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The effects of a hydroxyl group on some chemical and biological properties of n-pentyl ammonium salts

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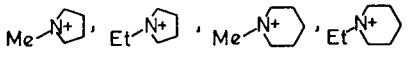
The effects of a hydroxyl group on the activity and affinity of compounds related to n-pentyltrimethylammonium have been studied on the guinea-pig ileum, frog rectus, acetylcholinesterase and on partitioning (Rm) and size (ϕ_v°, V_m). The hydroxyl group lowered affinity in all tests, confirming the importance of hydrophobic forces in binding to receptors. Activity on the ileum was lowered appreciably but on the rectus only slightly. The effects on Rm did not indicate any interactions between hydroxyl, onium group and water but the apparent size of the hydroxyl group ($\Delta\phi_v^\circ$) depends on the nature of the onium group.

The importance of the hydroxyl group for the activity of (+)-muscarine can be appreciated from its high stereospecificity and from the low activity of desoxymuscarine. Beckett, Harper & Clitherow (1963) found that the latter was about 1/20th as active as acetylcholine on the guinea-pig ileum so from the relative activities of acetylcholine and (+)-muscarine (Waser, 1961) it appears that the presence of the hydroxyl group increases activity about 60-fold. It does not follow, however, that the hydroxyl group increases the affinity for the receptors by this amount, because the compounds are agonists and the activity depends upon ability to activate receptors as well as on affinity.

It should be possible to study the effects of a hydroxyl group on affinity as well as on activity by testing series of compounds with onium groups of different size, because those with larger onium groups are often partial agonists or antagonists whose affinity can be measured. The present work describes an attempt to do this with simpler compounds by studying the effects on affinity and activity of introducing a 5-, 4-, or 3-hydroxy group into n-pentyl onium salts:

$$CH_3(CH_2)_4\overset{+}{N}R_3$$

where $\overset{+}{N}R_3$= $\overset{+}{N}Me_3$, $\overset{+}{N}Me_2Et$, $\overset{+}{N}MeEt_2$, $\overset{+}{N}Et_3$,



The compounds without a hydroxyl group had already been tested on the guinea-pig ileum by Abramson, Barlow & others (1969).

Although they lack the ring ether oxygen, these compounds bear some relation to (+)-muscarine (Fig. 1) and the distance between the hydroxyl group and the onium atom in muscarine, estimated to be

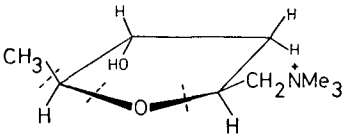


FIG. 1. (+)-Muscarine: 4-hydroxy-n-pentyl trimethylammonium can be derived by breaking the three bonds indicated.

between 5·0 and 6·0 Å (Waser, 1961), should lie within the range spanned by the three series.

The 5-hydroxy- and 4-hydroxy-trimethylammonium compounds were described by Jacob, Marszak & others (1952) who found them to have about 1% of the activity of acetylcholine at muscarine-sensitive receptors but activity comparable with that of acetylcholine at some nicotine-sensitive receptors. The series of compounds have therefore been tested on the frog rectus preparation and as inhibitors of electric eel acetylcholinesterase, as well as on the guinea-pig ileum.

The effects of the hydroxyl groups on the physical properties of the compounds has been estimated by measuring their Rm values from paper chromatography and the apparent molal volumes of some of them have been estimated and extrapolated to infinite dilution (ϕ_v°).

METHODS

Compounds

The 5-hydroxy compounds were obtained by heating the appropriate secondary amine with 5-chloropentylacetate prepared from tetrahydropyran and acetylchloride (Synerholm, 1947). The product was hydrolysed with concentrated hydrochloric acid and the tertiary amino-alcohol was isolated, distilled, and treated with methyl or ethyl iodide. The 4-

hydroxy compounds were obtained from the appropriate secondary amine and 5-chloropentan-2-one (Aldrich); the aminoketone was isolated, distilled, and reduced with sodium borohydride. The tertiary aminoalcohol was isolated, distilled, and treated with methyl or ethyl iodide. The 3-hydroxy compounds were obtained in a similar way from 1-chloropentan-3-one (Aldrich). The 4-hydroxy- and 3-hydroxy- compounds were racemic. Melting points and analyses are shown in Table 1.

