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Enthalpy-entropy relationship in drug-cholinoceptor interaction: a new approach

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1 The partial molal volume at infinite dilution, $\bar{v}_2$, was determined in toluene, benzene and acetonitrile for fifteen different drug molecules comprising muscarinic agonists, partial agonists and antagonists.

2 The difference in $\bar{v}_2$ between a given drug, X, and hyoscine, expressed as $(\bar{v}_{2x} - \bar{v}_{2h})$ was then multiplied by the internal pressure of the holding phase ($P_i \sim$cohesive energy density) in order to obtain an estimate of the excess enthalpy (ΔH) over hyoscine in the interaction of drug molecule X with a common cholinoceptor. As a working hypothesis, ΔH for hyoscine is taken as zero, hyoscine having the lowest $\bar{v}_2$/affinity ratio of any drug in the series investigated.

3 The corresponding change in entropy (ΔS) was then calculated from the relationship: $RT \ln K_x = P_i(\bar{v}_{2x} - \bar{v}_{2h}) - T\Delta S$, where K_x is the affinity constant of drug molecule X to the common cholinoceptor, obtained independently.

4 Linear regression of $P_i(\bar{v}_{2x} - \bar{v}_{2h}) \simeq \Delta H$ from the data in acetonitrile over ΔS gave a satisfactory isoequilibrium plot, $r^2 = 0.954$, slope (β) = 231°K.

5 The present approach offers a new course for the study of the enthalpy-entropy relationship in the interaction of drug molecules in a given series with a common receptor. It could provide an alternative to the Van't Hoff procedure for the estimation of relative ΔH, and is independent of the free energy of binding (ΔG).

Introduction

In a recent article (Cohen & Haberman, 1984) we advanced the view that the difference in affinities for the muscarinic cholinoceptor between hyoscine and atropine could be accounted for by the difference in molal volumes between the two drugs. When these volumes were determined by an accurate densitometric procedure in each of eight different model solvents, the partial molal volume at infinite dilution, $\bar{v}_2$, of hyoscine proved to be invariably smaller than the corresponding value for atropine. A similar result had been found for the hydrochloride salts of these two drugs in aqueous solution (Barlow & Winter, 1981). We reasoned that the fit of the larger molecule, namely atropine, with a common cholinoceptor should entail a relative expansion with respect to the same process with hyoscine. Under isothermal conditions, such expansion must be coupled with the absorption of heat, hence an increase in enthalpy would result compared with the corresponding process with hyoscine. If this increase in enthalpy is not compensated by an increase in entropy, then it must be extracted from the free energy of binding. Thus, association of atropine with the cholinoceptor would be characterized by an equilibrium constant K that must be correspondingly smaller than that for hyoscine. This hypothesis has found ample corroboration in published experimental data (Yamamura & Snyder, 1974; Kloog *et al.*, 1979). In effect, we could show that a difference in log K of 0.2 to 0.3 units between hyoscine and atropine matches the excess enthalpy of atropine over hyoscine, about 600 cal mol^{-1}, derived independently from their respective $\bar{v}_2$ values.

It has remained to be seen whether the same approach could be applied generally to drugs known to associate reversibly with the cholinoceptor. This is the subject of the present investigation which is concerned with two main issues: first, further validation is being sought for the hypothesis that differences in partial molal volume among drug molecules, as determined in a model phase, could in fact account for differences in affinity towards a common receptor;

second, if such is the case, could one reach an estimate of the relative values of the enthalpy and entropy changes associated with this process and hence an insight into the relative 'goodness of fit' with the cholinoceptor?

