Br. J. Pharmac. (1984), **83**, 807–811

The molal volumes of atropine and hyoscine in relation to their respective potencies

Sasson Cohen & Frank Haberman*

Department of Physiology and Pharmacology, Tel Aviv University Sackler School of Medicine, Tel Aviv 69978, and Institute for Biological Research* Ness Ziona 70450, Israel

1 The partial molal volumes, $\bar{v}_2$, at infinite dilution of atropine and hyoscine were determined in each of eight different solvents having cohesive energy densities in the range 64 to 144 cal cm^{-3}.

2 $\bar{v}_2$ for hyoscine in a given solvent was invariably and significantly smaller than that of atropine in the same solvent. The difference being 1.58 cm^3 mol^{-1} in the least polar solvent (*n*-propylbenzene) and 4.29 cm^3 mol^{-1} in the most polar one (acetonitrile) in the series studied.

3 It is proposed that the lower affinity of atropine relative to that of hyoscine for the muscarinic cholinoceptor could be accounted for by the relative increase in enthalpy in the adsorption of atropine to the receptor with respect to the same process with hyoscine.

Introduction

In a study concerned with the effect of size on affinity of muscarinic antagonists for postganglionic acetylcholine receptors in the guinea-pig ileum, Barlow & Winter (1981) found that the apparent molal volume of hyoscine cation is appreciably smaller than that of hyoscyamine cation in aqueous solution of their respective hydrochloride salts. This finding is not self-evident because these two compounds have very close structures, but hyoscine has a larger molecular weight than hyoscyamine, the increment arising from the presence of an oxirane oxygen in the former. Another distinction between the two compounds is the higher affinity of hyoscine cation relative to that of hyoscyamine cation for the receptors mentioned and for muscarinic receptors elsewhere (Inch *et al.*, 1973).

Barlow & Winter (1981) suggested that the smaller volume of hyoscine salt relative to that of the corresponding hyoscyamine salt in aqueous solution is due to a correspondingly smaller entropy of solution. In other words, hyoscine ought to be more water-soluble than hyoscyamine because it disrupts less hydrogen bonds among water molecules than the latter. This contention is supported by the observation that the oil/water partition coefficient of the respective uncharged species is almost ten times larger for hyoscyamine than for hyoscine (Weinstein *et al.*, 1977). On the other hand, absorption of hyoscine to the receptor is associated with a positive entropy change larger than occurs with hyoscyamine, the difference being almost of the same size as that between entropies in aqueous solution.

The smaller volume of hyoscine with respect to hyoscyamine cation in aqueous solution could be a consequence of the configuration of the N-methyl group. A shift from equatorial to axial was reported to favour binding (Barlow & Franks, 1973). In turn, such a shift could arise as a consequence of an interaction of water molecules with oxirane oxygen in hyoscine (Weinstein *et al.*, 1977; Barlow & Winter, 1981), or as a permanent feature of molecular structure, irrespective of the presence of water, as suggested from crystallographic data of the respective salts (Pauling & Petcher, 1969; 1970).

Distinction between the two cases is important for a proper understanding of the more favourable binding of hyoscine than hyoscyamine. Thus, if hyoscine is the more water-interacting of the two and, at the same time owes its higher affinity for the receptor to this capacity, then one faces the controversy of a drug partitioning between receptor and medium opposite to expectation. If, on the other hand, the molal volume of hyoscine is smaller than that of hyoscyamine also under conditions excluding the participation of water molecules, then one could argue that such being the case, the excess volume of hyoscyamine over hyoscine is likely to impose a corresponding expansion in the fit with the receptor. Under isothermal conditions, such expansion must be coupled with the absorption of heat, hence an increase in enthalpy with respect to the analogous reaction with hyoscine. Indeed, an increase in ΔH with size was shown to occur in the reaction of various ligands with acetylcholinesterase (Belleau & Lavoie, 1968). In

the present approach we asssume that protonation is not the critical issue in determining relative size and potency. In fact, the non-protonated form of hyoscine has about one-tenth the affinity of the protonated form (Barlow & Winter, 1981), which is still considerable.

