Apparent molal volumes were determined from density measurements made at 25.00 ± 0.01° with a 35-cm³ bicapillary pycnometer on solutions with concentrations in the range of 0.02–0.2 mole kg⁻¹. All solutions were made up by weight, and the weighings were corrected to values *in vacuo*. The precision of the solution densities was estimated from the error in reading the position of the menisci in the capillaries to be $\pm 3 \times 10^{-5}$ g cm^{-3}. This was confirmed by duplicate measurements on solutions prepared from the salts listed in Table 2 and by the reproducibility of the pycnometer calibration, which was based on a water density $d_0 = 0.997046$ g cm^{-3}.

The apparent molal volumes, ϕ_v, were calculated with the equation

$$\phi_v = \frac{1}{m}\left(\frac{1000 + mM}{d} - \frac{1000}{d_0}\right)$$

in which m is the solute concentration in moles per kilogram of solvent, M is the molecular weight of the solute, and d and d_0 are the densities of the solution and solvent, respectively. The values obtained for ϕ_v are most sensitive to errors in the measured density when the solute concentration is low, and for the concentration range 0.02–0.25 M the uncertainty in ϕ_v varies from 1.5 to 0.15 cm^3 mole^{-1} for an error in density of 3×10^{-5} g cm^{-3}.

RESULTS

For dilute salt solutions the apparent molal volume varies with concentration according to the equation

$$\phi_v = \phi_v{}^0 + S_v c^{1/2} + jc$$

in which $\phi_v{}^0$ is the value of ϕ_v at infinite dilution, S_v and j are constants, and c is the salt concentration in moles per liter. The theoretical basis for the $S_v c^{1/2}$ term has been given by Redlich and Rosenfeld (12), and this has been experimentally confirmed for tetraalkylammonium salts by Franks and Smith (13) and by Dunn (14). The constant S_v can be calculated from the physical properties of the solvent. For uni-univalent salts in water at 25°, Redlich and Meyer (15) obtained $S_v = 1.868$ cm^3 mole$^{-3/2}$ liter$^{-1/2}$. For quaternary ammonium salts j is small and negative. For example, for a 0.1 M solution of tetraethylammonium iodide jc is approximately -0.6 cm^3 mole^{-1}, and for tetramethylammonium iodide it is 0.0 cm^3 mole^{-1}; the corresponding figures for the bromides are -1.0 and -0.05 cm^3 mole^{-1} (16).

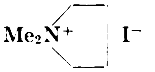
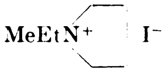
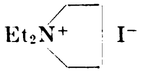
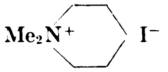
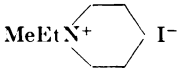
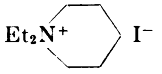
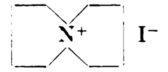
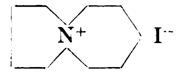
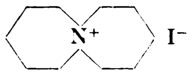
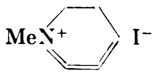
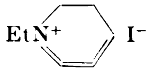
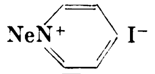
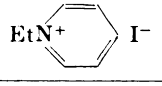
To obtain $\phi_v{}^0$ from the experimental ϕ_v values it is necessary to extrapolate ($\phi_v - S_v c^{1/2}$) to infinite dilution. However, in view of the increase in the error in ϕ_v which occurs with increased dilution of the solution, this procedure is not entirely satisfactory unless the apparent molal volumes are derived from density measurements accurate to approximately 1 part in 10^6. This cannot be achieved by simple pycnometry, and consequently we have also estimated $\phi_v{}^0$ by calculating the weighted (according to c^2) mean value of $\phi_v - 1.868c^{1/2}$. The values obtained by the two procedures rarely differed by more than 0.5 cm^3; those obtained by the second method are believed to be slightly more reliable and are given in Tables 1, 2, and 3. Since j is probably negative for all the compounds investigated, these values may be underestimated. However, this effect is well within the over-all limits of error, which we estimate to be ± 0.8 cm^3 mole^{-1}. For n-pentyltriethylammonium iodide the concentration dependence of $\phi_v - S_v c^{1/2}$ was much larger than for the other salts, and the extrapolated and weighted estimates of $\phi_v{}^0$ differed by 1.1 cm^3 mole^{-1}. The value obtained for $j(-7.1 \pm 1.1)$ is consistent with the values obtained for other tetraalkylammonium iodides by Conway, Verrall, and Desnoyers (16), and for this compound the extrapolated value of $\phi_v{}^0$ (Table 2) is considered to be more reliable than the average value.

