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AFFINITIES OF THE PROTONATED AND NON-PROTONATED 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

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1 At 37°C in 0.1 M NaCl the pK_a of hyoscine (10 mM) is 7.53; the non-protonated form has about one-tenth of the affinity (log K = 8.58) of the protonated form (log K = 9.58) for muscarine-sensitive receptors of the guinea-pig ileum at 37°C.

2 In the same conditions the pK_a of hyoscine *N*-oxide is 5.78 and the non-protonated form is inactive on the ileum whereas the protonated form is highly active with log K estimated to be 9.9, at least as active as hyoscine methobromide (log K = 9.85).

3 Hyoscine methobromide appears to occupy less space in water than atropine methobromide; hyoscine hydrochloride occupies less space than hyoscyamine hydrochloride: the non-protonated forms are slightly bigger. Hyoscine *N*-oxide hydrobromide is slightly smaller than hyoscine methobromide but the removal of the proton is accompanied by a reduction in volume, such as is seen with other zwitterions.

4 These differences in volume indicate a reduction in entropy on solution which may allow a greater increase in entropy on binding to receptors and hence greater affinity. The higher activity of hyoscine itself could also be due to the presence of the *N*-methyl group in the axial position, rather than equatorial as in hyoscyamine or atropine.

5 The different position of the *N*-methyl group may partly explain why the pK_a of hyoscine is 2 units lower than that of hyoscyamine or atropine. It is also probable that the unionized form of hyoscine is stabilized by hydration.

6 Although hyoscine *N*-oxide is only weakly active at pH 7.6, it is present in a highly active form in the acid environment of the stomach and so might be expected to act selectively at this site.

Introduction

Values for the pK_a of hyoscine quoted in the review by Perrin (1965) indicate that it is a much weaker base than hyoscyamine or atropine and that at physiological pH possibly as little as half is in the ionized form. The affinity of hyoscine for postganglionic acetylcholine receptors in the guinea-pig ileum is, nevertheless, greater than that of atropine (Barlow, Franks & Pearson, 1973a) so it is possible that the unionized form may have appreciable activity. The first part of this work describes the measurement of the separate affinities of the ionized and unionized forms for these receptors by observing the effects of changes in pH on the affinity of hyoscine. The quaternary salt, hyoscine methobromide, was used as a control to monitor any changes in affinity which might arise from the effects of changes in pH on the receptors. Experiments of this type were used by Burgen (1965) to assess the acetylcholine-like activities of the ionized and unionized forms of dimethylaminoethyl acetate and arecoline.

Similar experiments were also made with hyoscine *N*-oxide hydrobromide. This is apparently only weakly active but it is also a very weak base and at physiological pH it is almost entirely in the neutral (zwitterionic) form. The protonated form corresponds to hyoscine methobromide with one methyl group replaced by OH (Figure 1) and it was of great interest to know how this change in structure altered affinity. Although amine-oxides derived from acetylcholine, methacholine and related compounds were found by Canon, Smith, Fischer, Long & Benz (1971) to be virtually inactive, the tests were made at around pH 7: their methoxy analogues, however, with

$$\geqslant\overset{+}{N}\text{—Me replaced by } \geqslant\overset{+}{N}\text{—OMe}$$

had muscarinic and nicotinic activity comparable with the compounds from which they were derived (Darko, Cannon, Long & Burks, 1965).