Our working hypothesis dwells on the following concept: since the size and shape of the drug-binding site of the cholinoceptor cannot be directly determined, we shall assume it to be complementary to the size and shape of hyoscine. The choice of hyoscine is not arbitrary, in view of the fact that this molecule has proved to show the highest affinity to size ratio of any of the drugs in this series investigated. The same assumption would also imply that the fit of hyoscine into this site should not entail any absorption of heat. Therefore, $\Delta H_h = 0$ in the free energy equation (1), the subscript 'h' denoting hyoscine:

$$\Delta G_h = \Delta H_h - T\Delta S_h = -T\Delta S_h \quad (1)$$

Thus, in this particular case the free energy of binding (ΔG) arises totally from the change in entropy. Now, for any other molecule X, $\Delta H_x \neq 0$, being either positive or negative with respect to hyoscine, thus:

$$\Delta G_x = \Delta H_x - T\Delta S_x \quad (2)$$

Subscript '$\times$' denoting a particular drug molecule. ΔG_x may be derived from the relationship

$$\Delta G_x = RT \ln K_x \quad (3)$$

K_x being the equilibrium association constant of drug X with the cholinoceptor as determined in a convenient assay.

ΔH is related to ΔE as follows:

$$\Delta H = \Delta E + p\Delta V \quad (4)$$

E being the internal energy and p and V being pressure and volume, respectively.

For isobaric processes conducted at atmospheric pressure, $p\Delta V$ is negligible (Hildebrand *et al.*, 1970), so that

$$\Delta H = \Delta E \quad (5)$$

The enthalpy change in the adsorption of drug X to the cholinoceptor, relative to this process with hyoscine would now be

$$\Delta H_x = \Delta E_x = P_i(\bar{v}_{2x} - \bar{v}_{2h}) \quad (6)$$

where P_i is the internal pressure of the holding phase in units of cal cm^{-3}, and $\bar{v}_2$ is the partial molal volume at infinite dilution in this phase of either drug X or hyoscine as denoted by the appropriate subscript (Cohen & Haberman, 1984). Equation (2) can now be written as follows:

$$RT \ln K_x = P_i(\bar{v}_{2x} - \bar{v}_{2h}) - T\Delta S_x \quad (7)$$

The proposed approach is unconventional in two ways: first, it derives the entropy term from knowledge of the affinity constant K and an enthalpy term obtained in a totally unrelated system; it will be recalled that the conventional approach derives enthalpy from the relationship between T and K in the usual Van't Hoff equation, ΔH not being obtained independently from ΔG. Second, the present approach assumes a similarity between ΔH_x as defined in equation (6) and factual ΔH_x as would indeed be obtained in the association of the drug X with the cholinoceptor. Unfortunately, factual ΔH_x values for drugs in the system considered are available for comparison only for a few cases (Barlow *et al.*, 1976; 1979; Barlow & Burston, 1979). Therefore, other means will be used in order to verify the relevance of the term $P_i(\bar{v}_{2x} - \bar{v}_{2h})$ to factual ΔH_x.

At this point, some limitations of the proposed approach must be indicated. First, ΔH in the interaction of hyoscine with the receptor is not likely to be infinitesimal. In the absence of an alternative 'standard' state of the receptor, the latter is assigned the size and shape of hyoscine. Second, size alone is not indicative of shape; but shape, i.e., conformation and configuration, must have an impact on size. The extreme case is represented by a pair of enantiomers which must have the same theoretical size *in vacuo*, yet would seem to occupy space of different dimensions in an asymmetric environment as the cholinoceptor is presumed to be. Thus, for ΔH derived from $\bar{v}_2$ to be of relevance to the analogous situation in the receptor, $\bar{v}_2$ ought to be determined in a suitable anisotropic phase for each of the two configurations of an asymmetric molecule. The less rigorous approach which is being currently proposed dwells on the premise that $\bar{v}_2$ values in a suitable isotropic phase could still be indicative of relative ΔH, provided all the compounds investigated are known *a priori* to associate with a given receptor, whether as agonists or antagonists, hence to embody the pertinent pharmacopedia which, in turn, results from shape. The problem of size-configuration relationship does not arise in an isotropic phase, since enantiomeric pairs of compounds are expected to have the same $\bar{v}_2$ value. At the same time, the problem of configuration-activity relationship could perhaps be neglected, if one assumes that the activity of a racemic molecule is largely due to one of its enantiomers. These approximations must be borne in mind when considering the results and conclusions of this study.