The apparent molal volume at infinite dilution is the sum of the contributions from the cation and the anion. It is difficult to make an unambiguous separation of the two components, and estimates of the apparent molal volume of the iodide ion range from 36.6 [Owen and Brinkley (19)] to 43.9 cm^3 mole^{-1} [Stokes and Robinson (20)]. The difference between the apparent molal volumes of a pair of anions is readily determined from measurements on salts with identical cations; this procedure yields $\phi_v{}^0(\mathrm{I}^-) - \phi_v{}^0(\mathrm{Br}^-) = 11.46$ [Dunn (21)] and 11.6 cm^3 mole^{-1} [Conway, Verrall, and Desnoyers (16)]. For measurements made on bromides the values obtained for $\phi_v{}^0$ have been converted to iodide values

TABLE 1

Comparison of apparent molal volumes and the activity and affinity of quaternary ammonium salts at acetylcholine receptors of frog rectus muscle (Rana pipiens) (7)

The affinity of antagonists and partial agonists is expressed as the logarithm of the affinity constant. The activity of agonists is expressed as the logarithm of the equipotent molar ratio relative to tetramethylammonium, i.e., in terms of the molar amounts producing comparable biological effects. Positive values indicate that more of the compound is needed than of the standard (the compound is weaker than the standard). The results shown in Table 3 refer to experiments in which β-pyridylmethyltrimethylammonium was used as the standard; this is stronger than tetramethylammonium, and to convert values in this table relative to the standard used in Table 3 it is necessary to add 1.59 (the equipotent molar ratio for tetramethylammonium relative to β-pyridylmethyltrimethylammonium is 38.5). Values for the spiro compounds (IV), not previously tested, are the mean of the number of results in parentheses ± the standard error. Apparent molal volumes of compounds in series I, II, III, and VI are those obtained by Lowe and Rendall (6).

Series	Compound	ϕ_v^0	Log equipotent molar ratio	Log K
		cm³ mole⁻¹		
I (alkyl)	$Me_4N^+I^-$	126.0	0.0	
	$Me_3EtN^+I^-$	140.3	0.437	
	$Me_2Et_2N^+I^-$	155.9	1.018	
	$MeEt_3N^+I^-$	170.3		2.45
	$Et_4N^+I^-$	185.0		2.87
II (pyrrolidinium)		144.5	0.320	
		160.2	0.826	
		174.9		3.24
III (piperidinium)		159.1	1.049	
		173.9		2.87
		188.3		3.49
IV (spiro)		162.1[a]	Partial agonist	2.86 ±0.05 (7)
		176.8[a]	Weak partial agonist	3.14 ±0.05 (4)
		191.5[b]	Antagonist	3.41 ±0.06 (6)
V (quinuclidinium)		162.0	0.788	
		177.1		3.02
VI (pyridinium)		126.9		2.54
		143.9		2.49

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[a] Estimated values (see the text).

[b] ϕ_v^0 was measured for the bromide; the value given for the iodide was obtained by the addition of 11.5 cm³ mole⁻¹ (see the text).

TABLE 2

Comparison of apparent molal volumes and the activity and affinity of quaternary ammonium salts for guinea pig ileum (4, 11, 17)

The affinity of antagonists is expressed as the logarithm of the affinity constant, but there are also estimates for the agonists *n*-pentyltrimethylammonium and ethoxyethyltrimethylammonium. The activity of agonists is expressed as the logarithm of the equipotent molar ratio relative to acetylcholine.