It seemed, therefore, that this issue could be conveniently addressed from a knowledge of the molal volumes of either compound in non-aqueous media. Neither molecule is capable of a change of conformation, hence size, in view of their common rigid skeleton and evidence from nuclear magnetic resonance spectra of solutions of their respective salts (Feeney *et al.*, 1977).

Methods

Partial molal volumes from density measurements

The procedure used for high-precision density measurements was described at length in an earlier publication (Liron & Cohen, 1983) and will be reviewed here only to the extent of providing an understanding of the working principle involved. The apparatus used consisted of an Anton-Paar DMA-602 and DMA-60 units and adequate thermostatic controls. For each solution tested, at least eight different samples were weighted, each in volumetric flask. The desired weight of solvent was then added. The solute mass fraction which was in the range of 10^{-4} to 10^{-2} was determined for each solution to the nearest 10^{-5}. The density meter was calibrated by measuring the oscillatory period for air and distilled water, then the specific volumes, $\overline{V}_s$, of each of eight solutions of varying mass fraction of solute was determined, $\overline{V}_s$, being the reciprocal of density. Thus, a set of eight values of $\overline{V}_s$ were obtained each corresponding to a predetermined mass fraction of the solute in a given solvent. The data thus generated for the two compounds under consideration, each in eight different solvents may be represented by a single example, that of atropine in benzonitrile (Table 1). At vanishingly low concentrations of solute as used here, a plot of $\overline{V}_s$

Table 1 Experimental example: the mass fraction and specific volume of solutions of atropine in benzonitrile at 25°C

Sample no.	*Weight of atropine* (mg)	*Mass fraction*	*Specific volume*
1	5.08	0.00100	0.99929
			0.99929
2	10.50	0.00206	0.99915
			0.99915
3	15.98	0.00313	0.99901
			0.99901
4	20.97	0.00410	0.99889
			0.99889
5	26.07	0.00509	0.99876
			0.99876
6	30.69	0.00599	0.99865
			0.99865
7	37.80	0.00739	0.99847
			0.99846
8	52.53	0.01022	0.99811
			0.99811
Solvent	0	0.00000	0.99942
			0.99942

The indicated weight of atropine was dissolved in about 5 g of benzonitrile then the mass fraction was calculated. The specific volume was determined as described in the text by taking density measurements of two successive runs from the same sample. A plot of mass fraction (x) against specific volume (y) has the form: $y = -0.12814x + 0.99941$ ($r^2 = 0.99993$). At $x = 0$, y corresponds to $\overline{v}_{s1}$, the partial specific volume of the solvent; at $x = 1$, y corresponds to $\overline{v}_{s2}$, the partial specific volume of solute at infinite dilution.

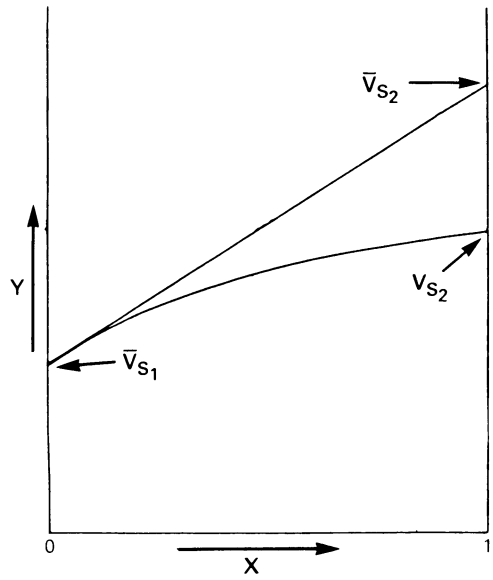

Figure 1 Relationship between solute mass fraction X and the specific volume of its solution, Y, in a given solvent. V_{s2} is the specific volume of the pure solute. When $X = 0$, $Y = \overline{V}_{s1}$, the partial specific volume of solvent; when $X = 1$, $Y = \overline{V}_{s2}$, the partial specific volume of solute at infinite dilution. In practice, measurements of Y are made for a range of X extending from 0 to 10^{-2}, i.e., not exceeding 0.01 of the full scale of X. The slope of the tangent drawn at $X = 0$ could be positive or negative. In a mixture of two liquids, 'real' behaviour follows the plot $\overline{V}_{s1}$ to V_{s2}. The tangent $\overline{V}_{s1} - \overline{V}_{s2}$ represents its extrapolation from infinite dilution of component 2.