Methods

Partial molal volumes from density measurements

The apparatus consisted of an Anton-Paar 602 and DMA-60 units and adequate thermostatic controls. The procedure used and the principle involved were similar to those described in two recent publications (Liron & Cohen, 1983; Cohen & Haberman, 1984).

Essentially, the specific volumes $\bar{V}_{s2}$, of a solution of a given solute-solvent pair were determined at eight different mass fractions of solute within the range 10^{-4} to 10^{-2}. The corresponding values of $\bar{V}_s$ were then regressed over mass fraction of solute. From the linear regression equation obtained, $\bar{V}_{s2}$, the specific volume of solute at mass fraction = 1 could be calculated. The coefficient of determination, r^2, was usually better than 0.9995 and the standard deviation not greater than 2×10^{-5}. The product of $\bar{V}_{2s}$ by the molecular weight gave the partial molal volume $\bar{v}_2$, at infinite dilution in the solvent used.

Compounds

All the compounds used in this study were free bases, either derived from their commercially available salts or prepared by synthesis according to published procedures. Their choice was guided by a desire to avoid the presence of an anion such as Cl^- as would necessarily be found in a quaternary amine salt. The following is a brief guide to their respective sources: 3-quinuclidyl benzilate (QNB), m.p. 164–165°C, was prepared according to procedure 'C' of Sternback & Kaiser (1952). Hyoscine was obtained from its hydrobromide salt (Sigma) and atropine, m.p. 114–116°C, from its sulphate salt (Fluka) as described earlier (Cohen & Haberman, 1984). 3-Quinuclidyl diphenylacetate, m.p. 95°C, was prepared according to procedure 'B' of Sternbach & Kaiser (1952). 3-Quinuclidyl acetate, butyrate and propionate were prepared according to Grobe *et al.* (1957) and purified by distillation. Arecoline and pilocarpine were prepared from their hydrobromide and nitrate salts (Sigma), respectively. Benactyzine, m.p. 51–53°C, was obtained from its hydrochloride salt (Sigma). Nortriptyline, m.p. 59–60°C, and imipramine were prepared from their hydrochloride salts (Teva). Oxotremorine (Sigma) was distilled before use. Benztropine was prepared from its methanesulphonate salt. AF-14 which is 1-aza-4-phenyltricyclo (6.2.2.$0^{2,7}$)-dodecan-5-one, m.p. 126°C, was prepared according to Cohen & Fisher (1978). Purity of all compounds was checked by means of t.l.c. on Merck 5581 alumina plates, GCMS for volatile compounds and direct inlet MS for non-volatile ones. The solvents used were of the highest purity commercially available and were dehydrated before use by distillation over sodium metal (toluene and benzene) or phosphorus pentoxide (acetonitrile).

Results and Discussion

The partial molal volumes, $\bar{v}_2$, of fifteen representative drugs, each in three different solvents, are given in Table 1. In view of the high precision of measurement, variations in $\bar{v}_2$ for a given drug among the three solvents used is real and not due to scatter. The solvent-dependence of $\bar{v}_2$ could possibly arise from a change in shape (conformation) or packing (solvation)

Table 1 Partial molal volumes ($\bar{v}_2$) of drugs, derived from densitometric measurements at 25°C of their dilute solutions in each of three solvents

	$\bar{v}_2$ (cm^3 mol^{-1})		
Drug	*Toluene*	*Benzene*	*Acetonirile*
Benactyzine	297.84	299.75	293.89
QNB	282.19	281.28	278.52
Benztropine	279.17	279.35	279.20
3-Q diphenylacetate	277.40	276.74	276.86
Imipramine	265.78	266.29	266.77
Atropine	250.81	251.28	246.76
Nortriptyline	246.89	247.23	247.95
Hyoscine	249.48	247.19	242.47
AF-14	216.10	215.59	213.52
Oxotremorine	189.11	188.90	190.14
3-Q butyrate	187.74	189.14	188.86
Pilocarpine	171.58	171.45	172.38
3-Q proprionate	169.69	170.47	171.39
3-Q acetate	154.20	153.62	153.09
Arecoline	148.22	148.94	147.35

Abbreviations used in this and following Tables: AF-14 = 1-aza-4-phenyltricyclo (6.2.2.$0^{2,7}$) dodecane-5-one; 3-Q = 3-quinuclidyl; QNB = 3-quinuclidyl benzilate. All quinuclidyl derivatives were racemates.