Series	Compound	ϕ_v^0	Log equipotent molar ratio	Log K
		cm³ mole⁻¹		
VII (*n*-pentyl)	*n*-Pent-$N^+Me_3I^-$	189.2	1.51	3.733
	n-Pent-$N^+Et_3I^-$	233.6[a]		4.588
VIII (ethoxyethyl)	$CH_3CH_2OCH_2CH_2N^+Me_3I^-$	178.6	1.03	4.074
	$CH_3CH_2OCH_2CH_2N^+Et_3I^-$	222.5		3.974
IX (acetoxyethyl)	$CH_3COOCH_2CH_2N^+Me_3I^-$	175.4	0.0	
	$CH_3COOCH_2CH_2N^+Et_3I^-$	219.7	~3	
X	$CH_3COOCH_2CHMeN^+Me_3I^-$	189.9	0.25	
	$CH_3COOCHMeCH_2{}^+Me_3I^-$	192.7[b]	1.66	

[a] Extrapolated value (see the text).

[b] ϕ_v^0 was measured for the bromide; the value given for the iodide was obtained by the addition of 11.5 cm³ mole⁻¹ (see the text).

TABLE 3

Comparison of apparent molal volumes and the activity of quaternary ammonium salts at acetylcholine receptors of frog rectus muscle (Rana pipiens) (5)

The activity of agonists is expressed as the logarithm of the equipotent molar ratio relative to β-pyridylmethyltrimethylammonium. Molar volumes, V_m, are for toluene and the corresponding *meta*-substituted toluenes and are based on densities (18) at 20°. The value for *m*-methoxytoluene is for 25°. The increments ΔV_m and $\Delta\phi_v^0$ are relative to toluene and benzyltrimethylammonium iodide, respectively.

Compound	ϕ_v^0	V_m	$\Delta\phi_v^0$	ΔV_m	Log equipotent molar ratio
		cm³ mole⁻¹			
$PhCH_2N^+Me_3I^-$[a]	187.5	106.3			1.97
p-Hydroxybenzyl-$N^+Me_3I^-$	191.1	104.6	3.6	−1.7	2.37
p-Chlorobenzyl-$N^+Me_3I^-$[a]	199.0	118.1	11.5	11.8	1.80
p-Nitrobenzyl-$N^+Me_3I^-$[a]	201.2	118.5	13.7	12.2	1.67
p-Bromobenzyl-$N^+Me_3I^-$[a]	202.6	122.1	15.1	15.8	1.64
p-Methoxybenzyl-$N^+Me_3I^-$[a]	208.9	126.0	21.4	19.7	1.62

[a] ϕ_v^0 was measured for the bromide; the value given for the iodide was obtained by the addition of 11.5 cm³ mole⁻¹ (see the text).

by the addition of 11.5 cm³ mole⁻¹ before inclusion in Tables 1, 2, and 3.

In addition to the apparent molal volumes, the tables include results for the biological activity of the compounds. For the antagonists, the values are the log of the affinity constant (high values indicate strong binding). For agonists it is usually impossible to measure the affinity; all that can be done is compare concentrations producing comparable responses and to express the result as an equipotent molar ratio, one agonist being taken as standard. For the results in Table 1 the standard is Me_4N^+ and the values represent the log of the equipotent molar ratio, i.e., of the number of molecules of the compound producing the same effect as that of Me_4N^+ (for compounds weaker than the standard, more material is needed and the log of the equipotent molar ratio is positive; for compounds more active than the standard, it is negative). For the results on the guinea pig ileum in Table 2 the standard is acetylcholine. For the results on the frog rectus in Table 3 the standard is β-pyridylmethyltrimethylammonium, and these can be related to the results on the same receptors shown in Table 1 by adding 1.59 to values of the log of the equipotent molar ratio relative to Me_4N^+ (the equipotent molar ratio for Me_4N^+ relative to β-pyridylmethyltrimethylammonium is 38.5).