Table 1. *Melting-points and analyses.*

n-Pentyl	$HO(CH_2)_5$-	(±)-CH_3-$CHOH(CH_2)_3$-	(±)-CH_3CH_2-$CHOH(CH_2)_2$-
$\overset{+}{N}Me_3$ 230°	131·7–132·5° 46·59 (46·46)	127·1–127·9° 46·55	178·5–179·4° 46·51
$\overset{+}{N}Me_2Et$ 178°	Sinters 60·7, melts 66·5° 44·09 (44·19)	80·0–81·0° 44·10	124·0–125·0° 44·01
$\overset{+}{N}MeEt_2$ 150°	45·0–48·5° 42·20 (42·13)	41–43° 41·89	83–90° 42·20
$\overset{+}{N}Et_3$ 162°	92·7–94·0 40·26 (40·26)	95·3–96·2° 40·28	88·0–88·9° 40·38
Methylpyrrolidinium 180°	60–65° 42·88 (42·41)	76–79° 42·45	101–105° 42·52
Ethylpyrrolidinium 147°	65–70° 41·01 (40·52)	81·5–83·0° 40·57	84·0–85·0° 40·50
Methylpiperidinium 163°	65·5–67·0° 40·47 (40·52)	86·0–87·3° 40·77	50–58° 40·34
Ethylpiperidinium 187°	88·5–89·8° 38·66 (38·79)	66·6–68·4° 38·90	87·1–88·1° 38·59

All the compounds are iodides and the analyses are gravimetric with samples of 50–250 mg: theoretical values are shown in parentheses. Melting-points were measured with Mettler FP instruments, coupled to a potentiometric recorder. Values given to 0·1° were obtained with a rate of heating of 0·2° min^{-1}; for the others the rate was 1° min^{-1}. Nearly all the compounds were very hygroscopic and the melting-points were measured in sealed tubes. Results for the n-pentyl compounds obtained by Abramson & others (1969) are included for comparison.

The guinea-pig isolated ileum was set up at 37° in oxygenated Tyrode solution containing hexamethonium ($2{\cdot}76 \times 10^{-4}$ M) as described by Edinburgh Staff (1972). Responses were recorded isotonically and the load was about 0·5 g. The activity of the agonists was expressed as the equipotent molar ratio relative to n-pentyltrimethylammonium, estimated in 2 + 2 dose assays of Latin square design. The doses were added manually once every 2 min and allowed to act for 25 s. The affinity of most of the partial agonists was estimated by the reciprocal plot method (Barlow, Scott & Stephenson, 1967) and approximate equipotent molar ratios were also calculated by comparing concentrations producing roughly half-maximal responses from the preparation. With one compound the addition method was used for estimating affinity (Stephenson, 1956) and another was so weak that it could be treated as an antagonist.

The affinities of the antagonists were measured as described previously (Abramson & others, 1969; Barlow, Franks & Pearson, 1973). Carbachol was the agonist, allowed to act for 30 s and given by a machine once every 90 s. Because the compounds were all weak it was not possible to test a range of concentrations and all the dose-ratios measured were less than 10.

The frog rectus preparation (*Rana temporaria*) was set up at room temperature (18–23°) in aerated frog-Ringer solution as described by Edinburgh Staff (1972). Responses were recorded isotonically and the load was about 1 g. Doses were made up in a 10 ml measure and poured onto the preparation, allowed to act for 5 min and given once every 16 min. The activities of agonists were expressed as equipotent molar ratios relative to pentyltrimethylammonium as in experiments on the ileum. In these assays of agonist activity responses were obtained at least in duplicate with two doses of each compound and several compounds were tested on each preparation. The affinities of 8 of the partial agonists were measured by the reciprocal plot method, with responses at least in duplicate to at least three different concentrations of each compound; carbachol was used as the full agonist. Approximate equipotent molar ratios were also calculated, as in similar experiments on the ileum, and were converted into ratios relative to n-pentyltrimethylammonium by dividing by the ratio for n-pentyltrimethylammonium relative to carbachol, which had been found in separate experiments to be 4·19 ± 0·13 (s.e.; 4 estimates). The remaining 4 partial agonists were very weak and could be treated as antagonists. The affinities of antagonists were estimated in experiments with carbachol as the full agonist and because the compounds were very weak the dose-ratios measured were usually less than 5.

Electric eel acetylcholinesterase was bought from Sigma (batch number 125C–8000). Stock solutions

were made in a mixture of 0·14M NaCl and 0·04M $MgSO_4$, prepared as described previously (Barlow, Bremner & Soh, 1977), and stored at 4°. The reaction was followed spectrophotometrically by the method of Ellman, Courtney & others (1961) and performed in 0·1 M phosphate buffer, pH 8·1. The total volume was 3·2 ml and the concentrations of acetylthiocholine were 0·1, 0·2, 0·3 and 0·4 mM in the controls made in the absence of inhibitor. The very small amount of spontaneous hydrolysis was neglected. Each concentration was tested at least in duplicate and values of K_m and V_{max} were calculated by fitting the values of velocity (V) and substrate concentration (S) to the expression $V = V_{max}\frac{S}{(K_m + S)}$ by the method of least squares. The estimates of K_m varied slightly from one set of experiments to another and the mean value was 0·096 mM ± 0·001 (s.e., 41 estimates).