against mass fraction gives a straight line which intercepts the ordinate at a point corresponding to the specific volume, $\overline{V}_{s1}$, of the pure solvent when the mass fraction equals zero; and at a point corresponding to the specific volume, $\overline{V}_{s2}$, of the pure solute when the mass fraction equals one (Figure 1). In practice, both $\overline{V}_{s1}$ and $\overline{V}_{s2}$ were derived from the linear regression equation by use of a desk computer. A double check on the accuracy and precision of determination was provided by the coefficient of determination, r^2, and by the calculated value of $\overline{V}_{s1}$ which should correspond to that of the pure solvent obtained independently. The partial molal volume was then calculated from the relationship

$$\overline{v}_2 = M_2.\overline{V}_{s2}$$

where M_2 is the molecular weight of solute.

In this model, the underlying hypothesis requires that the partial molal volume of solute, $\overline{v}_2$ does not change with increasing concentration as long as its mass fraction is kept within low limits ($< 10^{-2}$). In the Redlich & Rosenfeld (1931) model used by Barlow & Winter (1981) for the estimation of the apparent molal volumes of hyoscine and hyoscyamine, no such assumption is necessary but due correction must be made for the effect of concentration on volume. A critical comparison of the two procedures is beyond the scope of this presentation. Nevertheless, any difference that may exist does not invalidate direct comparison of values derived by either procedure because the partial and apparent molecular volumes of the solute are identical at infinite dilution (Moelwyn-Hughes, 1961).

Compounds

Atropine, which is the racemic form of hyoscyamine, was prepared from its sulphate salt by extraction with chloroform from an aqueous solution at pH 10. After dehydration and removal of solvent, the extract was recrystalized from acetone, m.p. 114–116°C. Hyoscine was likewise prepared from its hydrobromide salt and was further purified by distillation under high vacuum, b.p. 170–175°C at 0.1 mmHg. Both compounds were chromatographically pure when assayed by thin layer chromatography on neutral alumina and a selection of mobile phases.

The solvents used were all of the highest purity available commercially and were redistilled before use, some over phosphorus pentoxide, others over sodium metal, for complete removal of moisture.

Results and Discussion

The partial molal volumes, $\overline{v}_2$, at infinite dilution of atropine in nine different solvents are given in Table 2. The corresponding values for hyoscine in the same solvents, except cyclohexane are given in Table 3, hyoscine being relatively insoluble in cyclohexane. Parameters relating to the determination of $\overline{v}_2$ are shown in these Tables for inspection of the accuracy achieved. A graphic presentation of results is given in Figure 2 where $\overline{v}_2$values have been plotted against solubility parameter (δ) of the corresponding solvent, as given in a review article by Barton (1975). Solubility parameter is a measure of the cohesive energy density of a liquid, expressed as (Hildebrand *et al.*, 1970):

$$\delta = \frac{(-E)^{1/2}}{v} = \frac{(\Delta H^v - RT)^{1/2}}{v} \text{ cal}^{1/2}.\text{ cm}^{-3/2}$$

where E is the energy of the liquid expressing the molal heat of vaporization to the gas state at zero pressure, v the molal volume of the liquid and ΔH^v is the heat of vaporization at low vapour pressure.