DISCUSSION

Assessment of ion size. The apparent molal volumes of the compounds in Tables 1 and 2 are shown in Fig. 1 as a function of the number of CH_2 groups in the cations. The slope of the lines in Fig. 1 is therefore a measure of the change in ϕ_v^0 per CH_2 group; the values obtained for this increment, $\Delta\phi_v^0$, are given in Table 4. Those for the tetraalkylammonium, pyrrolidinium, piperidinium, and pyridinium ions were obtained and discussed by Lowe and Rendall (6). The value of $\Delta\phi_v^0$ (14.7 cm³ mole⁻¹) given for the spiro ions is an average of the values for the tetraalkylammonium and for the pyrrolidinium and piperidinium ions (taken together); this value has been used to estimate the apparent molal volumes of 1,1-spirobipyrrolidinium iodide and spiropiperidine-1,1-pyrrolidinium iodide from the experimental value of ϕ_v^0 for 1,1-spirobipiperidinium iodide (see Table 1). The values of $\Delta\phi_v^0$ based on the ϕ_v^0 values of *n*-pentyltrimethylammonium, ethoxyethyltrimethylammonium, and acetylcholine ions and their triethylammonium analogues are in excellent agreement with each other and with the value obtained for the tetraalkylammonium ions, as is also the value for the quinuclidinium compounds. This provides strong support for the reliability

TABLE 4

Change in apparent molal volume per added CH_2 group in a series of quaternary ammonium salts

Errors are estimates based on errors in individual ϕ_v^0 values of ±0.8 cm³ mole⁻¹; i.e., for two compounds differing by one methylene group the estimate is 1.6 cm³ mole⁻¹, but for two compounds differing by three methylene groups it is one-third of this. The results for the alkyl (I) and pyrrolidinium and piperidinium (II and III) compounds, however, are those of Lowe and Rendall (6).

Series	ϕ_v^0
	cm³ mole⁻¹
Alkyl (I)	14.8 ± 0.7
Pyrrolidinium and piperidinium (II + III)	14.6 ± 0.7
Spiro (IV)	14.7[a]
Quinuclidinium (V)	15.1 ± 1.6
Pyridinium (VI)	17.0 ± 1.6
n-Pentyl (VII)	14.8 ± 0.5
Ethoxyethyl (VIII)	14.6 ± 0.5
Acetoxyethyl (IX)	14.8 ± 0.5

[a] Estimated from series I and series II + III.

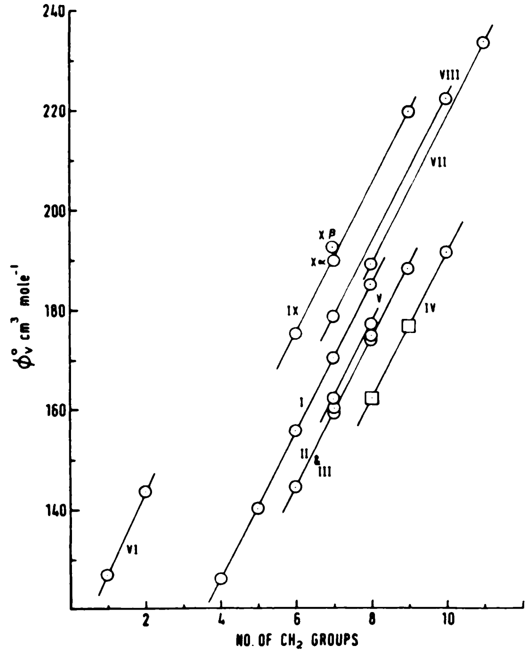

FIG. 1. *Apparent molal volumes of salts in Tabels 1 and 2, plotted against number of methylene groups in the cation*

Lines join members of series: I, alkyl; II, pyrrolidinium; III, piperidinium; IV, spiro; V, quinuclidinium; VI, pyridinium; VIII, *n*-pentyl; VIII, ethoxyethyl; IX, acetoxyethyl (see Tables 1 and 2).

of the apparent molal volumes given in Tables 1 and 2.