When the inhibitors were present the concentrations of substrate were increased and for each concentration of inhibitor (I) the values of rate and substrate concentration were fitted to the hyperbola, as with the controls. If the antagonism is competitive the 'apparent' K_m obtained in the presence of the inhibitor $= K_m\left(1 + \frac{I}{K_i}\right)$, and V_{max} should be unaltered. The ratio of the estimates of K_m in the presence and in the absence of the inhibitor is equivalent to the dose-ratio and has been used to calculate K_i and the log affinity constant ($-\log K_i$). As in the experiments on the ileum and rectus the range of concentration which could be studied was small and the dose-ratios were less than 5.

Apparent molal volumes at infinite dilution (ϕ_v°) were calculated from the densities of solutions of known composition by weight, measured with an Anton Paar Precision Density Meter, DMA 02D, as described previously (Lowe, MacGilp & Pritchard, 1973; Barlow & Franks, 1973). Some molal volumes (V_m) for pure liquids have been measured as described by Barlow & Tubby (1974). The temperature was 25·00 ± 0·01°.

Values of Rm (Bate-Smith & Westall, 1950) were calculated from chromatography of the compounds on Whatman No. 1 paper with the solvent system butanol–ethanol–water (5:5:2 by volume). The spots were developed with the Dragendorff reagent (Thies & Reuther, 1954). Ten samples were run on each sheet with each compound tested in duplicate and n-pentyltrimethyl-ammonium iodide always included. The estimates of Rm varied appreciably but the variation in ΔRm relative to n-pentyltrimethylammonium iodide was much less.

RESULTS

The results obtained on the guinea-pig ileum are shown in Table 2 and illustrated in Fig. 2. The hydroxyl group consistently reduces affinity and the effect is greatest when it is attached in the 5-position and least in the 3-position. In the compounds which are antagonists the average effect of a 3-hydroxyl group is to reduce log K by 0·7 units (K is reduced 5-fold) and in the 4- and 5-positions the average reductions are 1·06 and 1·17 log units, respectively (K is reduced just over 10-fold). The effect appears to be less in the compounds which are partial agonists. Because the 3- and 4-hydroxy compounds are racemates the effect may have been underestimated but even if one enantiomer is completely inactive the estimates of log K for the more active enantiomer will only be reduced by 0·3. The sequen-

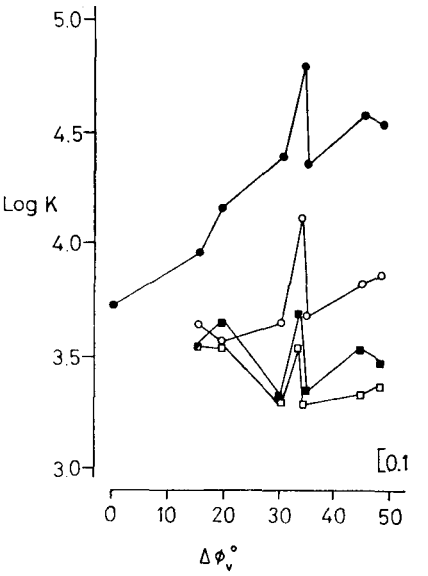


FIG. 2. The effect of hydroxyl groups on affinity for postganglionic acetylcholine receptors of the guinea-pig isolated ileum at 37° in the presence of hexamethonium ($2·76 \times 10^{-4}$ M). Values of log K (ordinate) are plotted against the size of the onium group ($\Delta\phi_v^\circ$; $cm^3\ mol^{-1}$ estimated from results obtained by Barlow & others, 1971, see text) (abscissa). Differences between mean values less than 0·1 log units (indicated) are not considered to be significant. Values for the n-pentyl series (●) are from the results of Abramson & others (1969). Note that the affinity of the 3-hydroxy compounds (○) is greater than that of the 4-hydroxy (■) and 5-hydroxy (□) compounds.

