Br. J. Pharmac. (1980), **71**, 31–34

THE SIZE OF THE HYDROXYL GROUP AND ITS CONTRIBUTION TO THE AFFINITY OF ATROPINE FOR MUSCARINE-SENSITIVE ACETYLCHOLINE RECEPTORS

R.B. BARLOW & SHENAZ RAMTOOLA

Department of Pharmacology, Medical School, Bristol BS8 1TD

1 From measurements of the affinity constants of hydratropyltropine and its methiodide for muscarine-sensitive acetylcholine receptors in the guinea-pig ileum, the increment in log K for the hydroxyl group in atropine is 2.06 and in the methiodide it is 2.16. These effects are slightly bigger than any so far recorded with these receptors.

2 The estimate of the increment in apparent molal volume for the hydroxyl group is 1.1 cm^3/mol in atropine and 1.0 cm^3/mol in the methobromide.

3 The large effect of the group on affinity may be linked to its small apparent size in water as suggested in the previous paper.

Introduction

The high stereospecificity of hyoscyamine (Figure 1: R = OH, atropine is the racemate) indicates that one enantiomer fits much more strongly than the other to muscarine-sensitive acetylcholine receptors. Barlow, Franks & Pearson (1973) estimated the log affinity constants for muscarine-sensitive receptors in the guinea-pig isolated ileum to be 9.38 for the S(−) form and 6.86 for the R(+) form, so the difference in the free energy of adsorption is at least 3.6 kcal (14.9 kJ)/mol. If the receptor interacts with the hydroxyl group of the S form but not that of the R form, this may indicate the contribution made by a hydrogen bond between the receptor and the hydroxyl.

It should be better assessed, however, by a direct comparison between compounds with and without the hydroxyl group, i.e. from the affinities of the enantiomeric forms of hyoscyamine and those of hydratropine (Figure 1: R = H). The racemic form of hydratropine was tested by Cushny (1926) and found to be only about 0.5% as active as atropine in antagonizing the effects of pilocarpine on salivary secretion in the dog but no measurements of its affinity appear to have been made. This paper describes estimates of the log affinity constant of hydratropine and its methiodide and of analogous *pseudo*tropine derivatives for the muscarine-sensitive receptors of the guinea-pig ileum. The pK_as of the tertiary bases have been measured and indicate that the compounds are predominantly ionized in physiological conditions. Measurements of apparent molal volumes have also been made in order to assess the apparent size of the hydroxyl group.

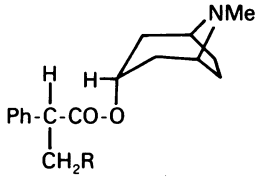

Figure 1

Methods

The guinea-pig isolated ileum was set up as described by Edinburgh Staff (1974) in aerated Tyrode solution containing 0.28 mM hexamethonium. Experiments were carried out at 30° and 37°C and 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 (at 37°C) or 40 s (at 30°C), as in previous work (Barlow & Burston, 1979).

Apparent molal volumes were calculated from measurements of density as described in the previous paper.

pK_as were measured by electrometric titration in water at 37°C as described by Armstrong & Barlow (1976) and Barlow & Burston (1980).

0007-1188/80/130031-04 $01.00

© Macmillan Publishers Ltd 1980

Compounds

Carbachol chloride, hexamethonium bromide and (−)-hyoscyamine hydrochloride were obtained from Sigma. Atropine methobromide was recrystallized from ethanol and had m.p. 213.1 to 214.1°C, found, Br^- 20.93; theory Br^-, 20.79%.