The difference between the apparent molal volumes of *n*-pentyltrimethylammonium iodide and ethyltrimethylammonium iodide corresponds to an increment per methylene group of 16.3 cm^3 $mole^{-1}$. This is in good agreement with the value of 15.8 cm^3 $mole^{-1}$ based on the results of Conway, Verrall, and Desnoyers (16) for tetra(*n*-pentyl)ammonium bromide and tetraethylammonium bromide, and is significantly larger than the increment (14.8 cm^3 $mole^{-1}$) obtained for the replacement of methyl by ethyl on the quaternary nitrogen of the compounds in Table 4. It appears that the increment in apparent molal volume per methylene group is larger when the group is farther from the quaternary nitrogen atom. The apparent molal volume of *n*-pentyltrimethylammonium iodide, for instance, is bigger by 4.2 ± 1.6 cm^3 $mole^{-1}$ than that of its isomer, tetraethylammonium iodide, in which all the methylene groups are as close to the quaternary nitrogen as possible.

The introduction of a methyl group on the α- and β-carbon atoms of acetylcholine leads to changes in ϕ_v^0 of 14.5 ± 1.6 and 17.3 ± 1.6 cm^3 $mole^{-1}$, respectively. The former substitution is analogous to the conversion of a methyl group to an ethyl group, and the volume change is in good agreement with those given in Table 4. Substitution on the β-carbon atom may be compared with the replacement of an ethyl group by a propyl group, and the volume change is in agreement with that (16.4 cm^3 $mole^{-1}$) calculated for this process from the values of Conway, Verrall, and Desnoyers (16) for tetrapropylammonium iodide and tetraethylammonium iodide.

The results in Table 2 show that for acetylcholine, ethoxyethyltrimethylammonium, and *n*-pentyltrimethylammonium ions the replacement of $—CH_2—CH_2—$ by $—CH_2O—$ and $—CO—O—$ leads to a reduction in ϕ_v^0 of 10.6 ± 1.6 and 13.8 ± 1.6 cm^3 $mole^{-1}$, respectively. The corresponding figures for the triethylammonium compounds are 11.1 ± 1.6 and 13.9 ± 1.6 cm^3 $mole^{-1}$. It is possible that part of the decrease in ϕ_v^0 may be due to hydration of the ether and acetyl groups, but the corresponding reductions in the molar volumes of liquid organic compounds are 9.8 and 14.1 cm^3 $mole^{-1}$, respectively. These values, which are based on an analysis by Exner (22) of the additivity properties of molar volumes, are sufficiently close to the changes in apparent molal volumes to suggest that both are due almost entirely to differences in the intrinsic volumes of $—CH_2—$, $—O—$, and $—CO—$.

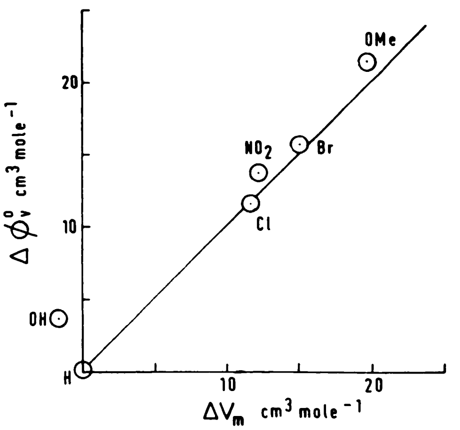

FIG. 2. *Increment in apparent molal volume due to para substitution in benzyltrimethylammonium iodide, plotted against increment in molar volume due to meta substitution in toluene*
See Table 3.

The differences between the apparent molal volumes of the benzyltrimethylammonium compounds (Table 3), too, may be explained in terms of the size of the substituents without invoking the possibility of interaction with the solvent. Figure 2 shows the change in apparent molal volume produced by a substituent in the benzyl group as a function of the corresponding difference in molar volume between toluene and substituted toluenes. Since not all the *para*-substituted toluenes are liquids, the molar volumes used for Fig. 2 and given in Table 3 are for the *meta*-substituted compounds. The difference between the molar volumes of the isomers is expected to be small; e.g., *p*- and *m*-chlorotoluenes, which are both liquids, have V_m = 118.3 and 118.1 cm^3 $mole^{-1}$, respectively. If the changes in apparent molal volume and molar volume are due only to the different intrinsic volumes of the substituents, the points in Fig. 2 should lie on a line of unit slope. This is found for all except the hydroxy-substituted compounds. In this case the molar volume of *m*-hydroxytoluene is less than that for toluene even though its intrinsic volume is of necessity larger, while the apparent molal volume of *p*-hydroxybenzyltrimethylammonium iodide is larger than that for benzyltrimethylammonium iodide by 3.6 ± 1.6 cm^3 $mole^{-1}$.