Table 2. *Affinity for postganglionic acetylcholine receptors of the guinea-pig ileum, 37°.*

		Guinea-pig ileum			
	n-Pentyl	5-Hydroxy-n-pentyl	4-Hydroxy-n-pentyl	3-Hydroxy-n-pentyl	$\Delta\phi^{o}_{v}$
$\overset{+}{N}Me_3$	*1·0* (3·733)	*12·9* ±*0·7* (4)	*18·3* ±*1·1* (4)	*27·8* ±*2·5* (4)	0
$\overset{+}{N}Me_2Et$	3·970 *A*	3·548 *A* ±0·109 (6)	3·630 *A* ±0·111 (5)	3·650 *A* ±0·097 (6)	15·0
	19·4	*132* ±*6* (6)	*242* ±*17* (5)	*243* ±*20* (6)	
Methyl pyrrolidinium	4·165 *A*	3·547 *A* ±0·053 (4) *112* ±*11* (4)	3·663 *B* ±0·102 (3)	3·569 *C* ±0·017 (4)	19·2
$\overset{+}{N}MeEt_2$	4·399	3·308 ±0·006 (4)	3·319 ±0·061 (4)	3·663 ±0·035 (4)	30·0
Methyl piperidinium	4·815	3·556 ±0·052 (4)	3·704 ±0·114 (5)	4·127 ±0·034 (4)	33·4
Ethyl pyrrolidinium	4·370	3·295 ±0·025 (4)	± 3·364 ±0·099 (4)	3·692 ±0·028 (4)	34·4
$\overset{+}{N}Et_3$	4·588	3·343 ±0·028 (4)	3·536 ±0·075 (4)	3·839 ±0·005 (4)	44·5
Ethyl piperidinium	4·546	3·378 ±0·056 (4)	3·476 ±0·049 (6)	3·880 ±0·017 (6)	48·0

Values in italics are the equipotent molar ratios relative to pentyltrimethylammonium and results for partial agonists are only approximate (see text): all other values are estimates of log affinity constant. Mean values are shown with the standard error and number of estimates. Results for the n-pentyl series, included for comparison, are taken from Abramson & others (1969), who measured the affinity of pentyltrimethylammonium by using an irreversible blocking agent and the value of log K is included in parentheses. The letter *A* indicates a partial agonist whose affinity constant was measured by the reciprocal plot method (Barlow, Scott & Stephenson, 1967), *B* indicates the use of the addition method (Stephenson, 1956) and *C* indicates that the compound was only a very weak partial agonist and the affinity constant was measured as if it were an antagonist. The increment in the size of the onium group in cm^3 mol^{-1} is indicated by $\Delta\phi^{o}_{v}$, calculated from results obtained by Barlow & others (1971).

tial increase in the effect of the hydoxyl group from the 3-position to the 4- and 5-positions in fact suggests that there is not likely to be appreciable stereospecificity, and that the reduction in affinity is due to disturbance of hydrophobic binding which will be greater when there are hydrophilic groups, onium and hydroxyl, at both ends of the molecule.

The estimates of the agonist activity show that the hydroxyl group markedly reduces activity but the effect is greatest when the group is in the 3-position and least when it is in the 5-position. The results for the 4- and 5-hydroxy pentyltrimethylammonium compounds agree reasonably with those of Jacob & others (1952), who also found the 5-hydroxy compound more active than the 4-isomer on rabbit intestine. Efficacy is reduced by the introduction of a 3-hydroxy group because the reduction in affinity is insufficient to account for the reduction in activity. With the ethyldimethylammonium compounds, for example, log K is reduced by 0·32 but the activity drops over 10-fold and the compound is a partial agonist. With the 5-hydroxy group the effect on efficacy is less: the reduction in log K is 0·42 (i.e. 2·6-fold) which accounts for much of the drop in activity (132/19·4 = 6·8-fold). The effect of position on efficacy can also be seen with the methylpyrrolidinium compounds; the affinity of the 5-hydroxy compound, like that of the unsubstituted analogue, could be measured by the reciprocal plot method, whereas that of the 4-hydroxy compound was measured by the addition method and the 3-hydroxy compound was so weak a partial agonist that it could be tested as an antagonist.

The results on the frog rectus are summarized in Table 3 and illustrated in Fig. 3. The hydroxyl group again reduces affinity and the average effects in the antagonists are 0·78, 0·92 and 0·84 log units for the 3-, 4- and 5-hydroxyl group respectively, but the differences between these values are not significant. Affinity is less than for the receptors of the guinea-pig ileum by roughly 0·5 log units and the structure-affinity and structure-activity patterns are quite different. The trimethylammonium compounds have appreciable nicotine-like activity, again in agreement with the results of Jacob & others (1952) but they are weaker than pentyltrimethylammonium itself. The methylpyrrolidinium compounds also have appreciable activity, relatively more than on the ileum. The hydroxyl group clearly has least effect on activity when it is in the 4 position and in the partial agonists this causes the smallest decrease in affinity. The activity may again be underestimated because the 3- and 4- compounds are racemates but their equipotent molar ratios should not be less than half those in the Table unless one enantiomer is actually an antagonist.