(±)-Hydratropic acid (Alrich) was treated in chloroform with thionyl chloride and the acid achloride was isolated and distilled, b.p. 95 to 96°C/0.12 mm. This was added to a slight excess of tropine (Aldrich), dissolved in chloroform; the chloroform was removed and the residue treated with aqueous NaOH (2 M) and extracted with ether. The extract was dried with magnesium sulphate and distilled, b.p. 130 to 134°C/0.3 mm, N_D^{19} 1.5269. This was converted to the hydrochloride which was recrystallized from a mixture of acetone, ethanol and ether, and had m.p. 179.0 to 180.5°C; found C, 65.5; H, 8.23; N, 4.33: $C_{17}H_{24}O_2NCl$ requires C, 65.9; H, 7.81; N, 4.52%. The methiodide was recrystallized from methylethylketone and ethanol; m.p. 260.0 to 262.0°C (d); found C, 51.5; H, 6.54; N, 3.16; I^-, 30.3: $C_{18}H_{26}O_2N$ I requires C, 52.0; H, 6.31; N, 3.37; I^-, 30.6%. The methobromide, recrystallized from methylethylketone and ethanol had m.p. 262.0 to 262.8°C (dec); found, Br^-, 21.99; theory, Br^-, 21.69%. The ester with pseudotropine (T. and H. Smith Ltd., Edinburgh) was prepared similarly but not distilled. The hydrochloride, crystallized from acetone and ethanol, had m.p. 216.5 to 217.5°C (d); found C, 65.8; H, 8.28; N, 4.50%. The methiodide, recrystallized from methylethylketone and ethanol, had m.p. 164.7 to 165.4°C; found C, 51.8; H, 6.27; N, 3.20; I^-, 30.1%. Microanalyses (C, H, N) are by Mr M. West, University of Bristol; halide analyses are gravimetric and m.ps were recorded with a Mettler FP5 instrument coupled to a potentiometric recorder and at a rate of heating of 0.2°C/min.

Table 1 Log affinity constant (A), apparent molal volume at infinite dilution (B) and effects of the hydroxyl group on affinity and size (C).

A		*Hydrochloride*		*Methiodide*	
		30°	37°	30°	37°
(±)-Hydratropyltropine		6.96 ±0.03 (10)	6.95 ±0.02 (12)	7.29 ±0.01 (6)	7.29 ±0.02 (9)
(±)-Hydratropyl*pseudo*tropine		6.35 ±0.08 (11)	6.26 ±0.04 (13)	6.53 ±0.04 (4)	6.44 ±0.06 (4)
(−)-S-Hyoscyamine			9.38		9.67
Atropine			9.01		9.45
(+)-R-Hyoscyamine			6.86		7.73

B		$\emptyset_v^0$	j		$\emptyset_v^0$	j
(±)-Hydratropyltropine	15–65 mM (4)	260.0 ±0.3	−22	5–14 mM (12)	290.7 ±1.6	108
(±)-Hydratropyl*pseudo*tropine	22–61 mM (7)	258.5 ±0.5	−13	7–26 mM (3)	290.6 ±0.3	24
(−)-S-Hyoscyamine				Atropine methobromide		
	19–73 mM (4)	261.1 ±0.3	−24	8–52 mM (9)	280.7 ±0.1	13
				(±)-Hydratropyltropine methobromide		
				8–35 mM (6)	279.7 ±0.4	6

C			
	Δ log *K*	2.06	2.16
	Δ $\emptyset_v^0$ (cm^3/mol)	1.1	1.0

In section A mean values of log affinity constant are shown with the standard error and number of estimates for the temperatures indicated. The results for atropine and hyoscyamine were obtained by Barlow, Franks & Pearson (1973). In section B the apparent molal volume at infinite dilution ($\emptyset_v^0$; cm^3/mol) is shown with the standard error and an estimate of j in the equation $\emptyset_v = \emptyset_v^0 + 1.868c^{1/2} + jc$, where c is the concentration; the range in which measurements were made is shown with the number of concentrations in parentheses. Section C shows the effect of the hydroxyl group on affinity and size.

Results and Discussion

The electrometric titrations showed that the pK_a of hyoscyamine hydrochloride (used in preference to atropine sulphate) in water at 37°C is slightly less than the values listed by Perrin (1965) at 21°C; the values listed for atropine at 18°C are higher still. Eight titrations were made in the range 5 to 20 mM and, as in previous work (Armstrong & Barlow, 1976), the estimates of pK_a were fitted by least-squares to the equation $pK = pK_0 + mc^{1/2}$. The interpolated pK_a at 10 mM was 9.53; pK_0 was 9.05 and m was 0.15. With the esters of hydratropic acid the base came out of solution halfway through the experiments at 10 mM. With titrations in duplicate at 5 and 10 mM (up to the point of precipitation) the pK_a of hydratropyltropine lay in the range 9.2 to 9.4 and for the *pseudo*-tropine isomer the range was 8.9 to 8.9. Even this compound, therefore, is well over 90% ionized at pH 7.6. The absence of the hydroxyl group greatly reduces solubility and it is likely that the effects on pK involve water; there is a bigger reduction in pK_a in the ester with *pseudo*tropine in which the residue is equatorial and closer to the ionizing group than in the ester with tropine in which it is directed away from the ionizing group.