The following findings and their possible implica-

Table 2 Partial molal volume, $\overline{v}_2$, of atropine at infinite dilution in various solvents at 25°C

Solvent	$\overline{V}_{s1}$ Calcd.	$\overline{V}_{s1}$ Exptl.	$\overline{V}_{s2}$	r^2	*s.d.* 10^5	$\overline{v}_2$ ($cm^3\,mol^{-1}$)
Cyclohexane	1.29239	1.29238	0.89902	0.9994	2	260.157
n-Propylbenzene	1.16612	1.16610	0.87360	0.9999	1	252.801
Toluene	1.15994	1.15994	0.86672	0.9998	2	250.811
Benzene	1.14471	1.14470	0.86835	0.9990	3	251.282
Chlorobenzene	0.90829	0.90828	0.87207	0.9997	2	252.358
Benzonitrile	0.99941	0.99942	0.87128	0.9999	0	252.129
1,2-dichloroethane	0.80276	0.80276	0.87441	0.9999	0	253.036
1-Octanol	1.21714	1.21710	0.86854	0.9999	1	251.624
Acetonitrile	1.28771	1.28769	0.85272	0.9999	2	246.758

$\overline{v}_{s1}$ (calcd), the partial specific volume of solvent, $\overline{v}_{s2}$, the partial specific volume of solute and r^2 were derived by linear regression analysis from the plot of mass fraction against specific volume of solution, as in the example given in Table 1. $\overline{v}_{s1}$ (exptl) is the patial specific volume of solvent measured independently. $\overline{v}_2$ is the partial molal volume of atropine at infinite dilution obtained from $\overline{v}_{s2} \times 289.378$.

Table 3 Partial molal volume, $\bar{v}_2$, of hyoscine at infinite dilution in various solvents at 25°C

Solvent	$\bar{V}_{s1}$ Calcd.	$\bar{V}_{s1}$ Exptl.	$\bar{V}_{s2}$	r^2	*s.d.* 10^5	$\bar{v}_2$ ($cm^3\,mol^{-1}$)
Cyclohexane						
n-Propylbenzene	1.16607	1.16607	0.82156	0.9994	2	249.221
Toluene	1.15992	1.15993	0.82243	0 9997	2	249.484
Benzene	1.14473	1.14472	0.81487	0.9999	1	247.192
Chlorobenzene	0.90831	0.90830	0.82185	0.9993	1	249.308
Benzonitrile	0.99941	0.99941	0.82345	0.9995	1	249.793
1,2-Dichloroethane	0.80276	0.80275	0.82233	0.9891	1	249.453
1-Octanol	1.21708	1.21708	0.82218	0.9999	1	249.408
Acetonitrile	1.28768	1.28767	0.79929	0.9999	1	242.466

Symbols have the same meaning as in Table 2. $\bar{v}_2$ was obtained from $\bar{V}_{s2} \times 303.350$

tions offer themselves for consideration. The partial molal volume of atropine varies with solvent cohesive energy density over a range of about 260 to 247 cm^3 mol^{-1}. The same is true for hyoscine with a range of 250 to 242 cm^3 mol^{-1}. In either molecule, there is an obvious tendency to decrease in size with increasing δ of the holding phase; but the course of change over the range studied is not regular. Nevertheless, in any given solvent among the series tested, $\bar{v}_2$ for hyoscine is invariably smaller than that for atropine, $\Delta\bar{v}_2$ ranging from 1.3 cm^3 mol^{-1} in toluene to 4.3 cm^3 mol^{-1} in acetonirile. Thus, the decrease in molal volume of hyoscine with respect to atropine does not necessarily arise following preferential hydrogen bonding of the former with water molecules or protic media in general. Rather, it seems to reflect an intrinsic property of the respective molecule, which could partly arise from the configuration of the N-methyl group, partly from factors as yet unexplored. In aqueous media where hydrogen bonding must play a role, this property appears to be enhanced even further. In this context, comparison of the present data with values of apparent molal volume φ_v recorded by Barlow & Winter (1981) is instructive. According to these authors, φ_v for hyoscine HCl in water at 25°C is 254.1 cm^3 mol^{-1}, and that for hyoscyamine HCl is 261.9 cm^3 mol^{-1}. To reach an estimate of φ_v for the uncharged species, one must add to these values 4.7 cm^3 mol^{-1} and 3.3 cm^3 mol^{-1} respectively to account for the loss of a proton, then further subtract 18.1 cm^3 mol^{-1} which is the apparent molal volume of Cl^-. The results are now $\varphi_v = 240.7$ for hyoscine and 247.1 cm^3 mol^{-1} for hyoscyamine in water, as compared to 242.47 and 246.76 cm^3 mol^{-1} respectively in acetonitrile which is the most polar solvent used in the present series. To use a simile borrowed from Barlow (1980), atropine seems to be shielded by a larger umbrella than hyoscine also under conditions where dispersion forces constitute a major interaction with the solvent.