Table 2 Excess enthalpy (ΔH) over hyoscine, of drugs in dilute solution in each of three solvents

	ΔH (kcal mol^{-1})		
Drug	*Toluene*	*Benzene*	*Acetonitrile*
Benactyzine	4.0	4.6	6.9
QNB	2.7	3.0	4.8
Benztropine	2.5	2.8	4.9
3-Q diphenylacetate	2.3	2.6	4.6
Imipramine	1.4	1.7	3.3
Atropine	0.1	0.3	0.6
Nortriptyline	− 0.2	0	0.7
Hyoscine	0	0	0
AF-14	− 2.8	− 2.8	− 3.9
Oxotremorine	− 5.0	− 5.2	− 7.0
3-Q butyrate	− 5.1	− 5.1	− 7.2
Pilocarpine	− 6.5	− 6.7	− 9.4
3-Q proprionate	− 6.6	− 6.8	− 9.5
3-Q acetate	− 7.9	− 8.3	− 1.2
Arecoline	− 8.4	− 8.7	− 1.3

ΔH is expressed as $P_i(\bar{v}_{2\times} - \bar{v}_{2h})$ where $\bar{v}_2$ is partial molal volume of either drug (×) or hyoscine (h) in solvent having an internal pressure (P_i) as follows: benzene, 88.4; toluene, 83.0; acetonitrile, 133.7 cal cm^{-3}.

Table 3 Affinity constants (K) of drugs for the cholinoceptor in the guinea-pig ileum preparation and their corresponding values of Gibbs free energy calculated as $\Delta G = RT \ln K$

Drug	*log* K*	ΔG (kcal mol^{-1})	*Source*	$\bar{v}_2/\Delta G$**
Benactyzine	8.0	11.3	Rehavi *et al.* (1978)	26
QNB	11.0	15.6	Abramson *et al.* (1974)	20, 18
	10.0	14.2		
Benztropine	9.1	12.9		
	8.5	12.1	Farquharson & Johnson (1959)	23, 22
3-Q diphenylacetate	9.3	13.2	Abramson *et al.* (1974)	21
			Fisher *et al.* (1980)	
Imipramine	6.8	9.6	Rehavi *et al.* (1977)	28
Atropine	9.0	12.8	Barlow & Ramtoola (1980)	19
Nortriptyline	6.7	9.5	Rubinstein *et al.* (1984)	26
Hyoscine	9.5	13.5	Barlow & Winter (1981)	18
AF-14	5.8	8.2	Fisher *et al.* (1976)	26
Oxotremorine	7.8	11.1	Kloog & Sokolovsky (1977)	22, 17
	6.2	8.8	Takeyasu *et al.* (1979)	
3-Q butyrate	5.6	7.9	Talitman (1976)	24
Pilocarpine	5.7	8.1	Takeyasu *et al.* (1979)	24, 21
	5.1	7.2	Waud (1969)	
3-Q proprionate	4.0	5.7	Talitman (1976)	30
3-Q acetate	6.4	9.1	Kloog *et al.* (1979)	19, 17
	5.6	7.9	Talitman (1976)	
Arecoline	6.5	9.2	Birdsall *et al.* (1978)	16

* Where two values of log K are shown, these represent the highest and lowest values recorded. For most values of K, the reported s.e is of the order of $\pm$ 0.05 ($n = 4-7$). The estimated corresponding 95% confidence limits of ΔG are 14.5–14.1 kcal mol^{-1} for K = 10, and 7.0–7.3 for $K = 5$.
** In units of cm^3. kcal^{-1} for $\bar{v}_2$ in acetonitrile.