The X-ray crystallographic studies on salts of atro-

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pine and hyoscine (Pauling & Petscher, 1969; 1970) and on hyoscine *N*-oxide hydrobromide (Huber, Fodor & Mandava, 1971), and the nuclear magnetic resonance (n.m.r.) studies of aqueous solutions of salts of atropine and hyoscine (Feeney, Foster & Piper, 1977) show that, as indicated in Figure 1, the *N*-methyl group is equatorial in hyoscyamine and atropine but is forced into an axial position in salts of hyoscine because of interactions between the proton on the nitrogen and the epoxy oxygen atom. In the protonated form of hyoscine *N*-oxide there is actually a weak hydrogen bond between this oxygen atom and the proton of the hydroxyl group attached to the nitrogen. In hyoscine itself the distance is too great for direct hydrogen bonding and the interaction involves intervening water molecules. The hydration of hyoscine is accordingly very different from that of hyoscyamine or atropine and an attempt has therefore been made to see whether there are detectable differences in apparent molal volumes. Accurate measurements have also been made of the pK_as of hyoscine and hyoscine *N*-oxide at 37°C in 0.1 M NaCl as well as in water at 25°C.

Methods

The guinea-pig isolated ileum was set up as described by Edinburgh Staff (1974) in Tyrode solution containing 0.28 mM hexamethonium or in a modification of it (see below). The temperature was 37°C and the responses were recorded isotonically with a load of about 0.5 g. Carbachol was used as agonist, added by machine once every 90 s and allowed to act for 30 s, as in previous work (Barlow & Burston, 1979). Alternate small and large control responses were obtained, usually with 0.1 and 0.2 mM carbachol, and when these were regular the ileum was exposed to a solution of antagonist and the concentration of carbachol was increased to try to obtain responses which roughly matched the controls. When these were regular, usually after about 45 min, the size of the responses could be used to obtain an estimate of the exact dose-ratio (Edinburgh Staff, 1974).

In the first group of experiments, dose-ratios were obtained for 50 nM solutions of hyoscine and hyoscine methobromide. Both were tested on each preparation with adequate time allowed for the equilibration of the second antagonist; pairs of preparations were usually tested simultaneously with hyoscine tested first on one but second on the other. Sets of these experiments were made at different pH (see below).

In the second group of experiments dose-ratios were obtained for 1 mM hyoscine *N*-oxide, tested at the two extremes of pH possible. After the measurement at one pH, the physiological salt solution was changed and a fresh set of control responses to carbachol was obtained. It was usually necessary to wait 2 h before these were clearly regular and the estimation of the dose-ratio at this altered pH could proceed. The direction of the pH changes, from more acid to more alkaline or *vice versa*, was alternated between experiments.

Physiological salt solutions with a range of pH

Attempts were made to use the range of modified Krebs-Henseleit solutions described by Burgen (1965). To avoid precipitation of phosphates in the more acidic solutions, Burgen reduced the concentrations of calcium and magnesium ions to one-fifth of normal and, for consistency, used these low concentrations over the whole range, even when the buffering system involved Tris or glycine. In previous work (Barlow & Burston, 1980b) we have used these solutions satisfactorily in the more alkaline range on the guinea-pig ileum but when we attempted to use the more acid solutions in this work the responses of the ileum did not appear normal. The contractions faded badly and the tissue was much less sensitive than with ordinary Tyrode solution. We have therefore used Tyrode solution and modifications of it with the normal concentrations of calcium and magnesium ions. All the solutions contained (mM): NaCl 137, KCl 2.7, $MgSO_4$ 1.0, $CaCl_2$ 1.8, glucose 5, and hexamethonium 0.28; normal Tyrode solution (Edinburgh Staff, 1974) contains also NaH_2PO_4 0.42, and $NaHCO_3$ 11.9, and has a pH of about 7.6. Our most

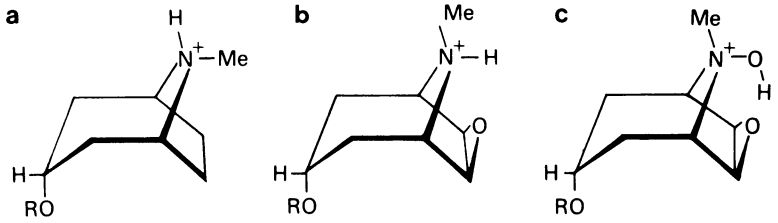

Figure 1 Structure of the protonated forms of (a) hyoscyamine (or atropine); (b) hyoscine; (c) hyoscine *N*-oxide. R indicates the tropyl group (PhCH(CH$_2$OH)—CO—)

acidic solution contained NaH_2PO_4 0.84 and $NaHCO_3$ 5.95 mM and had a pH of about 7.3. In more alkaline solutions the phosphate and bicarbonate were replaced by 50 mM glycine and either 1.6 mM or 5 mM sodium hydroxide; these had a pH of about 8.0 and 8.5 respectively.

