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RELATIONSHIPS BETWEEN CHEMICAL STRUCTURE AND AFFINITY FOR POSTGANGLIONIC ACETYLCHOLINE RECEPTORS OF THE GUINEA-PIG ILEUM

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1 Some phenylacetyl, diphenylacetyl, benziloyl and (±)-cyclohexylphenylglycolloyl esters have been made with 2- and 3-hydroxymethylpyrrolidines, 3-hydroxymethyl-*N*-methylpiperidine, piperidin-3-ols, piperidin-4-ols, 2,2,6,6-tetramethyl-*N*-methylpiperidin-4-ol, tropine, pseudotropine and quinuclidin-3-ol, and the affinity of these compounds and of their metho- and etho- derivatives has been measured for postganglionic acetylcholine receptors of the guinea-pig isolated ileum.

2 Some of the compounds were very active indeed; the benziloyl esters of *N*-methylpiperidin-4-ol methiodide, tropine methiodide, and quinculidin-3-ol, and the (±)-cyclohexylphenylglycolloyl esters of *N*-methylpiperidin-4-ol and its methiodide had affinity constants greater than 10^{10}.

3 The effects of inserting an additional methylene group onto the nitrogen were extremely variable, ranging from a decrease in log K of 1.64 units to an increase of 0.97 units. The effects of replacing hydrogen by phenyl in the acid portion ranged from an increase of 1.04 units to an increase of 3.06 units and of replacing hydrogen by hydroxyl from a decrease of 0.09 units to an increase of 1.94 units.

4 The extent of the variation in the effects of a particular change in structure on affinity does not appear to be any different in these relatively rigid compounds from that observed with the same changes in open-chain aminoalcohols.

5 Reasons for the variable effects of groups on affinity are discussed. If differences in effects on preferred conformations of these particular compounds in solution are of secondary importance, the effect of a group on affinity will be the net result of what it could contribute to binding, offset by the disturbance it causes to existing binding. The maximum effect observed in a large number of comparisons may indicate the contribution in the absence of disturbance and for groups containing only carbon and hydrogen it appears to be related to size, assessed from the increments in apparent molal volume at infinite dilution. The variation in the effects of these groups also appears to be related to size. Changes involving groups containing oxygen can produce bigger contributions to binding, and a bigger variation in contribution, than would be expected from their size.

6 It is difficult to predict the extent to which groups may fail to produce their maximum effects. Variation is greatest with groups which could produce the biggest changes and so are of the greatest interest.

7 The relevance of the results to the successful prediction of biological activity is discussed.

Introduction

Many compounds have been tested for their ability to block the actions of acetylcholine or carbachol on the guinea-pig isolated ileum. Estimates of affinity constants in conditions in which the antagonist has had time to come into equilibrium with the tissue, however, have mostly been restricted to relatively flexible molecules, containing structures such as

$\geq C-CO-O-CH_2CH_2-\overset{+}{N}\leq$, $\geq CCH_2OCH_2CH_2\overset{+}{N}\leq$, or $\geq CCH_2CH_2CH_2CH_2\overset{+}{N}\leq$

(Barlow, Scott & Stephenson, 1963; Abramson, Barlow, Mustafa & Stephenson, 1969; Brimblecombe, Green, Inch & Thompson, 1971; Barlow, Franks & Pearson, 1973). This study describes

measurements made with some esters of cyclic aminoalcohols (pyrrolidinols, piperidinols, tropine, pseudotropine, and quinuclidin-3-ol) with phenylacetic, diphenylacetic, benzilic, and cyclohexylphenylglycollic acids.

In the work of Barlow *et al.* (1963) it was assumed that the binding of competitive antagonists to receptors was made up of additive contributions from the substituent groups. If this is true, values of log affinity constant should be directly related to these contributions and should be predictable from equations of the Hansch type (Hansch, Maloney, Fujita & Muir, 1962; review by Tute, 1971). Further work (Abramson *et al.*, 1969), however, showed that the effects of a particular change in structure were variable and it was suggested that the contribution which a substituent made to affinity depended on the extent to which it disturbed existing binding as well as on the contribution which it could make in an ideal situation. The extent to which affinity could be predicted by equations of the Hansch type would therefore depend on the extent to which the disturbance in binding caused by sustituents could be predicted, as well as on the predictability of the contributions which a substituent could make in an ideal situation.