For all the salts in Table 1 except the quinuclidinium and spiro compounds, Lowe and Rendall (6) have determined the viscosity *B*-coefficients and the limiting equivalent conductances in aqueous solution. Both the viscosity *B*-coefficients and the reciprocals of the limiting ionic conductances were found to be linearly related to the apparent molal volumes. Thus, for these compounds, both types of measurement place the salts in the same order as the apparent molal volumes.

Although it is not possible to determine how closely the apparent molal volumes in Tables 1, 2, and 3 represent the intrinsic volumes of the salts, it is clear that they may be used to give reliable estimates of the relative sizes of the cations. It therefore seems worthwhile to consider how far the biological results may be interpreted in terms of differences in apparent molal volumes.

Ion size and biological activity. The graph of the log affinity constant for the acetylcholine receptors of the frog rectus [from the results of Barlow, Scott, and Stephenson (7)] plotted against ϕ_v^0 (Fig. 3, lower section) appears to show only a trend to increasing affinity with increasing size. Although ion size affects affinity, it is not the only factor involved, because ions of comparable size have very different affinities. The receptors, for instance, can clearly distinguish among diethylpyrrolidinium (ϕ_v^0 174.9), methylethylpiperidinium (ϕ_v^0 173.9), and methyltriethylammonium ion (ϕ_v^0 170.3). It appears that particular groups in the ion must be capable of interacting with groups in the receptor.

The upper section of Fig. 3 shows the relationship between ϕ_v^0 and the activity of the agonists, expressed as the logarithm of the equipotent molar ratio relative to tetramethylammonium (and plotted with the scale inverted so that the more active compounds appear at the top).

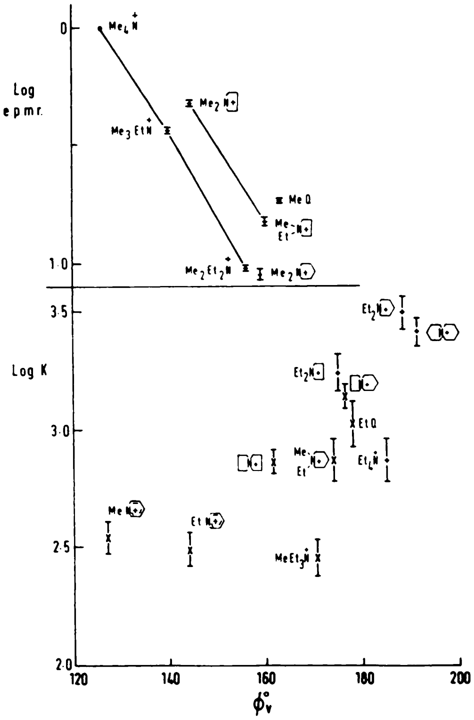

FIG. 3. *Graphs of* ϕ_v^0 *against log equipotent molar ratio (e.p.m.r.) relative to* Me_4N^+ *(upper) and against log affinity constant for acetylcholine (nicotine and tetramethylammonium)-sensitive receptors of frog rectus muscle (Rana pipiens)*

In the upper graph, the scale has been inverted so that the more active compounds appear at the top.

Increasing the size clearly decreases the activity of the compounds, and this must indicate a decrease in efficacy unless increasing the size decreases the affinity of the agonists. Increasing the size of the antagonists and partial agonists increases their affinity (see above), and so it would seem likely that increasing the size of the agonist decreases their activity because it decreases their efficacy. Because activity depends on both affinity and efficacy, however, the relatively high activity of methylquinuclidinium and of the pyrrolidinium compounds (Fig. 3, upper section) may be explained by supposing that these have relatively high affinity for their size. This is certainly true for their higher homologues (Fig. 3, lower section).