All the solutions were aerated with 95% O_2 and 5% CO_2; in each experiment the pH was measured after the fluid had been in contact with the ileum and was found to be appreciably more alkaline than initially. The pH of Tyrode solution after contact with the ileum was usually around 8.0; our most acid modified solution, initially pH 7.3 was subsequently found to be 7.6; the solution pH 8.0 changed to around 8.2 and even the more alkaline solution altered slightly, from pH 8.5 to 8.6. These changes probably indicate excretion of bases by the ileum, whose environment is normally alkaline.

Measurement of pK_a

pK_as were determined electrometrically with a Metrohm model E500 pH meter, as in previous work (Armstrong & Barlow, 1976; Barlow & Burston, 1980a). Measurements were made over a range of concentrations (C) and, as before, estimates were fitted by least squares to the expression $pK = pK_o + mC^{1/2}$. Experiments were done in water and in 0.1 M NaCl and the temperatures were 25.0 ± 0.1° and 37.0 ± 0.1°C.

Measurements of density and calculations of apparent molal volume

Densities were determined with an Anton Paar Precision Density Meter 02D, calibrated with solutions of sodium chloride, exactly as in previous work (Lowe, MacGilp & Pritchard, 1973; Barlow & Franks, 1973; Barlow, 1980). The calculations of apparent molal volume (ϕ_v) and of the volume change following the addition of alkali (ΔV) were made as described by Barlow (1980):

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

where m is the molality, M is the molecular weight, d is the density of the solution and d_w is the density of water:

$$\Delta V = \frac{M}{w_1}\left(\frac{w_1 + w_2}{d} - \frac{w_1}{d_1} - \frac{w_2}{d_2}\right)$$

where ΔV is the change in volume following the solution of 1 mol of solute in 1 kg of solvent, w_1 is the weight of solute with a density d_1 and w_2 is the weight of solvent with a density d_2. For the solution of an acid in alkali the net change is obtained by deducting the amount of water formed and the amount of hydroxyl ion removed; for complete reaction of one acidic group these total 22.11 cm^3/mol (Dunn, Stokes & Hepler, 1965).

Compounds

Carbachol chloride, hexamethonium bromide, (−)-hyoscine hydrochloride, (−)-hyoscine methobromide and (−)-hyoscyamine hydrochloride were obtained from Sigma. These hydrochlorides appeared to be reasonably anhydrous; gravimetric analysis gave for hyoscine hydrochloride Cl^- 10.60 (theory 10.43), for hyoscine methobromide Br^- 20.06 (theory 20.06) and for hyoscyamine hydrochloride Cl^- 10.40 (theory 10.88%). In some of the pK_a measurements a sample of hyoscine hydrobromide was used which was obtained from BDH Ltd. and nominally contained $3H_2O$ but analysis indicated that the composition was closer to $2H_2O$ (found Br^- 19.28, theory for $2H_2O$ 19.01%). Hyoscine *N*-oxide hydrobromide was purchased from Sigma; found Br^- 19.20 (theory 19.96; for $1H_2O$ 19.11%). Another sample was kindly given to us by Laboratories Amido S.A. which had Br^- 19.4%.

Results

The estimates of pK_a are summarized in Table 1. These confirm that the hyoscine is a much weaker base than hyoscyamine or atropine. The results in 0.1 M NaCl at 37°C can be used to calculate the extent of ionization in the biological experiments and the interpolated values for 10 mM were taken, i.e. 7.53 for hyoscine and 5.78 for hyoscine *N*-oxide.