and will be the subject of a forthcoming publication. These $\bar{v}_2$ values were then used to calculate ΔH, which is, by definition, the excess enthalpy of a given compound over hyoscine in a common solvent, expressed as $P_i(\bar{v}_{2x} - \bar{v}_{2h})$ (equation 6). Now, we have assumed that these ΔH values represent the corresponding changes in enthalpy, relative to hyoscine, associated with the cholinoceptor-drug interaction, ΔH for hyoscine being equal to zero. Such being the case, the entropy change $T\Delta H$ in the same process can be simply calculated from the difference between ΔH (Table 2) and ΔG (Table 3). The results for ΔS are given in Table 4 in entropy units, the experimental temperature of most bioassays being 310°K. The disparity between this temperature and the one at which ΔH was determined by the densitometric procedure, 298°K, should not detract from the applicability of equation (2), since ΔH is not as sensitive to changes in temperature as $T\Delta S$ obviously is.

Verification of the present results will now proceed in several courses. First, ΔH and ΔS values of Tables 3 and 4 need to be compared with corresponding values derived by more conventional methods as, for example, in the use of the Van't Hoff equation (8). This equation establishes the relationship between the rate of change of affinity (K), rate of change of temperature (T) and enthalpy ΔH, the latter being constant

$$\ln K_2/K_1 = \frac{\Delta H(T_2 - T_1)}{R \quad T_1 T_2} \tag{8}$$

Determination of K in the guinea-pig ileum preparation at widely different temperatures is the obvious course, but the procedure has not proved practical, because the response of this preparation is confined to the narrow range between 29°C and 27°C. Nevertheless, Barlow *et al.* (1976) and Barlow & Burston (1979) used this procedure to derive ΔH and ΔS values for a number of muscarinic antagonists, but also duly cautioned that the error involved could be as high as $\pm$ 5 kcal mol^{-1}. With this reservation in mind, one finds that ΔH for hyoscine, given as -17.1kcal mol^{-1}, is still perhaps the lowest of the ΔH values found of the nine antagonists studied, with the exception of hyoscine methiodide, its ΔH being -36.4kcal mol^{-1}. The latter value seems to us paradoxical in view of the expectation that an increase in molecular size following incorporation of an additional methyl group in hyoscine should entail an increase rather than a decrease in enthalpy. Beyond this, further comparison of data proved fruitless, first because the com-

Table 4 Entropy, ΔS, calculated from $\Delta G = \Delta H - T\Delta S$, using values from Table 3 and ΔH values calculated in each of three solvents in Table 2

	ΔS (e.u.)		
Drug	*Toluene*	*Benzene*	*Acetonitrile*
Benactyzine	50	52	59
QNB	59	60	66
	55	56	61
Benztropine	50	51	58
	47	48	55
3-Q diphenylacetate	50	51	57
Imiprine	36	37	42
Atropine	42	42	43
Nortriptyline	30	31	33
Hyoscine	44	44	44
AF-14	17	17	14
Oxotremorine	20	19	13
	12	12	6
3-Q butyrate	9	9	3
Pilocarpine	5	5	−4
	3	2	−7
3-Q proprionate	−3	−4	−12
3-Q acetate	4	3	−9
	0	−1	−13
Arecoline	3	2	−11

Where two values of ΔS are shown, these correspond to the highest and lowest values of log *K* indicated in Table 3. e.u. = entropy units as cal $K^{-1}\,mol^{-1}$