The aim of the present work was to see, by studying a large number of closely related compounds, the extent to which the effects of substituents on affinity varied and the extent to which this variation might be predictable. This should indicate how far it might be possible to predict the affinity of new compounds.

A preliminary account of some of the results has been given at meetings of the British Pharmacological Society in Bristol (Abramson & Barlow, 1964, unpublished) and in Florence (Barlow & Mustafa, 1968).

Methods

Affinity for postganglionic acetylcholine receptors

The methods were those used in earlier work (Barlow *et al.*, 1963; Abramson *et al.*, 1969; Barlow *et al.*, 1973a). Estimates of log affinity constant were made with the guinea-pig isolated ileum suspended in aerated Tyrode solution (pH 7.7) at 37° C in the presence of 2.76×10^{-4} M hexamethonium with carbachol as the agonist, allowed to act for 30 s, given once every 90 seconds. Each antagonist was tested in at least two concentrations which usually differed by a factor of 10. The results are presented as mean values of log K, based on the number of preparations used.

Compounds

Carbachol was obtained from British Drug Houses Ltd and hexamethonium bromide from Koch-Light Ltd.

The benzilic esters of tropine and pseudotropine were kindly supplied by Dr E.W. Gill from material prepared by transesterification by Foster & Ing (1956). Samples of the benzilic ester of quinuclidin-3-ol were kindly supplied by Dr T.D. Inch and Dr J.M. Osbond and the latter also supplied the methobromide (Ro 2-3773) and the (+)- and (−)-methocamphorsulphonates (Ro 2-5044 and 5109, respectively). These had M_D +175, −187 and M_{300} +904 and −916, $c = 10^{-2}$ M in water, 20° C, and the corresponding value of α_D for Ro 2-5044 was +29.7° compared with +30.5°, recorded by Sternbach & Kaiser (1953). The benzilic ester of piperidin-3-ol was prepared from 3-chloropiperidine and benzilic acid (Biel, Friedman, Leiser & Sprengeler, 1952) but the ester with piperidin-4-ol could only be prepared by transesterification (Foster & Ing, 1956) and this method was also used for preparing the ester with cyclohexylphenylglycollic acid.

The esters of phenylacetic and diphenylacetic acids were prepared from the acid chlorides and the appropriate aminoalcohol.

The esters were purified by distillation under reduced pressure or by recrystallization and their infra-red spectra showed the presence of the required functional groups (phenyl, carbonyl, −O−, etc.).

Salts

Hydrochlorides of tertiary bases were prepared by treating them with hydrogen chloride dissolved in ethanol. Quaternary salts were obtained by adding excess methyl or ethyl iodide to the tertiary base dissolved in ethylmethylketone. In the preparation of etho-salts it was usually necessary to heat the mixture under reflux, sometimes for as long as 3 hours. Most of the salts could be recrystallized from ethylmethylketone but with some it was necessary to add ethanol and with others ethylacetate. With the derivatives of benziloyltropine and benziloylpseudotropine it was necessary to use methanol.

Analyses and melting points are shown in Table 1.

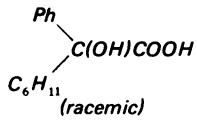
Results

In Table 2 the mean values of the estimates of log K (±s.e. mean) for the compounds are shown with the number of results. As in previous work