The results of the experiments with acetylcholinesterase are shown in Table 4 and illustrated in Fig.

Table 3. *Affinity for acetylcholine receptors of the frog rectus at room temperature (18–23°).*

	n-Pentyl	5-Hydroxy-n-pentyl	4-Hydroxy-n-pentyl	3-Hydroxy-n-pentyl
$\overset{+}{N}Me_3$	*1·0*	*2·08*	*1·22*	*2·01*
		±0·11	*±0·03*	*±0·11*
		(10)	(10)	(10)
$\overset{+}{N}Me_2Et$	3·64 *A*	3·13 *A*	3·25 *A*	2·98 *A*
	±0·08	±0·02	±0·08	±0·13
	(4)	(4)	(5)	(4)
	8·56	*22·9*	*11·5*	*14·1*
	±0·07	*±1·9*	*±1·1*	*±0·2*
	(6)	(4)	(7)	(5)
Methyl pyrrolidinium	3·41 *A*	2·98 *A*	3·39 *A*	3·10 *A*
	±0·10	±0·20	±0·12	±0·24
	(3)	(4)	(7)	(4)
	5·31	*8·29*	*5·44*	*7·18*
	±0·39	*±0·62*	*±0·90*	*±0·47*
	(4)	(4)	(7)	(4)
$\overset{+}{N}MeEt_2$	4·50	3·37	3·36	3·60
	±0·11	±0·04	±0·04	±0·10
	(6)	(4)	(4)	(4)
Methyl piperidinium	4·29 *C*	3·43 *C*	3·48 *C*	3·84 *C*
	±0·07	±0·08	±0·05	±0·12
	(4)	(6)	(4)	(6)
Ethyl pyrrolidinium	4·54	3·54	3·43	3·38
	±0·08	±0·02	±0·09	±0·10
	(4)	(4)	(4)	(4)
$\overset{+}{N}Et_3$	4·25	3·62	3·45	3·56
	±0·09	±0·03	±0·03	±0·06
	(6)	(4)	(4)	(6)
Ethyl piperidinium	4·17	3·57	3·42	3·48
	±0·08	±0·08	±0·09	±0·02
	(8)	(4)	(6)	(4)

Values in italics are the equipotent molar ratios relative to pentyltrimethylammonium and results for partial agonists are only approximate (see text): all other values are estimates of log affinity constant. Mean values are shown with the standard error and number of estimates. The letter *A* indicates a partial agonist whose affinity constant was measured by the reciprocal plot method (Barlow & others, 1967) and *C* indicates that the compound was only a very weak partial agonist and the affinity constant was measured as if it were an antagonist. The onium groups are arranged in increasing order of size, as with the results for the guinea-pig ileum.

4. The hydroxyl group again reduces affinity but, as on the frog rectus, the position has little effect. Possibly with the smaller onium groups the hydroxyl group reduces affinity less in the 3 position than in the 4- or 5-positions. The affinities for the enzyme are of the same order as for the receptors in the frog rectus but the structure-activity pattern is again different. All the compounds depressed V_{max} to some extent and so are reversibly non-competitive rather than strictly competitive. The depression appeared to depend on the dose-ratio, rather than on chemical structure. When the trimethylammonium

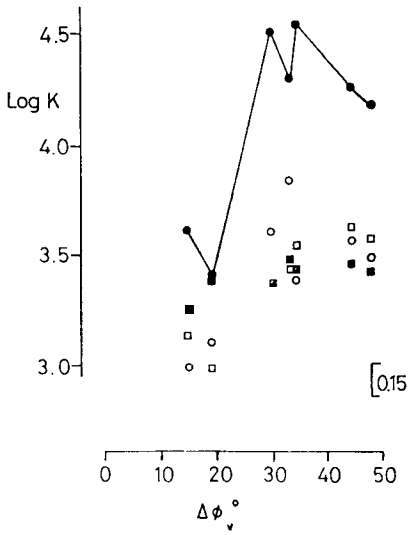


FIG. 3. The effect of hydroxyl groups on affinity for acetylcholine receptors in the frog rectus (*Rana temporaria*) at room temperature (18–23°). Values of log K (ordinate) are plotted against an estimate of the size of the onium group ($\Delta\phi_v^\circ$; $cm^3\ mol^{-1}$, as in Fig. 1) (abscissa) Differences between mean values less than 0·15 log units (indicated) are not considered to be significant. The affinities of the 3-hydroxy (○), 4-hydroxy (■) and 5-hydroxy (□) compounds are all less than those of the n-pentyl compounds (●). The position of the hydroxyl group makes little difference and the points have not been joined up, to avoid confusion.

and triethylammonium compounds were tested in concentrations which produced roughly the same dose-ratio they all produced similar reductions in V_{max}.