The values of the dose-ratios for (−)-hyoscine and (−)-hyoscine methobromide and the pH measured after contact with the tissue are shown in Table 2A. In each experiment the activity of hyoscine relative to its methobromide was expressed as the ratio, y, calculated from (dose-ratio for hyoscine − 1)/(dose-ratio for the methobromide − 1). This clearly decreases in more alkaline conditions and should be directly proportional to the fraction ionized, x. A least-squares fit of y on x gives $y = 0.475x + 0.066$ with $r = 0.74$, $P < 0.01$. This indicates that the ionized form is 0.541/0.066 = 8.45 times as active as the unionized form.

The dose-ratios for hyoscine methobromide vary considerably, but this was expected with these very dilute solutions. A least-squares fit of (dose-ratio − 1) on pH gives (DR−1) = −5.70 (pH) + 397.6 with r = −0.026. The dose-ratio for pH 8 (352) corresponds to log K = 9.85 compared with 9.70 obtained by Barlow *et al.* (1973a). A shift of 1 pH unit in a more alkaline direction, however, appears to reduce the dose-ratio by only 5.7. If this is neglected, the affinities of the ionized and unionized forms of hyoscine can be cal-

Table 1 Estimates of pK_a

	0 mM	*10 mM*	*m*	*n*
(−)-Hyoscyamine HCl in water, 37°C, 5–20 mM	9.05	9.53	0.151	8
(−)-Hyoscine HCl in water, 25°C, 5–20 mM	7.25	7.56	0.098	7
(−)-Hyoscine HBr in 0.1 M NaCl, 37°C, 5–20 mM	7.45	7.53	0.026	8
(−)-Hyoscine *N*-oxide HBr in water, 25°C, 5–15 mM	5.13	5.52	0.122	3
in 0.1 M NaCl, 37°C, 5–15 mM	6.07	5.78	−0.093	6

The range of concentrations is shown, the number of titrations (n), and the slope (m) for the expression $pK = pK_0 + mC^{½}$ to which the values of pK_a were fitted by least-squares, weighted according to reciprocal of the variance of each titration (which involves from 9 to 15 measurements of alkali added and pH). The values of pK at 0 mM and 10 mM calculated from the expression are indicated.