The effect of size on efficacy can be seen more clearly from the distribution of partial agonists (indicated by ×; antagonists are indicated by +) in the lower part of Fig. 3. There is a reasonably sharp division between partial agonists and true antagonists when ϕ_v^0 is about 170 cm^3 mole^{-1}. The division is not absolute, however; ethylquinuclidinium ion (ϕ_v^0 = 179 cm^3 mole^{-1}) is a partial agonist, the spiro compound with 5- and 6-membered rings (ϕ_v^0 = 176 cm^3 mole^{-1} estimated) is only a weak partial agonist, and diethylpyrrolidinium ion (ϕ_v^0 = 175 cm^3 mole^{-1}) is an antagonist. These differences lie outside the experimental error, and so it may be concluded that although size may be important in limiting efficacy, it is probably not the only factor which determines efficacy. Indeed it cannot be, because of the big differences in the biological properties of *ortho*, *meta*, and *para* isomers in the substituted phenyl and pyridyl compounds studied by Barlow and Thompson (5).

The relationships between size and affinity and efficacy at the muscarine-sensitive receptors of the guinea pig ileum seem to be qualitatively similar to those at the receptors in the frog rectus. Replacement of trimethylammonium by triethylammonium leads to an increase in the affinity of the *n*-pentyl compounds and a very slight increase in that of the ethoxyethyl compounds. There is a loss in efficacy, however, shown by the change from agonist to antagonist (Table 2). It seems likely, therefore, that the marked drop in activity when trimethylammonium is replaced by triethylammonium in acetylcholine is due to a similar marked drop in efficacy, rather than to a drop in affinity as implied by Burgen (23).

The effect of size on efficacy at the muscarine-sensitive receptors can also be seen by comparing the *n*-pentyl, ethoxyethyl, and acetoxyethyl series studied by Abramson, Barlow, Mustafa, and Stephenson (4). The decrease in size produced by replacing —$(CH_2)_2$— by —CH_2O— and by —CO—O— leads to increased activity, and this is probably due to increased efficacy rather than to increased affinity. The *n*-pentyl and ethoxyethyl compounds do not differ greatly in affinity, but whereas in the series studied by Abramson *et al.* (4) five of the eight *n*-pentyl compounds were antagonists and one compound was a partial agonist, only three of the eight ethoxyethyl analogues were antagonists and two were partial agonists. None of the acetoxyethyl analogues was an antagonist; all appeared to be full agonists (24).

There is evidence, therefore, that both at the acetylcholine receptors of the frog rectus and at the muscarine-sensitive receptor of the guinea pig ileum efficacy is associated with small apparent molal volume. Burgen (23) has suggested that the ability of ions to activate the muscarine-sensitive acetylcholine receptor depends upon the ability of their positively charged group to approach closely to a negatively charged group within the receptor. Although his evidence for this is open to criticism (4), the results we have obtained, which indicate that size limits efficacy, support this view and suggest that it may also apply to the receptors of the frog rectus.

Small apparent molal volume appears to be associated with low affinity, as well as with ability to activate receptors. This is consistent with the rate theory of Paton (25), although in the absence of information about the rate constants for association and dissociation it is not possible to see whether either of these is related directly to ion size or to ability to activate receptors. Another possibility suggested by the limitations apparently imposed by size on efficacy is that the transition from agonist to antagonist with increasing size follows from limitations on access. Small ions might be able to penetrate to sites within the receptor and disturb interactions between groups in the receptor protein (possibly between carboxyl and amino), so leading to a change in conformation. This process would be adequately described by occupation theory unless the agonist had a particularly high affinity for the receptor in the modified state or the return of the modified receptor to its original state took a relatively long time. Substances which lack the ability to penetrate and produce a change in conformation but which have an affinity for sites which restrict the access of agonists, and for which the agonists themselves have affinity, will behave as competitive antagonists. The affinity of the agonist for the "outer" site need not necessarily be less than its affinity for the inner site, because combination with the inner site should lead to a change in conformation. There would be limits, however, beyond which greater affinity for the outer site would lead to loss of ability to produce a change in conformation, i.e., to loss of ability to activate receptors.