The results of the chromatography experiments are shown in Table 5. If the effects on Rm are plotted against the mass of onium group estimated from the increment in molecular weight, it is clear that the trialkylammonium, pyrrolidinium and piperidinium compounds fall on separate lines (Fig. 5A). If they are plotted against an estimate of the changes in apparent molal volume (based on average values for methyl- and ethyl-onium salts obtained by Barlow, Lowe & others, 1971) the results fit more closely to a single line (Fig. 5B), indicating that partitioning is related to size in solution, rather than to mass.

The apparent molal volumes of the trimethylammonium and triethylammonium compounds and molal volumes of the corresponding pentyldiethylamines are shown in Table 6. In the liquid tertiary bases the hydroxyl group has a size reasonably commensurate with its mass but is smallest when it is at the end of the molecule and increases as it is moved nearer to the middle. In solution however, the hydroxyl group has a very small size, as was

Table 4. *Affinity for electric eel acetylcholinesterase, 25°.*

	n-Pentyl	5-Hydroxy-n-pentyl	4-Hydroxy-n-pentyl	3-Hydroxy-n-pentyl
$\overset{+}{N}Me_3$	3·383 ±0·053 (4)	2·576 ±0·054 (4)	2·576 ±0·063 (4)	2·767 ±0·033 (4)
$\overset{+}{N}Me_2Et$	3·507 ±0·014 (5)	2·829 ±0·033 (4)	2·825 ±0·032 (5)	2·998 ±0·027 (4)
Methyl pyrrolidinium	3·695 +0·030 (4)	3·066 ±0·057 (4)	3·181 ±0·029 (4)	2·965 ±0·043 (4)
$\overset{+}{N}MeEt_2$	3·662 ±0·032 (4)	3·069 ±0·043 (5)	3·233 ±0·039 (4)	3·161 ±0·036 (4)
Methyl piperidinium	3·551 ±0·018 (4)	3·292 ±0·103 (5)	3·197 ±0·033 (5)	3·185 ±0·029 (5)
Ethyl pyrrolidinium	3·915 ±0·020 (4)	3·517 ± 0·036 (6)	3·509 ±0·026 (4)	3·411 ±0·039 (4)
$\overset{+}{N}Et_3$	3·759 ±0·058 (4)	3·283 ±0·043 (4)	3·276 ±0·043 (4)	3·154 ±0·067 (4)
Ethyl piperidinium	4·074 ±0·025 (5)	3·577 ±0·031 (4)	3·552 ±0·023 (4)	3·574 ±0·022 (4)

Values are mean estimates of log affinity constant ($1/K_i$) for the enzyme with acetylthiocholine as substrate, together with the standard error and number of estimates.

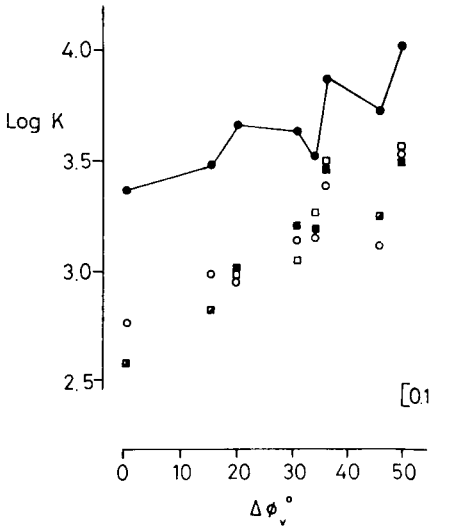


FIG. 4. The effect of hydroxyl groups on affinity for electric eel acetylcholinesterase at 25°C. Values of logK (ordinate) are plotted against an estimate of the size of the onium group ($\Delta\phi_v^\circ$; $cm^3\ mol^{-1}$, as in Fig. 1) (abscissa). Differences between mean values less than 0·1 log units (indicated) are not considered to be significant. The affinities of the 3-hydroxy (○), 4-hydroxy (■), and 5-hydroxy (□) compounds are all less than those of the n-pentyl componds (●). The position of the hydroxyl group makes little difference when the onium groups are large and the points have not been joined up, to avoid confusion.

found in other compounds by Abramson, Barlow & others (1974); with the trimethylammonium compounds the group is largest when at the end of the molecule but with the triethylammonium compounds it appears to be smallest at the end.