Table 1 Analyses and melting-points

Aminoalcohol	*Acid*		
	$PhCH_2COOH$	*$Ph_2CHCOOH$*	*$Ph_2C(OH)COOH$*
N-methyl-2-pyrrolidylmethanol			
Methiodide		178-9°C	
C		55.9 (56.0)	
H		5.77 (5.82)	
I		28.0 (28.2)	
Ethiodide		153-4°C	
C		56.5 (56.8)	
H		5.66 (6.06)	
I		27.1 (27.3)	
N-methyl-3-pyrrolidylmethanol			
Methobromide		147-8°C	
C		62.5 (62.4)	
H		6.65 (6.48)	
Br		20.2 (19.8)	
Ethobromide		90-92°C	
C		62.9 (63.1)	
H		6.52 (6.74)	
Br		19.4 (19.1)	
N-methyl-3-piperidylmethanol			
Methiodide		215-6°C	
C		56.6 (56.8)	
H		6.09 (6.06)	
I		27.6 (27.3)	
N-methyl-2 : 2 : 6 : 6-tetramethylpiperidin-4-ol			
Base		89-90°C	
C		79.4 (78.9)	
H		9.21 (8.49)	
N		3.91 (3.84)	
Methiodide*		53-57°C	
C		60.0 (59.2)	
H		7.73 (6.75)	
N-methylpiperidin-3-ol			
Hydrochloride	130.8-131.8°C	191.0-191.8°C	216.0-216.2°C*d*
Cl	13.08 (13.15)	10.33 (10.51)	9.83 (9.81)
Methiodide	136.5-138.2°C	185-6°C	218.3-218.5°C*d*
C		55.9 (56.0)	
H		5.77 (5.82)	
I	33.88 (33.84)	28.4 (28.2)	27.36 (27.15)
Ethiodide		68-70°C*d*	208.4-209.4°C*d*
C		56.7 (56.8)	
H		6.33 (6.06)	
I		27.0 (27.3)	26.43 (26.36)
N-ethylpiperidin-3-ol			
Hydrochloride			181.8-182.8°C*d*
Cl			9.376 (9.445)
Methobromide			171.0-171.6°C*d*
Br			18.38 (18.40)
Ethiodide			188.6-189.2°C*d*
I			25.63 (25.62)
N-methylpiperidin-4-ol			
Hydrochloride	177.3-178.2°C	135.5-137.0°C	
Cl	13.20 (13.15)	10.53 (10.51)	
Base			162.6-163.3°C
C			74.0 (73.9)
H			6.98 (7.14)
Methiodide	137.5-139.0°C	213-214°C	201.4-202.4°C
I	33.98 (33.83)	28.5 (28.2)	27.07 (27.15)
C		55.9 (56.0)	
H		5.75 (5.82)	
Ethiodide		184-5°C	175.0-177.0°C
I		27.7 (27.3)	26.55 (26.36)
C		57.1 (56.8)	
H		6.02 (6.06)	

Table 1—*continued*

Aminoaclohol	*Acid*		
	$PhCH_2COOH$	*$Ph_2CHCOOH$*	*$Ph_2C(OH)COOH$*
N-ethylpiperidin-4-ol			
Base			89-93°C
C			74.1 (74.3)
H			7.52 (7.42)
Ethiodide		161-2°C	203.4-203.8°C
I		26.2 (26.5)	25.23 (25.62)
C		57.5 (57.6)	
H		6.38 (6.31)	
	 *(racemic)*		
N-methylpiperidin-4-ol			
Hydrochloride	236.5-237.2°C		
Cl	9.640 (9.80)		
Methiodide	165-170°C		
I	26.75 (26.80)		
Ethiodide	168-174°C		
I	26.01 (26.03)		
	$PhCH_2COOH$		
(±)-Quinuclidin-3-ol			
Hydrochloride	178.0-179.4°C		(Gift)
Cl	12.57 (12.60)		
Base		92-93°C** (95-6°C)	
Methiodide	163.0-168.0°C	187.4-188.4°C	(Gift)
I	32.79 (32.77)	27.21 (27.38)	
Ethiodide	92-100°C	188.0-189.0°C	
I	30.52 (31.63)	26.43 (26.59)	
Ethobromide			228-9°C** (229-230°C)
Tropine			
Hydrochloride	197.0-198.5°C*d*	213.5-214.2°C*d*	(Gift)
Cl	12.05 (12.00)	9.610 (9.548)	
Methiodide	238-244°C*d*	271-2°C	275-6°C*d*
I	31.71 (31.63)		25.5 (25.7)
C		58.0 (57.8)	55.8 (56.0)
H		6.27 (5.93)	5.64 (5.73)
Ethiodide	148-150°C	208-210°C	254-5°C*d*
I	30.80 (30.56)		25.1 (25.0)
C		58.5 (58.6)	57.1 (56.8)
H		5.76 (6.17)	5.98 (5.97)
Pseudotropine			
Hydrochloride	200.3-200.7°C	219.8-221.2°C*d*	(Gift)
Cl	11.90 (12.00)	9.802 (9.548)	
Methiodide	227.5-228.0°C*d*	264-5°C	257-8°C*d*
I	31.49 (31.63)		25.8 (25.7)
C		58.2 (57.8)	56.2 (55.9)
H		6.38 (5.93)	5.92 (5.73)
Ethiodide	138.3-138.8°C	232-3°C	253-4°C*d*
I	30.42 (30.56)		24.8 (25.0)
C		58.7 (58.6)	56.6 (56.8)
H		6.33 (6.17)	5.91 (5.97)