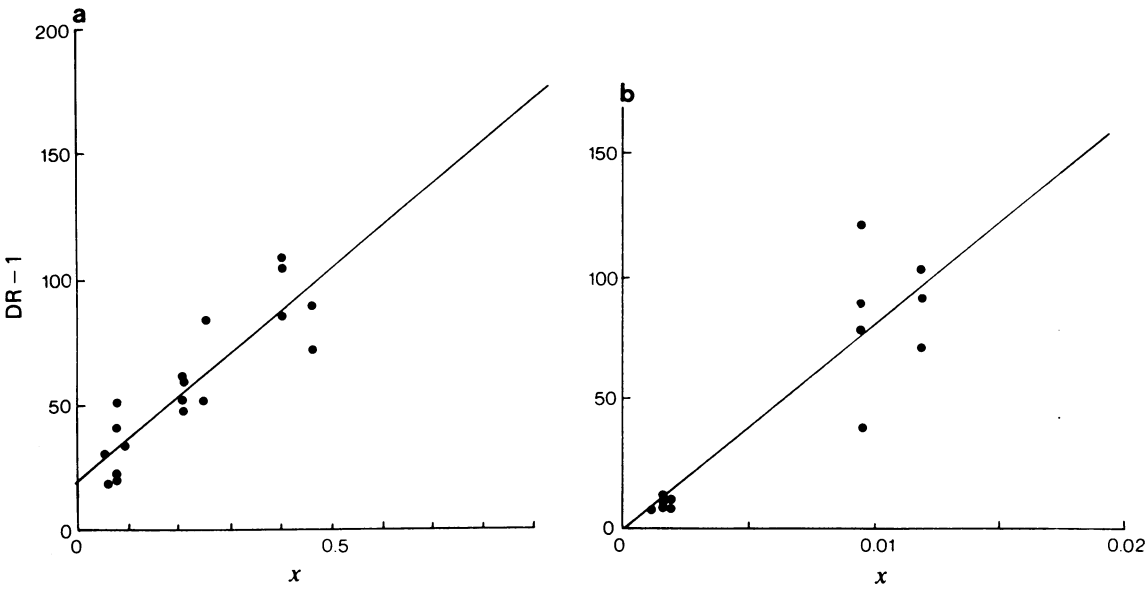

Figure 2 Effects of ionization of biological activity. Dose-ratio − 1 is plotted against the fraction ionized, x, for 50 nM hyoscine (a) and for 1 mM hyoscine *N*-oxide (b). Note the difference in the scales on the x-axis. The least-squares fit gives $(DR-1) = 171.9x + 19.0$ (r=0.88) for hyoscine and $(DR-1) = 8172x - 1.3$(r=0.91) for hyoscine *N*-oxide.

culated from the graph of (dose-ratio for hyoscine − 1) against the fractionized, x (Figure 2a). A least-squares fit gives

$$(DR-1) = 171.9x + 19.0$$

with $r = 0.88$. Accordingly log K for the unionized form appears to be 8.58, compared with 9.58 for the protonated form. This indicates a 10 fold difference in affinity which will be reduced when the slight effect of alkali is taken into account, so there is agreement with the original method of calculating the relative affinities of the two forms. As the percentage ionized in the experiments with pH > 8.5 is less than 10% the extrapolation for the unionized form should be reasonably accurate. There is more uncertainty about the extrapolation involved in the calculation for the ionized form but this appears to be just over half as active as the methobromide, which seems reasonable.

The results for hyoscine *N*-oxide are shown in Table 2B. Although the proportion which is protonated is only small at pH 7.7, the change of 1 pH unit reduces it still further with a marked reduction of the

Table 2 Dose-ratios and pH

A

pH	*(−)-Hyoscine (50 nM)* Fraction protonated	Dose-ratio	*(−)-Hyoscine methobromide (50 nM)* Dose-ratio
7.6	0.460	90.5	485
		73.5	480
7.7	0.403	107	275
		110	398
		86.6	280
8.0	0.253	84.6	360
		51.5	271
8.1	0.212	48.9	371
		59.7	288
		53.2	225
		62.6	275
8.5	0.097	34.7	529
8.6	0.078	22.1	291
		22.9	261
		41.8	396
		51.9	435
8.7	0.063	19.0	391
		31.3	326

B

	(−)-Hyoscine N-*oxide (1 μM)*	
7.7	0.0119	104
		93.1
		73.3
7.8	0.0095	79.7
		91.7
		41.5
		123
8.5	0.0019	12.4
		9.9
8.6	0.0015	11.4
		11.5
		11.0
		13.9
8.7	0.0012	9.6
		10.6

dose-ratio (Figure 2b). A least-squares fit of (dose-ratio −1) on fraction protonated, x, gives

$$(DR-1) = 8172x - 1.36$$

with $r = 0.91$. The non-protonated (zwitterion) form appears therefore to be virtually inactive, even when allowance is made for the effects of alkali on dose-ratio observed with hyoscine methobromide. The protonated form, however, is highly active and the dose-ratio with $x = 1$ gives an estimate of log K = 9.91. Although there must be large errors associated with the extrapolation, this high value does not seem impossible because it is consistent with the presence of the hydroxymethyl group in the *axial* position. Barlow, Harrison, Ison & Pearson (1973b) observed that etho-salts of atropine and hyoscyamine had considerably higher affinity when this group was axial rather than equatorial.