DISCUSSION

In all three tests the hydroxyl group has an adverse effect on affinity. This suggests that the binding of the n-pentyl chain is largely hydrophobic and therefore reduced by the introduction of a hydrophilic group. It appears that there is a larger hydrophobic region accessible in the muscarine-sensitive receptors than in the others, because the affinities of the n-pentyl compounds are higher and the position of the hydroxyl group is important. It lowers affinity most in the 5-position and least in the 3-position.

In these n-pentyl compounds the hydroxyl group usually also reduces efficacy, though the reduction is marginal among 5-hydroxy compounds acting on muscarine-sensitive receptors and among 4-hydroxy compounds acting on the receptors in the frog rectus.

It is evident that even though these compounds can be drawn to resemble (+)-muscarine they do not interact with the receptors in the same way. This may be because in spite of the apparent similarity shown in Fig. 1, the compounds are considerably different in shape. Possibly too much energy is needed for them to assume the required conformation but it is also possible that the hydrocarbon chain causes them to become attached to a more hydrophobic part of the receptor than that occupied by (+)-muscarine, which contains the more hydrophilic ether group. It is not possible to check this from the pharmacological experiments, because with a tissue containing spare receptors the results will not be distinguishable from competition in the range of concentrations which it is possible to test. The idea is however supported by the finding that although the compounds closely resemble acetylcholine, they do not bind to exactly the same site in acetylcholinesterase, because they depress V_{max}.

To assess the contribution of the hydroxyl group to the binding of muscarine it will therefore be necessary to work with compounds more closely related to it and containing the tetrahydrofuran ring.

Table 5. ΔRm *relative to n-pentyltrimethylammonium iodide: mean values are shown with the standard error and number of estimates.*

	n-Pentyl	5-Hydroxy-n-pentyl	4-Hydroxy-n-pentyl	3-Hydroxy-n-pentyl
$\overset{+}{N}Me_3$	0	0·46 ±0·06 (8)	0·32 ±0·02 (8)	0·23 ±0·01 (8)
$\overset{+}{N}Me_2Et$	−0·05 ±0·01 (8)	0·27 ±0·02 (8)	0·24 ±0·01 (8)	0·14 ±0·01 (8)
Methyl pyrrolidinium	−0·07 ±0·01 (8)	0·31 ±0·03 (8)	0·25 ±0·02 (8)	0·11 ±0·03 (8)
$\overset{+}{N}MEt_2$	−0·11 ±0·01 (10)	0·23 ±0·02 (8)	0·18 ±0·01 (8)	0·10 ±0·01 (8)
Methyl piperidinium	−0·10 ±0·01 (14)	0·24 ±0·03 (12)	0·19 ±0·02 (12)	0·07 ±0·02 (12)
Ethyl pyrrolidinium	−0·14 ±0·01 (8)	0·20 ±0·02 (8)	0·17 ±0·01 (8)	0·06 ±0·02 (8)
$\overset{+}{N}Et_3$	−0·19 ±0·03 (8)	0·14 ±0·01 (8)	0·10 ±0·02 (8)	0·09 ±0·01 (8)
Ethyl piperidinium	−0·16 ±0·02 (8)	0·19 ±0·03 (8)	0·16 ±0·02 (8)	0·01 ±0·02 (8)

Even then it may be objected that the change in structure which produces a partial agonist or antagonist, whose affinity can be measured, inevitably leads to binding at a different region in the receptor. This will clearly be true if the receptor is allosteric. It is therefore necessary to be cautious in attempting to estimate the contribution which a group makes to the binding of a drug to a receptor by comparing the properties of compounds with and without the group.

The measurements of Rm and ϕ_v° were made to see if there was any evidence for interactions between the hydroxyl group, onium nitrogen and water. Tait & Franks (1971) have drawn attention to the occurrence in many biologically interesting molecules of groups which can interact with water, such as hydroxyl or carbonyl, separated by about 4·8 Å, the distance between next-nearest neighbours in the ice I lattice. From measurements with Dreiding models this is about the distance between the hydroxyl group and onium nitrogen in the extended conformation of the 4-hydroxy-n-pentyl compounds. The Rm values, however, are intermediate between those of the 3-hydroxy and 5-hydroxy compounds. The interaction between the hydroxyl group and water can be seen by comparing the size in the liquid