The compounds were tested on the guinea-pig ileum in at least two concentrations and gave results consistent with competitive antagonism. The mean values of log affinity constant are shown in Table 1A, which includes values for atropine and atropinemethiodide obtained by Barlow, Franks & Pearson (1973). With the esters of hydratropic acid, temperature has little, if any, effect on affinity. This is noticeably different from the greater affinity at lower temperature seen with esters of tropic acid on this preparation (Barlow & Burston, 1979) and with isolated receptor preparations obtained from rat brain (Barlow, Birdsall & Hulme, 1979).

The affinity of hydratropyltropine is very close to that for (+)-R-hyoscyamine but that for hydratropyltropine methiodide is less than for R-hyoscyamine methiodide. Unfortunately it has not been possible to study the resolved forms of hydratropyltropine (extensive racemization has occurred during esterification with resolved hydratropic acid), so the exact contribution of the hydroxyl group to R- and S-hyoscyamine is not known. The increment in log K for the racemate (atropine), however, is 2.06 for the tertiary base and 2.16 for the methiodide. This is slightly less than the effect observed by Cushny (1926) on salivation in the dog, which corresponds to an increment of 2.3, but both results are slightly bigger than any previously seen on the ileum; the highest increment for hydroxyl so far recorded in 59 comparisons was 1.94 (Barlow, 1979).

Estimates of the size of the hydrochlorides may be slightly, but only slightly, affected by the presence of free base; estimates of the size of the methiodides, on the other hand, are complicated by the limited solubility of the compounds and the large values of j (Table 1B). The large standard error attached to the estimate of $\emptyset_v^0$ for hydratropyltropine methiodide is particularly noticeable but the result obtained with the methobromide indicates that the increment for replacing I^- by Br^- is -11.0 cm^3/mol, which compares reasonably with -11.5 cm^3/mol quoted by Millero (1971). The values in Table 1B are self-consistent and indicate that the increment in $\emptyset_v^0$ for the hydroxyl group in atropine is 1.1 cm^3/mol and in its methobromide it is 1.0 cm^3/mol, which is smaller than the increments observed in the compounds described in the previous paper.

Whether the small increment in $\emptyset_v^0$ for hydroxyl is connected with its large increment in log K is still an open question, but a mechanism for such a link has been suggested in the previous paper. Some slight support is afforded by the different effects of temperature on the affinity of compounds with and without the hydroxyl group.

We thank Mr K. N. Burston for help with the affinity measurements.

References

ARMSTRONG, J. & BARLOW, R.B. (1976). The ionization of phenolic amines, including apomorphine, dopamine and catecholamines and as assessment of zwitterion constants. *Br. J. Pharmac.*, **57**, 501–516.

BARLOW, R.B. (1979). The use of affinity constants for studying interactions between pharmacodynamic groups and the muscarinic receptor. In *Advances in Pharmacology and Therapeutics* Vol. 3, ed, Stoclet, J.C. pp. 253–260, Oxford: Pergamon Press.

BARLOW, R.B., BIRDSALL, N.J.M. & HULME E.C. (1979). Temperature coefficients of affinity constants for the binding of antagonists to muscarinic receptors in the rat cerebral cortex. *Br. J. Pharmac.*, **66**, 587–590.

BARLOW, R.B. & BURSTON, K.N. (1979). Temperature coefficients of affinity and entropies of adsorption for enantiomeric pairs of compounds acting at muscarinic receptors in the guinea-pig ileum. *Br. J. Pharmac.*, **66**, 581–585.

BARLOW, R.B. & BURSTON, K.N. (1980). The ionization of 5-hydroxytryptamine and related compounds and an appraisal of methods for the estimation of zwitterion constants. *Br. J. Pharmac.* **69**, 587–596.

BARLOW, R.B., FRANKS, F.M. & PEARSON, J.D.M. (1973). Studies on the stereo-specificity of closely related compounds which block postganglionic acetyl-choline receptors in the guinea-pig ileum. *J. med. Chem.*, **16**, 439–446.

CUSHNY, A.R. (1926). On optical isomers VIII. The influence of configuration on the activity of tropeines. *J. Pharmac. exp. Ther.*, **29**, 5–16.

EDINBURGH STAFF (1974). *Pharmacological Experiments on Isolated Preparations.* 2nd edition, pp. 2, 26–28, 58–62. Edinburgh: Churchill Livingstone.

MILLERO, F.J. (1971). The molal volumes of electrolytes. *Chem. Rev.*, **71**, 147–176.

PERRIN, D.D. (1965). *Dissociation Constants of Organic Bases in Aqueous Solution*, pp. 342 and 347. London: Butterworth.

(*Received February 13, 1980.*)