Estimates of apparent molal volume over a range of concentrations are shown in Figure 3, in which Φ_v is plotted against $(\text{conc})^{1/2}$. The values of Φ_v^0, calculated from a least-squares fit using the equation of Redlich & Rosenfeld (1931):

$$\phi_v = \phi_v^\circ + 1.868c^{1/2} + jc$$

with the values of j in parentheses, are (−)-hyoscine HCl 254.1 (−6.7); (−)-hyoscyamine HCl 261.9 (7.7); (−)-hyoscine methobromide 276.1 (−1.9); atropine methobromide 280.7 (13.3). The results for atropine methobromide are taken from Barlow & Ramtoola

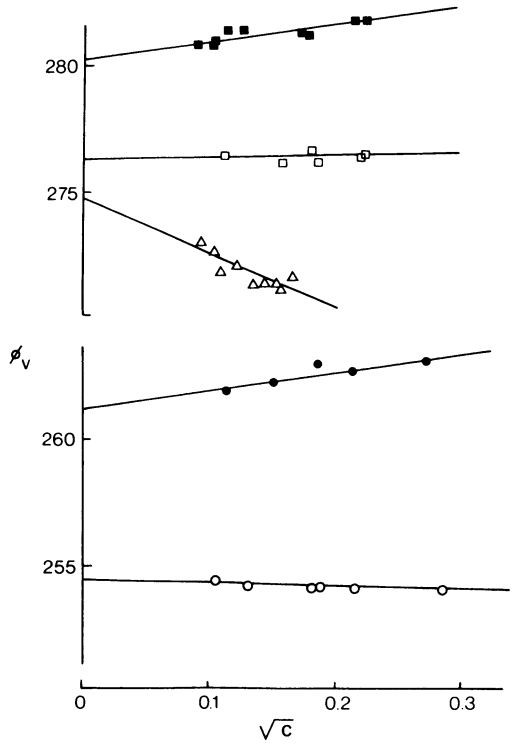

Figure 3 Apparent molal volumes (ϕ_v in cm^3/mol) plotted against (molar concentrations)$^{1/2}$ for hyoscine HCl (○), hyoscyamine HCl (●), hyoscine methobromide (□), atropine methobromide (■) and hyoscine *N*-oxide (△). Note the break in the y-axis. The lines represent the least-squares fit of Φ_v on $c^{1/2}$; these give slightly different values of Φ_v^0 from those calculated by the Redlich-Rosenfeld equation; for hyoscine HCl, 254.5 (254.1); for hyoscyamine HCl, 261.3 (261.9); for hyoscine methobromide, 276.3 (276.1); for atropine methobromide, 280.4 (280.7); for hyoscine *N*-oxide HBr, 274.4 (273.2). The slope according to Debye-Hückel theory, $1.868c^{1/2}$, is approximately indicated by the results for hyoscine methobromide (□; for which j is only −1.9).

Note that although it contains an additional oxygen atom, hyoscine is considerably smaller in solution than hyoscyamine and that hyoscine methobromide is also appreciably smaller than atropine methobromide. Hyoscine *N*-oxide HBr is also smaller than hyoscine methobromide (which contains —CH_3 in place of —CH_2OH); if allowance is made for hydration of the *N*-oxide the difference is slightly bigger than shown here.

(1981); their values for (−)-hyoscyamine HCl have been supplemented by further measurements. Allowance must be made for the difference between bromides and chlorides (6.88 cm^3/mol at 25°C; Millero, 1971).