Halide analyses shown to two decimal places are gravimetric with samples of 50-250 mg; all other analyses are micro- by Dr J.W. Minnis, Department of Biochemistry or by Drs Weiler and Strauss, Oxford. Theoretical values are shown in parentheses. Melting-points shown to one tenth of a degree are with a Mettler FP1 instrument coupled to a potentiometric recorder and at a rate of heating of 0.2°C/minute. All others are with a Kofler hot-stage microscope. All melting-points are uncorrected: *d* indicates decomposition. The sterically hindered methiodide marked with an asterisk was only obtained in low yield when the base was heated with methyl iodide in a metal bomb at 120°C for 6 hours. The double asterisks indicate that literature values are given in parentheses (Sternbach & Kaiser, 1952; 1953).

Table 2 Affinity for postganglionic acetylcholine receptors of the guinea-pig ileum

(a) Esters of diphenylacetic acid with:

	Methiodide	*Ethiodide*
2-hydroxymethyl-*N*-methylpyrrolidine	6.995 ± 0.006 (8)	7.187 ± 0.012 (6)
3-hydroxymethyl-*N*-methylpyrrolidine	7.480 ± 0.005 (6)	7.660 ± 0.004 (6)
3-hydroxymethyl-*N*-methylpiperidine	7.423 ± 0.015 (4)	

(b) Esters of:

	$PhCH_2COOH$	$Ph_2CHCOOH$	$Ph_2C(OH)COOH$	Ph, $C(OH)COOH$, C_6H_{11} (racemic)
with:				
N-methylpiperidin-3-ol HCl	4.823 ± 0.034 (7)	6.765 ± 0.031 (12)	8.703 ± 0.023 (7)	
Methiodide	5.109 ± 0.039 (8)	7.094 ± 0.006 (6)	8.849 ± 0.051 (5)	
Ethiodide		7.226 ± 0.010 (4)	8.856 ± 0.055 (5)	
N-ethylpiperidin-3-ol HCl			8.221 ± 0.022 (6)	
Methobromide			8.746 ± 0.037 (7)	
Ethiodide			8.502 ± 0.078 (6)	
N-methylpiperidin-4-ol HCl	5.586 ± 0.023 (7)	8.361 ± 0.033 (8)	9.934 ± 0.021 (6)	10.6-11? (10.52)
Methiodide	6.188 ± 0.028 (11)	9.064 ± 0.016 (4)	10.251 ± 0.102 (9)	10-11? (10.39)
Ethiodide		9.080 ± 0.014 (7)	9.570 ± 0.037 (7)	9.450 ± 0.134 (5)
N-ethylpiperidin-4-ol ethiodide		8.902 ± 0.006 (7)	8.813 ± 0.015 (6)	
N-methyl-2,2,6,6-tetramethylpiperidin-4-ol (base)		7.215 ± 0.041 (5)		
Methiodide		7.237 ± 0.018 (6)		
Tropine HCl	6.254 ± 0.077 (6)	8.110 ± 0.042 (10)	9.486 ± 0.041 (5)	
Methiodide	6.959 ± 0.037 (8)	8.669 ± 0.020 (6)	10.373 ± 0.050 (10)	
Ethiodide	6.825 ± 0.023 (5)	7.864 ± 0.029 (6)	9.085 ± 0.012 (8)	
Pseudotropine HCl	5.798 ± 0.026 (7)	7.511 ± 0.034 (9)	8.788 ± 0.032 (6)	
Methiodide	6.358 ± 0.025 (6)	8.231 ± 0.052	9.761 ± 0.058 (6)	
Ethiodide	5.729 ± 0.016 (6)	6.873 ± 0.039 (6)	8.119 ± 0.045 (8)	
(±)-Quinuclidin-3-ol HCl	6.760 ± 0.042 (9)	9.286 ± 0.023 (7)	10-11?	
Methiodide	5.502 ± 0.059 (7)	7.858 ± 0.020 (6)	9.433 ± 0.044 (5)*	
Ethiodide	5.263 ± 0.032 (8)	8.325 ± 0.046 (6)	9.339 ± 0.043 (6)*	

Mean values of log K are shown with the standard error and the number of pieces of intestine (in parentheses). Hexamethonium (2.76×10^{-4} M) was present in all experiments.