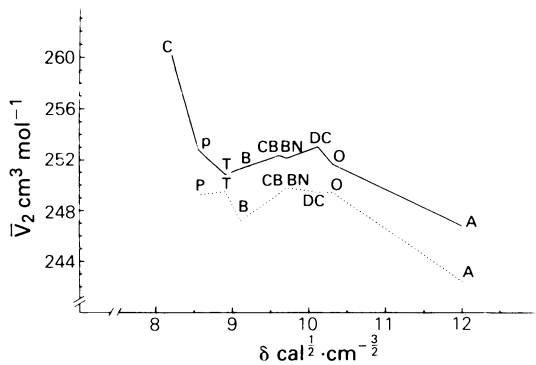

Figure 2 Variation of the partial molal volumes, $\bar{v}_2$, of atropine (——) and hyoscine (.....) with δ of the holding phase. δ is the multicomponent solubility parameter, shown in parentheses (Barton, 1975). C, cyclohexane (8.2); P, *n*-propylbenzene (8.55); T, toluene (8.9); B, benzene (9.1); CB, chlorobenzene (9.6); BN, benzonitrile (9.7); DC, 1, 2-dichloroethane (10.1); 0, 1-octanol (10.3); A, acetonitrile (12.0). The segments joining adjacent $\bar{v}_2$ values have been drawn for convenience and do not necessarily represent the exact course of $\bar{v}_2$ with δ.

Excess enthalpy, ΔE, of atropine solution over hyoscine solution in a given solvent can be simply derived as the product of partial molal volume differences, $\Delta\bar{v}_2$, but the internal pressure, P_i of that solvent (Hildebrand *et al.*, 1970):

$$E = P_i(\bar{v}_{2\,\text{atropine}} - \bar{v}_{2\,\text{hyoscine}}) \sim \delta^2.\,\Delta\bar{v}_2$$

Accordingly, ΔE in acetonitrile is about 618 cal mol^{-1} which could account for a difference in free energy (ΔG) corresponding to a difference in affinity of about 0.44 log units. For comparison, log K_A at 37°C in the guinea-pig ileum preparation has been give as 9.58 for hyoscine cation (Barlow & Winter, 1981) and 9.38 for (−)-S-hyoscyamine cation (Barlow & Ramtoola, 1980), the difference in affinity

being 0.2 log units or about 280 cal mol^{-1}. The data from radioligand binding are somewhat equivocal because most authors neglected to account for the relative abundance for the protonated and unprotonated forms of hyoscine under the specific binding conditions used. Nevertheless, a fair estimate can be reached by considering the data from a typical study (Kloog *et al.*, 1979). In this work, log K_A values at 25°C in mouse brain homogenates are as follows: for hyoscine, 9.2 (cortex, caudate-putamen), 9.1 (hippocampus), 8.6 (medulla-pons, cerebellum); for atropine, 8.9 (cortex), 9.0 (caudate-putamen), 8.8 (hippocampus), 8.3 (medulla-pons), 8.4 (cerebellum). If one would ascribe all the binding with atropine to the cation of the more active enantiomer, then further assume that hyoscine cation constitutes almost half the concentration of total hyoscine used, then through a fortuitous cancellation of errors, the above results become directly comparable. In all cases Δ log K_A is 0.2 to 0.3, in fair agreement with the results derived by the dose-ratio approach in the guinea-pig ileum.