It is clear that although hyoscine has a bigger molecular weight it has an apparently smaller size in water ($\Delta\phi_v^\circ$ = 7.8 cm^3/mol). With the methobromides the difference is slightly smaller (4.6 cm^3/mol). The negative value of j for hyoscine may partly be due to hydrolysis, which will lead to reaction with hydroxyl ions and the formation of water and will be greater in more dilute solutions (the fraction hydrolysed $\fallingdotseq \sqrt{K_a/c}$), so ϕ_v will appear bigger. With hyoscine the fraction is about 0.1% but with hyoscine *N*-oxide HBr it is between 1 and 2 % in these experiments, and should produce an increase in volume of 0.2 to 0.4 cm^3/mol. The appropriate amount has therefore been deducted from the estimates of ϕ_v. The *N*-oxide HBr (ϕ_v° = 273.2 cm^3/mol;) appears to be smaller than the corresponding methobromide, i.e. the replacement of Me by CH_2OH leads to a reduction in volume of about 2.9 cm^3/mol. It is difficult to obtain a precise estimate because ϕ_v for the *N*-oxide decreases greatly with increasing concentration (Figure 3), the extrapolation of the graph of ϕ_v against $c^{1/2}$ gives ϕ_v° = 274.4 cm^3/mol, compared with 273.2 with the Redlich & Rosenfeld equation but in the range tested the values for the *N*-oxide are at least 3 cm^3/mol, less than those for the methobromide. Because there is also uncertainty about the extent of hydration the results were all recalculated to allow for the presence of $1H_2O$. This gave estimates of ϕ_v° of 269.4 cm^3/mol and 267.4 cm^3/mol by the two methods. It seems that the differences between the *N*-oxide and the methobromide shown in Figure 3 are probably underestimated.

The results of the experiments on the change in volume associated with the loss of a proton are shown in Figure 4, in which the change in volume on solution corrected for water formed the hydroxyl ions removed, ΔV^* is plotted against the fraction which has lost a proton, x. The lines represent the least-squares fit weighted according to the reciprocal of (concentration)^2 as in previous work (Barlow, 1980). The effect of concentration is indicated by the bars which show the extreme values; the spread is particularly marked with hyoscine *N*-oxide. The slope of the line is independent of the errors in the estimate of the density of the solid and is only slightly affected by uncertainties about the hydration of hyoscine *N*-oxide. An error of up to 5% in the molecular weight produces a similar error in ΔV^*.

For hyoscyamine the loss of a proton is associated with an increase of 3.3 cm^3/mol and for hyoscine the increase is 4.7 cm^3/mol. For the addition of a proton to N-methylpyrrolidine it has been estimated that there is a decrease in volume of 3.1 cm^3/mol and for the nicotine the decrease was estimated to be 4.0 cm^3/mol (Barlow, 1980). For the loss of a proton from hyoscine *N*-oxide there is a decrease in volume of 8.2 cm^3/mol, which is intermediate between the change for the loss of a proton from —COOH in β-alanine (7.8 cm^3/mol) and in γ-aminobutyric acid (10.3 cm^3/mol).

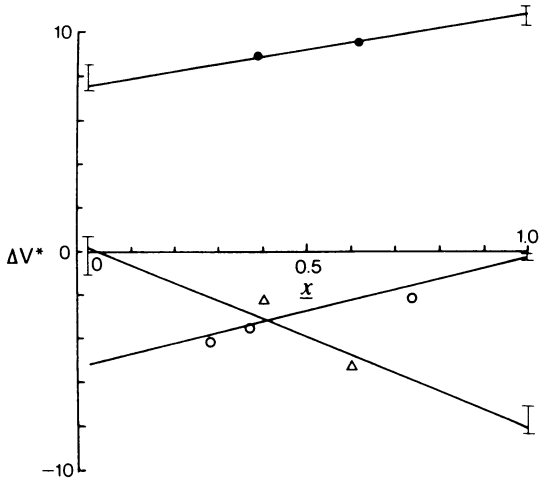

Figure 4 The change in volume on solution, corrected for water formed and loss of hydroxyl ions, ΔV^*, plotted against the fraction which has lost a proton, x, in the experiments with compounds dissolved in alkali. The loss of a proton from hyoscine HCl (○) and hyoscyamine HCl (●) is accompanied by an increase in volume but the loss of a proton from hyoscine *N*-oxide HBr (△) is accompanied by a decrease in volume. The bars indicate the range of values, which vary with concentration, and the line is a least-squares fit weighted with respect to $c^{1/2}$.