An asterisk indicates that the compound tested was a bromide. The resolved methocamphorsulphonates had: (−), log K, 9.618 ± 0.120 (6); (+), log K, 8.782 ± 0.054 (11). The compounds for which values are marked with a query produced a progressive antagonism which never became steady. Brimblecombe *et al.* (1971) obtained the values shown in parentheses for the hydrochloride and methiodide of the ester of *N*-methylpiperidin-4-ol with (±)-phenylcyclohexylglycollic acid.

each piece of ileum was considered to give a single result, because the variance of estimates made with different pieces of ileum is bigger than that of estimates obtained with the same piece. Even so, the variance seems to be an underestimate of the real error. In a separate group of experiments the mean values of log K for the methiodide and ethiodide of (±)-3-(diphenylacetyl)quinuclidine were 7.967 ± 0.027 (7) and 8.325 ± 0.046 (6), respectively, compared with the results shown in Table 2 (7.858 and 8.361, respectively). This confirms that in this type of experiment with guinea-pig ileum differences between mean values which are less than 0.1 log units are unlikely to be real because there are unknown systematic errors which exceed the statistical errors (Abramson *et al.*, 1969).

Values for the tertiary bases will also be complicated by the extent to which these are ionized but at pH 7.6 this should be extensive. Perrin (1965) lists the following values of estimates of pK_a: *N*-methylpyrrolidine, 10.32 or 10.46 (25°C); *N*-methylpiperidine, 10.07 (30°C); 1,2,2,6,6-pentamethylpiperidine, 11.19 (30°C); tropine, 10.33 or 11.02 (25°C); pseudotropine, 9.86 or 10.26 (25°C). The values for the esters may be lower than these; the pK_a of atropine, for instance, is listed as 9.85 (18°C) or 10.20 (16.5°C) and will be reduced by perhaps as much as 0.3 units (Albert & Serjeant, 1962) by raising the temperature from 25° to 37°C, but nevertheless most of the bases are likely to be about nine-tenths ionized. Even if the pK_a were as low as 7.6, the estimate of log K for the receptors will only be 0.3 log units below the correct value for the ion.

The values for some of the compounds will also be complicated by the existence of optical and/or geometrical isomers. Piperidin-3-ol, like quinculidin-3-ol, contains an asymmetric carbon atom but the affinities of the resolved forms of the benzilic ester of *N*-methyl-quinuclidin-3-ol camphorsulphonates (Table 2) differ less than 10-fold and it seems unlikely that enantiomeric forms of esters of piperidin-3-ol differ even as much as this. There is an additional possibility of isomerism when different substituents are attached to the quaternary nitrogen atom and the epimeric forms of atropine ethiodide, for instance, have been found to differ greatly in affinity for these receptors (Barlow, Harrison, Ison & Pearson, 1973). The result obtained with the ethiodide of the benziloyl ester of *N*-methylpiperidin-4-ol (8.849), however, does not differ greatly from that obtained with the methobromide of the benziloyl ester of *N*-ethylpiperidin-4-ol (8.746), though the nuclear magnetic resonance spectra of these compounds indicate that the proportions of the epimers present are substantially different in the two samples.

With some of the very active compounds it was impossible to obtain satisfactory estimates of log K, because the effects of the antagonist appeared to increase with time and never became steady. The value is therefore indicated as a range. However, estimates for two of the compounds, the cyclohexylphenylglycolloyl ester of *N*-methylpiperidin-4-ol and its methiodide, were obtained by Brimblecombe *et al.* (1971) and these are close to the range shown in Table 2. Although the failure to obtain a steady antagonism could be ascribed to the gradual entry of the antagonist into the cell, this seems an inadequate explanation because the phenomenon was observed with the methiodide as well as with the tertiary base.

Apart from these experiments where the result was expressed as a range, it seems likely that the effects of a change in structure on affinity can be calculated with an error of not more than about 0.2 log units.