pounds used by these authors were different from the ones used in our study; and, second, because we could not reconcile our hypothesis to their data which imply that receptor-binding of rather large antagonist molecules is entropy driven (i.e., $T\Delta S > \Delta H$) only in two cases out of seven, namely diphenylacetylcholine bromide and 4-benziloyloxy-N-methylpiperidine methiodide. To support our hypothesis further, we recall the work of Weiland *et al.* (1979) who showed that antagonist binding to the β-adrenoceptor was largely entropy-driven. In a subsequent investigation, Barlow *et al.* (1979) indeed showed that the specific binding of methylhyoscine and methylatropine to rat cortex homogenates was entropy-driven, $T\Delta S$ being respectively 9 and 10 kcal mol^{-1} against an estimated enthalpy change of about 4 to 5 kcal mol^{-}. At the current state of the art, it seems that the main difficulty in the application of the Van't Hoff equation for the derivation of enthalpy and entropy data from binding experiments in the muscarinic system lies in the low temperature coefficient of the association constant *K*. This is clearly a condition beyond any forseeable experimental remedy.

The alternative course is to seek a demonstration of the existence of a linear relationship between ΔH and ΔS, as in the so-called isoequilbrium plot (Leffler, 1966). This approach dwells on the concept that requires members of a series of reactants, in our case cholinoceptor specific drugs, that share a common interaction mechanism should abide by the relationship

$$\Delta H = \beta\Delta S \qquad (9)$$

where β, the isokinetic temperature, is that temperature at which the entropy change is fully compensated by the enthalpy change for all members of the series. If this provision is indeed fulfilled, a plot of ΔH against ΔS should be linear and with a slope equal to β. A short review of the application of the isoequilibrium plot may be found in the publication by Triggle (1971). The works of Belleau (1966; 1967) and Belleau & Lavoie (1968) should be singled out as being of particular relevance to this study. Using acetylcholinesterase as a model for a cholinoceptor and a series of trimethylammonium derivatives $R\text{-}N(CH_3)_3$ as inhibitors of enzyme activity, the latter authors showed that ΔS was linearly related to ΔH, β being 288°K. However, a plot of ΔH against estimated molar volume proved much less satisfactory. A much better correlation was obtained when δΔH which is the calculated ΔH value for the group R, was plotted against $\delta\Delta H/V$ which is the same value normalized with respect to molar volume *V* of the substituent R. This course of action is certainly open to criticism, since the two variables of the regression equation are now not independent of each other. Yet, we agree with the conclusion of these authors that 'ligand binding will occur only at the expense of an actual physical change in the molecular species concerned'. When these ligand molecules are rigid or almost so, as in our case, then the physical change should occur almost entirely at the expense of the cholinoceptor. The following results agree with this hypothesis.

Linear regression of ΔH, as found in acetronitrile solution (Table 2), with ΔS values, that were calculated from the data of Table 3 using the minimal *K* values described in the literature, is shown in Figure 1. The equation of the line has the form $y = 231x - 8118$ ($r^2 = 0.966$; $\beta = 231°K$). For ΔS values derived from the maximal *K* values reported, the regression line has the form $y = 231x - 8450$ ($r^2 = 0.976$, $\beta = 231°K$). Thus, the difference between the two sets of data is not significant in the particular context of demonstrating a linear relationship between ΔH and ΔS.

The correlation coefficient r^2 becomes less satisfactory if one uses the ΔH data found in toluene or benzene solution. Thus, for toluene and minimal *K* values, the regression equation is $y = 197x - 7131$ ($r^2 = 0.940$; $\beta = 197°K$). For benzene, it is $y = 202x - 7231$ ($r^2 = 0.943$; $\beta = 202°K$). Given the nature and diverse origin of log *K* used to derive ΔS on the one hand, and the independent origin of ΔH on the