We thank Professor R.B. Barlow, University of Bristol Medical School and Professor H. Weinstein, Mount Sinai Medical Center, New York, for reading the manuscript and helpful criticism. This work was partly supported by the Recanati Fund for Medical Research and by the Mauerberger Chair of Neuropharmacology provided to S.C.

References

BARLOW, R.B. (1980). The size of hydroxyl groups in solution and the changes in size associated with the ionization of phenolic, carboxylic and amino groups in phenolic quaternary ammonium salts, nicotine and some amino acids: possible implications for drug-water and drug-receptor interactions. *Br. J. Pharmac.*, **71,** 17–30.

BARLOW, R.B. & FRANKS, F.M. (1973). A comparison of phenylalkyl and phenoxyalkyl trimethylammonium and triethylammonium salts; their apparent molal volume at infinite dilution and effects on the frog rectus and guinea-pig ileum preparations. *Br. J. Pharmac.*, **49,** 480–489.

BARLOW, R.B. & RAMTOOLA, S. (1980). The size of the hydroxl group and its contribution to the affinity of atropine for muscarinic-sensitive acetylcholine receptors. *Br. J. Pharmac.*, **71,** 31–34.

BARLOW, R.B. & WINTER, E.A. (1981). Affinities of the protonated and nonprotonated forms of hyoscine and hyoscine N-oxide for muscarinic receptors of the guinea-pig ileum and a comparison of their size in solution with that of atropine. *Br. J. Pharmac.*, **72,** 657–664.

BARTON, A.F.M. (1975). Solubility parameters. *Chem. Reviews*, **75,** 731–753.

BELLEAU, B. & LAVOIE, J.L. (1968). A biophysical basis of ligand-induced activation of excitable membranes and associated enzymes. A thermodynamic study using acetylcholinesterase as a model receptor. *Can. J. Biochem.*, **46,** 1397–1409.

FEENEY, J., FOSTER, R. & PIPER, F.A. (1977). Nuclear Magnetic resonance study of the conformations of atropine and scopolamine cations in aqueous solution. *J. Chem. Soc.* (Perkin II), 2016–2020.

HILDEBRAND, J.H., PRAUSNITZ, J.M. & SCOTT, R.L. (1970). *Regular and Related Solutions.* New York: Van Nostrand Reinhold.

INCH, T.D., GREEN, D.M. & THOMPSON, P.B.J. (1973). The central and peripheral activities of anti-acetylcholine drugs. Some concepts of practical relevance. *J. Pharm. Pharmac.*, **25,** 359–370.

KLOOG, Y., EGOZI, Y., & SOKOLOVSKY, M. (1979). Characterization of the muscarinic acetylcholine receptors from mouse brain: Evidence for regional heterogeneity and isomerization. *Molec. Pharmac.*, **15,** 545–558.

LIRON, A. & COHEN, S. (1983). Densitometric determination of the solubility parameter and molal volume of compounds of medicinal relevance. *J. Pharmac. Sciences*, **72,** 499–504.

MOELWYN-HUGHES, E.A. (1961). *Physical Chemistry*, 2nd Edition, p. 814 New York: Pergamon Press.

PAULING, P. & PETCHER, T.J. (1969). The crystal structure of (−)-(S)-hyoscine hydrobromide. *J. Chem. Soc., Chem. Comm.*, 1001–1002.

PAULING, P. & PETCHER, T.J. (1970). Interaction of atropine with the muscarinic receptor. *Nature*, **228,** 673–674.

REDLICH, O. & ROSENFELD, P. (1931). Zur theorie des molvolumens geloster elektrolyte II. *Z. Elektrochemie*, **37,** 705–711.

WEINSTEIN, H., SREBRENIK, S., MAAYANI, S. & SOKOLOVSKY, M. (1977). A theoretical model study of the comparative effectiveness of atropine and scopolamine action in the central nervous system. *J. Theor. Biol.*, **74,** 295–309.

(Received April 18, 1984. Revised May 31, 1984.)