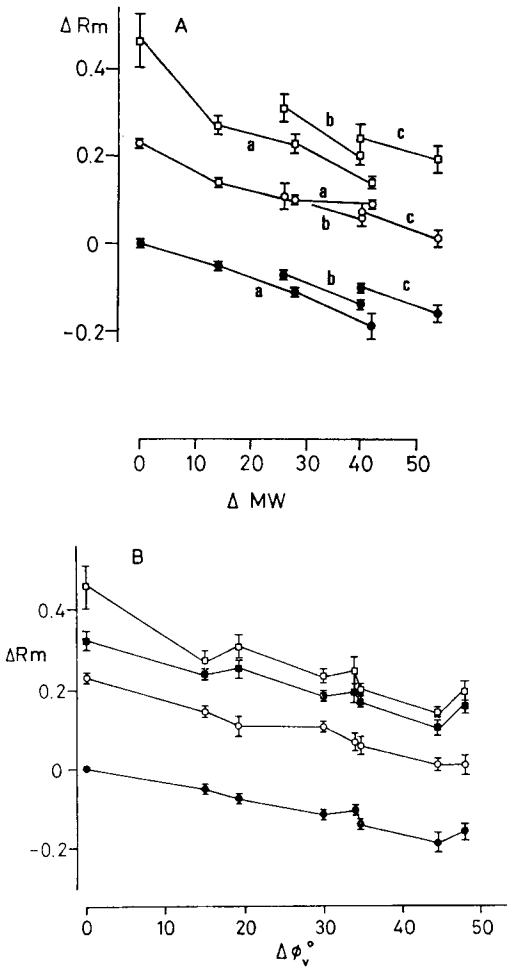


FIG. 5. The effect of hydroxyl groups on partitioning (paper chromatography with the solvent system butanol–ethanol–water (5:5:2)), expressed as ΔRm (ordinate) with reference to n-pentyltrimethylammonium iodide and plotted A against the increment in molecular weight (ΔMW) and B against an estimate of the size of the onium group ($\Delta\phi_v^\circ$, $cm^3\ mol^{-1}$, as in Fig. 1). The n-pentyl compounds (●) have greater lipophilicity so hydrophilicity is indicated by positive values of ΔRm. This increases in a regular way as the hydroxyl group is moved from the 3- (○) to the 4- (■) and 5- (□) positions. Bars indicate the standard errors. Note that in the method of plotting used in A results for the pyrrolidinium (b) and piperidinium (c) compounds fall on separate lines from the alkyl compounds (a) whereas in B all the results lie roughly on a single line.

(ΔV_m) with the remarkably small apparent size in water ($\Delta\phi_v^\circ$) but the results also indicate that the apparent size of the hydroxyl group depends on the size of the onium group. A hydrophilic hydroxyl group placed close to a small onium atom produces less disturbance than when it is at the far end of the

Table 6. *Apparent molal volumes, ϕ_v°, of n-pentyl quaternary ammonium iodides and molal volumes (V_m) of analogous n-pentyldiethylamines.* Mean values of ϕ_v° are shown with the standard error and number of concentrations tested; molal volumes (V_m) were estimated as described by Barlow & Tubby (1974). The column Δ indicates the increment for the hydroxyl group. All values are in $cm^3\ mol^{-1}$. Temperature, 25°.

n-Pent$\overset{+}{N}Me_3$	Δ	n-Pent$\overset{+}{N}Et_2$	Δ	n-Pent$\overset{+}{N}Et_2$	Δ
H 189·6 ±0·07(8)		233·1 ±0·04 (10)		170·1	
3-HO 189·6 ±0·11(14)	0·0	235·0 ± 0·10 (10)	1·8	187·0	16·9
4-HO 190·9 ±0·05 (7)	1·3	235·3 ± 0·18 (11)	2·2	184·4	14·3
5-HO 191·0 ±0·02 (10)	1·4	234·2 ±0·20 (10)	1·1	181·1	11·0

molecule. With a large onium group, however, the whole ion becomes extensively hydrophobic so a hydrophilic group may produce a bigger disturbance in the middle and therefore appear bigger than at one end. These interactions between hydroxyl and onium groups, however, need not depend on a critical distance between them such that they can fit into the structure of water. Somewhat similar effects may be seen with the melting points (Table 1). For smaller onium groups the 3-hydroxy compounds have higher m.p.s than 4- or 5-hydroxy compounds but with larger onium groups this difference disappears.

The results illustrate the need to consider the importance of water in the binding of antagonists to receptors, the extent to which it may be involved in ability to activate receptors, and the extent to which water structure around a drug molecule may be altered by the introduction of substituents.

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