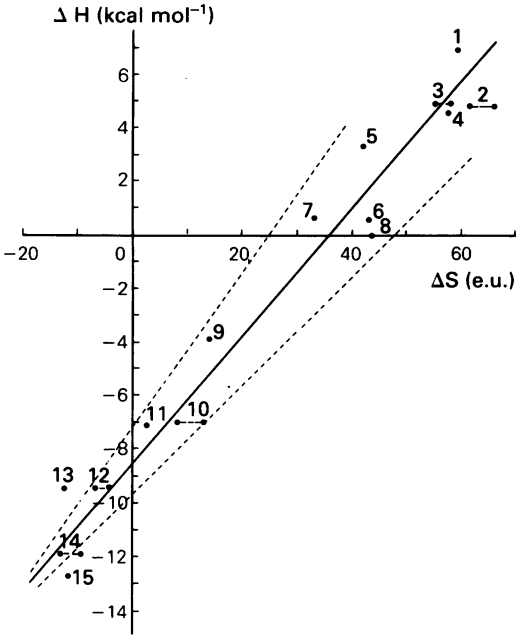

Figure 1 Isokinetic plot of ΔH (enthalpy; determined in acetonitrile solution) against ΔS (entropy) that has been derived from the higher values of log K (Table 3). The linear regression equation is $y = 230x - 8450$, $r^2 = 0.976$. The dashed lines represent the 95% confidence limits for slope (201–261°K), x intercept (30–42 entropy units) and y intercept(9–10 kcal mol^{-1}). Where two values of ΔS are shown for a given compound, these represent the two extreme limits computed from the corresponding K values reported. Compounds used: 1, benactyzine; 2, 3-quinuclidyl benzilate; 3, benztropine; 4, 3-quinuclidyl diphenylacetate; 5, imipramine; 6, atropine; 7, nortriptyline; 8, hyoscine; 9, AF-14; 10, oxotremorine; 11, 3-quinuclidyl butyrate; 12, pilocarpine; 13, 3-quinuclidyl propionate; 14, 3-quinuclidyl acetate; 15, arecoline. For ΔH determined in benzene solution, the regression equation is $y = 202x - 7230$, $r^2 = 0.943$; in toluene solution, $y = 197x - 7130$, $r^2 = 0.940$.

other, the fit to a linear plot is satisfactory enough to discount the likelihood of fortuity. At this point it is proper to recall that this extrathermodynamic approach has had its share of criticism (Exner, 1964; Petersen, 1964). It would seem that the linear regression of ΔH over ΔS is meaningless if the calculated isokinetic temperature β is close to experimental temperature. In our own case β is obviously much lower than the experimental temperature, abiding by the criteria for significance offered by Krug *et al.* (1976). In protein processes which are of immediate relevance to the present case, linear enthalpy-entropy relationship was proposed as a diagnostic test for the participation of water (Lumry & Rajender, 1970), in view of the possibility that such processes must be coupled to water via expansion and contraction of the protein, the compensation temperature being 250–315°K. Eftink *et al.* (1983) presented general thermodynamic models to account for enthalpy-entropy compensation in protein-ligand interactions. In the latter case, the source of compensation is attributed to a shift in equilbria involving transitions such as aggregation or conformational change of the protein, the response of the solvent (water) being a consequence of the ligand-induced change in the state of the protein.

Thus, the premise that $P_i(\bar{v}_{2x} - \bar{v}_{2h})$ could constitute a reasonable estimate of factual $\Delta H_x - \Delta H_h$ as would be obtained in the interaction with the cholinoceptor seems to be well founded, at least in the series of drugs studied. That acetonitrile may constitue a better model of the biophase involved than either benzene or toluene is, perhaps not surprising in view of its more polar character with a solubility parameter, δ, equal to 12 cal$^{1/2}$cm$^{-3/2}$, as compared to 8.9 (toluene) and 9.1 (benzene). But it must be admitted that its choice was guided more by practical convenience than by theoretical considerations.;