Discussion

Variable effects of changes in structure

In part A of Table 2 it can be seen that the diphenylacetyl esters of 2- and 3-hydroxymethylpyrrolidines and the 3-hydroxymethyl- and 3-hydroxy-piperidines all have much the same affinity for the receptors. The derivatives of 4-hydroxypiperidine, however, are about 100 times as active (log K is bigger by 2 units) and many have higher affinity than the corresponding derivatives of tropine. Although this suggests that the ethylene bridge present in the tropines does not contribute much to affinity, the ethylene bridge present in the quinuclidines clearly contributes greatly to affinity. In both instances it imparts rigidity to the moderately flexible piperidine ring. The low affinity of the diphenylacetyl ester of *N*-methyl-2,2,6,6-tetramethylpiperidin-4-ol, however, shows that the introduction of methyl groups in the region corresponding to the ethylene bridge of tropine has adverse effects on affinity and is of interest because of the very high mydriatic activity of the benzilic ester of *N*-methyl-2,2,6-trimethylpiperidin-4-ol methiodide (Ing, Dawes & Wajda, 1945).

By comparing the values of log K it is possible to calculate the effects on binding of increasing the size of the onium group and of replacing hydrogen by phenyl or hydrogen by hydroxyl at the opposite end of the molecule. These are shown in Table 3 and are very variable.

Table 3 Effects of changes in chemical structure on log affinity constant for postganglionic acetylcholine receptors of the guinea-pig ileum

(a) Introduction of $-CH_2-$ on the nitrogen atom

Base	*Acid*		
	$PhCH_2COOH$	*$Ph_2CHCOOH$*	*$Ph_2C(OH)COOH$*
Piperidin-3-ol			
$MeNH \rightarrow Me_2N$	0.286	0.329	0.146
$Me_2N \rightarrow MeEtN$*		0.132	0.007
$MeNH \rightarrow EtNH$			−0.482
$EtNH \rightarrow EtMeN$*			0.525
$EtMeN \rightarrow Et_2N$*			−0.244
Piperidin-4-ol			
$MeNH \rightarrow Me_2N$	0.602	0.703	0.317
$Me_2N \rightarrow MeEtN$*		0.016	−0.681
$MeEtN \rightarrow Et_2N$*		−0.178	−0.757
Tropine			
$MeNH \rightarrow Me_2N$	0.705	0.559	0.887
$Me_2N \rightarrow MeEtN$*	−0.134	−0.805	−1.288
Pseudotropine			
$MeNH \rightarrow Me_2N$	0.560	0.720	0.973
$Me_2N \rightarrow MeEtN$*	−0.629	−1.358	−1.642
Quinuclidin-3-ol			
$HN \rightarrow MeN$	−1.258	−1.428	
$MeN \rightarrow EtN$	−0.239	0.467	−0.094

Range: for base → $\geq$NMe, −1.428 to +0.973; for changes in quaternary compounds only, −1.642 to +0.467; Overall range: −1.642 to +0.973.

Replacement of:	(b) −H by −Ph	(c) −H by OH
N-methylpiperidin-3-ol		
Base	1.942	1.938
Methiodide	1.985	1.755
Ethiodide*		1.630
N-ethylpiperidin-3-ol		
Ethiodide		−0.089
N-methylpiperidin-4-ol		
Base	2.775	1.573
Methiodide	2.876	1.187
Ethiodide*		0.490
Tropine		
Base	1.856	1.376
Methiodide	1.710	1.704
Ethiodide	1.039	1.221
Pseudotropine		
Base	1.713	1.277
Methiodide	1.873	1.530
Ethiodide	1.144	1.246
Quinuclidine		
Base	2.526	
Methiodide	2.356	1.575
Ethiodide	3.062	1.014
Range:	1.039 to 3.062	−0.089 to 1.938

Values show the change in log K and can be converted to changes in the free energy of adsorption by multiplying by 1.417 (Kcal/mole) or by 5.923 (KJ/mole).

An asterisk indicates that the change involves a compound which may contain a mixture of epimers; for the derivatives of tropine and pseudotropine the ethyl group is predominantly, if not exclusively, equatorial and for the derivatives of piperidine it does not seem likely that the epimers differ much in affinity for the receptors (see text).