Because changes in receptor structure are likely to be all-or-none (the conformation is either altered or unaltered), compounds might be expected to be divided sharply into agonists and antagonists. Substances may differ in their ability to produce a change in conformation, however, and when the biological response depends on the sum of a number of all-or-none responses some compounds may not be able to produce a maximal response from the tissue. These will therefore behave as partial agonists on tissues containing smooth muscle or slow fibers.

It can be argued that the apparent molal volumes of ions in the environment of the receptor may be very different from what they are in aqueous solution. The results we have obtained, however, indicate that it is worth attempting to correlate the latter with affinity and efficacy, as we have done. Apparent molal volumes are easy to obtain, and in our present ignorance about the nature of receptors they appear to be the most suitable measure of ion size.

REFERENCES

1. R. A. Robinson and R. H. Stokes, "Electrolyte Solutions," Revised Ed., p. 124. Butterworths, London, 1965.
2. R. L. Kay and D. F. Evans. *J. Phys. Chem.* **70,** 2325 (1966).
3. R. H. Stokes and R. Mills, "Viscosity of Electrolytes and Related Properties." Pergamon Press, London, 1965.
4. F. B. Abramson, R. B. Barlow, M. G. Mustafa and R. P. Stephenson, *Brit. J. Pharmacol. Chemother.* **37,** 207 (1969).
5. R. B. Barlow, G. M. Thompson and (in part) N. C. Scott, *Brit. J. Pharmacol. Chemother.* **37,** 555 (1969).
6. B. M. Lowe and H. M. Rendall, *Trans. Farad. Soc.* (in press).
7. R. B. Barlow, N. C. Scott and R. P. Stephenson, *Brit. J. Pharmacol. Chemother.* **31,** 188 (1967).
8. A. H. Beckett, N. J. Harper and J. W. Clitherow. *J. Pharm. Pharmacol.* **15,** 349 (1963).
9. J. Thomas, *J. Med. Pharm. Chem.* **3,** 45 (1961).
10. R. B. Barlow and J. T. Hamilton, *Brit. J. Pharmacol. Chemother.* **18,** 510 (1962).
11. R. B. Barlow, K. A. Scott and R. P. Stephenson, *Brit. J. Pharmacol. Chemother.* **21,** 509 (1963).
12. O. Redlich and P. Rosenfeld, *Z. Physikal. Chem. A,* **155,** 65 (1931).
13. F. Franks and H. T. Smith, *Trans. Faraday Soc.* **63,** 2586 (1967).
14. L. A. Dunn, *Trans. Faraday Soc.* **64,** 2951 (1968).
15. O. Redlich and D. M. Meyer, *Chem. Rev.* **64,** 221 (1964).
16. B. E. Conway, R. E. Verrall and J. E. Desnoyers, *Trans. Faraday Soc.* **62,** 2738 (1966).
17. A. H. Beckett, N. J. Harper, J. W. Clitherow and E. Lesser, *Nature* **189,** 671 (1961).
18. "Handbook of Chemistry and Physics," Ed. 48. Chemical Rubber Co., Cleveland, 1967.
19. B. B. Owen and S. R. Brinkley, Jr., *Chem. Rev.* **29,** 461 (1941).
20. R. H. Stokes and R. A. Robinson, *Trans. Faraday Soc.* **53,** 301 (1957).
21. L. A. Dunn, *Trans. Faraday Soc.* **64,** 1898 (1968).
22. O. Exner, *Collect. Czech. Chem. Commun.* **32,** 1 (1967).
23. A. S. V. Burgen, *Brit. J. Pharmacol. Chemother.* **25,** 4 (1965).
24. F. B. Abramson, "The Synthesis and Pharmacological Properties of some Alicyclic Compounds Related to Acetylcholine," Ph.D. thesis, University of Edinburgh, 1964.
25. W. D. M. Paton, *Proc. Roy. Soc. Ser. B Biol. Sci.* **154,** 21 (1961).