It remains to be seen whether the thermodynamic parameters that have evolved from this work are reflected in any way in the structure-activity relationship of the molecules concerned. First, one finds that for all antagonists, including AF-14 which is smaller in size than hyoscine, $T\Delta S$ is invariably larger than ΔH. The reverse is true for the agonist molecules, with the exception of oxotremorine whose ΔH is invariably larger than $T\Delta S$. However, in the case of oxotremorine any increase in entropy ($13 \times 0.310 \simeq 4$ kcal mol^{-1}) is more than compensated for by a decrease in enthalpy (7.0), so that the emerging relationship between entropy and enthalpy changes in the context of agonist-antagonist activity has remained the same as those found by Weiland *et al.* (1979) with the β-adrenoceptive drugs, namely that antagonist binding is entropy-driven and agonist binding is enthalpy-driven. Further scrutiny of the present data reveals even more interesting aspects of drug action. For example, quinuclidyl benzilate and benztropine have close molal volumes in acetonitrile, both being in excess of the volume of hyoscine (Table 1). It follows that either of these molecules should impose a distortion on the cholinoceptor site in order to accommodate the excess bulk relative to hyoscine, both investing in enthalpy to a similar extent, ΔH being 4.8 and 4.9, respectively (Table 2). However, the entropy change with quinuclidyl benzilate is larger than with benztropine, being about 19 compared with 17 in the latter case. This extra gain in entropy with quinuclidyl benzilate could possibly reduce the activation energy required for cholinoceptor isomerization so often observed with this drug (Galper *et al.*, 1977).

The physical interpretation of such isomerization is simply transition to a state of permanent change in the size and shape of the cholinoceptor site following occupancy by a larger molecule than it would normally accomodate.

Another interesting example is nortriptyline. This molecule has a smaller volume than hyoscine in toluene, a similar one in benzene and a larger one in acetonitrile. Its excess enthalpy over hyoscine is correspondingly -0.2, 0 and $0.7\,kcal\,mol^{-1}$ (Table 2). Since 'fit' depends among other things on size, one can envisage a situation where a change in phase composition of the cholinoceptor site, in analogy with the change from toluene to acetonitrile, would entail different affinities for nortriptyline but not for hyoscine. This is perhaps the basis for relative receptor heterogeneity, often demonstrated with drugs such as pirenzepine (Hammer *et al.*, 1980) but not with hyoscine or atropine. Remarkably, nortriptyline was shown to differentiate, in terms of the affinity constant K, between cholinoceptor sites in the bladder and ileum (Rubinstein *et al.*, 1984).

The case of agonists is less straightforward to portray in tangible physical phenomena. By the same token that molecules larger than hyoscine are assumed to expand the cholinoceptor site, smaller molecules ought to contract it, such contracture reaching an amplitude as large as the difference in size between hyoscine and, say arecoline and which is about $95\,cm^3\,mol^{-1}$. Its physical interpretation is perhaps the formation of an ordered structure with the participation of structured water molecules which, upon 'freezing' would decrease the enthalpy of the system by release of latent heat. The excess enthalpy of hyoscine over arecoline is consistent with the transition of about eight molecules of water, not an unreasonable ratio to one unit of receptor site. Similar ideas, arrived at from different standpoints, were voiced by Belleau (1966; 1967) and Belleau & Lavoie (1968).

The question that necessarily arises in conjunction with agonist action is not that of affinity but of efficacy. To this end, the series of 3-quinuclidyl acetate, propionate and butyrate lends itself remarkably well to a study of this issue. The first member is a full agonist (Cho *et al.*, 1972), the second is a weaker agonist (Talitman, 1976) and the third is only a partial agonist (Talitman, 1976). The least enthalpy decrease and the most entropy increase among the three drugs is found for the butyrate (Tables 2 and 4). It might well be that the rate of change of enthalpy versus entropy is the factor that determines whether a given molecule will be a full agonist, a partial one or an antagonist, again in full agreement with Weiland *et al.* (1979). Thus, AF-14 ($\Delta H = -3.9$; $T\Delta S = 4.3$) seems to be on the antagonist side just above the line between measurable agonist and antagonist activity; but 3-quinuclidyl butyrate ($\Delta H = -7.2$; $T\Delta S = 0.5$) just below it on the agonist side.

There remains the significance of β in the linear regression of ΔH against ΔS to be considered. The value found in Figure 1, 231°K or -42°C cannot have a conceivable biological significance, but it might have one at the molecular level. We suggest that it represents the temperature at which concerted modulation of receptor site, size and shape, allowing for expansion or contraction, must cease to occur and the thermodynamic balance between enthalpy and entropy set at zero with whatever ligand might be present there.

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