Discussion

The results show that coulombic forces do not make a very big contribution to the binding of hyoscine to muscarine-sensitive acetylcholine receptors in the guinea-pig ileum. This is consistent with the calculations made by Burgen (1965) and with the comparisons between compounds containing quaternary nitrogen and quaternary carbon made by Barlow & Tubby (1974). It is therefore surprising that the neutral form of hyoscine *N*-oxide is so weakly active but this may be because it is a zwitterion rather than because it is neutral. In this form it interacts with water to a great extent, as can be seen from the decrease in volume associated with its formation from the protonated species. The measurements of apparent molal volume indicate that the epoxy bridge in hyoscine is also associated with ability to interact with water and from Figure 4 it can be seen that this occurs with the unionized species as well as with the ions. The difference between hyoscine and hyoscyamine extends to solubility; in the experiments with added alkali, hyoscyamine was precipitated as the base from solutions stronger than about 35 mM wheras hyoscine was not.

It has been suggested that the decrease in entropy associated with the reduction in volume such as is seen with compounds containing hydroxyl groups could lead to increased binding to receptors if this process involves the breakup of these drug-water interactions (Barlow, 1980). The high affinity of both ionized and unionized forms of hyoscine might therefore be partly due to such an effect; hyoscine may bind better than hyoscyamine because there is a bigger increase in entropy on adsorption. This would apply also to the protonated form of hyoscine *N*-oxide. The differences in volume actually suggest that entropy changes may account almost entirely for the differences in affinity between these compounds. From the estimate that a decrease of 1 cm^3/mol approximately corresponds to $T\Delta S$ = 0.3 kcal/mol (Hepler, 1965) a change of 4.7 cm^3/mol for the loss of a proton from hyoscine should indicate 1.4 kcal/mol less available energy for binding, which corresponds almost exactly to a difference in log K of 1.

However, the inactivity of the zwitterionic form of hyoscine may be because the interactions with water are accompanied by increases in enthalpy which offset the increase in entropy and make the hydrated form relatively more stable. It would be of great interest to know the enthalpies of hydration of these compounds.

The greater affinity of hyoscine than hyoscyamine might also be the consequence of the shift of the *N*-methyl group from equatorial to axial, in which position it should bind better (Barlow *et al.*, 1973b). This change might also account for the big difference in pK_a, because access by protons should be easier when no group projects axially. It seems doubtful whether this could account for a difference as big as 2 log units, however. Pauling & Petcher (1969) estimated the distance between the nitrogen and the epoxy oxygen atom in the crystal to be 2.47 Å. In the *N*-oxide there is a weak hydrogen-bond involving the —N—OH group and this epoxy oxygen, with the hydrogen atom 1.73 Å from it and 0.98 Å from the N-oxygen; the —N—O— bond length is 1.46 Å (Huber *et al.*, 1971). Interactions in hyoscine, therefore, must involve intervening water molecules and it is the unionized form which is stabilized. Perhaps a water molecule is placed with one proton hydrogen-bonded to nitrogen and the other to the epoxy-oxygen.

The very high affinity of the protonated form of hyoscine *N*-oxide together with its pK_a of 5.8 suggest a possible mechanism for selective action in the stomach. At physiological pH the compound is over 90% in the inactive form but in the stomach it should be almost 100% in the active form. If the uncharged form can cross the membranes of the gastrointestinal tract, or if the drug has been given orally, there should be a high concentration of this active form in the stomach but not elsewhere. The pH of ulcerous

areas may, of course, be nearer to that of the blood than that of the stomach contents but the conditions would seem likely to favour blockade of parasympathetic effects.

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