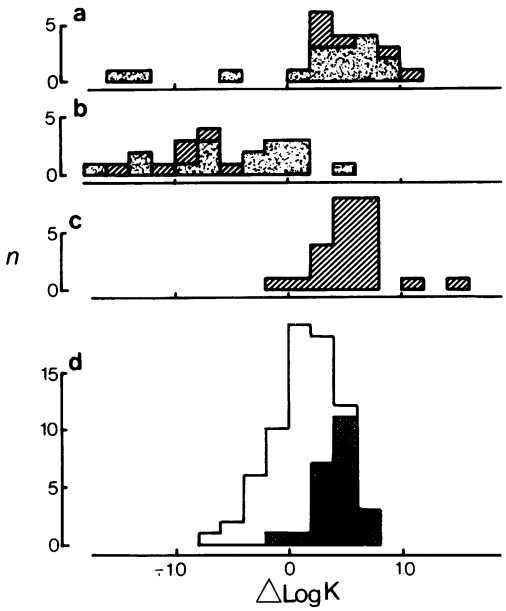


Fig. 1 Effects of altering the onium group on log affinity constant for postganglionic acetylcholine receptors of the guinea-pig ileum. The histograms indicate the number of comparisons (*n*) for which the change in structure produced a change in log K lying within the range shown.

(a) Methylation of cyclic tertiary bases.

(b) Replacement of methyl by ethyl in cyclic quaternary salts. Values for the present work (stippled) are compared with values for hyoscyamine, hyoscine and homatropine derivatives (Barlow *et al.*, 1973a; diagonal hatching).

(c) Methylation of open-chain tertiary bases (Barlow *et al.*, 1973a).

(d) Replacement of methyl by ethyl in open-chain quaternary compounds (combined results of Abramson *et al.*, 1969, and Barlow *et al.*, 1973a). Replacement of $-\overset{+}{N}Me_3$ by $-\overset{+}{N}Me_2Et$ is shown by cross hatching, the open area includes results for changing methyl to ethyl in pyrrolidinium and piperidinium compounds.

Note that the range of effects is greater in the cyclic compounds and that replacement of methyl by ethyl usually reduces affinity.

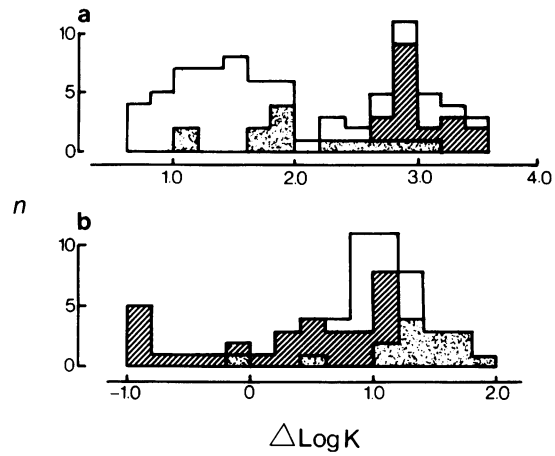


Fig. 2 Effects of changes in structure on log affinity constant for postganglionic acetylcholine receptors of the guinea-pig ileum. The histograms indicate the number of comparisons (*n*) for which the change in structure produced a change in log K lying within the range shown. Values for this work (stippled) are compared with values for open-chain compounds obtained by Abramson *et al.* (1969; open areas) and by Barlow *et al.* (1973a; diagonal hatching). (a) Replacing hydrogen by phenyl. (b) Replacing hydrogen by hydroxyl.

Note that the effects with the cyclic compounds are about as variable as with the open-chain compounds.

Because the cyclic structure of the compounds makes many of them relatively inflexible it might be expected that the variation in the effect might be greater than with the open-chain compounds, because some compounds might possibly be held in a conformation which has particularly high affinity for the receptors whereas others may be held in a particularly unsuitable conformation. The effects have therefore been compared with those of similar changes in the compounds studied by Abramson *et al.* (1969) and by Barlow *et al.* (1973a). The effects of methylating a tertiary base and of replacing methyl groups by ethyl appear to be more varied in the cyclic compounds and the latter change usually reduces affinity (Fig. 1), but the difference in the range is not large. The situation is further complicated because the increase in affinity caused by methylating a tertiary base may include an effect due to increased ionization and the effects of replacing methyl groups in the cyclic compounds may be complicated by the production of epimers (see above). The effects of replacing hydrogen by phenyl or hydrogen by hydroxyl appear to be about as variable in the open-chain compounds as in the cyclic ones (Figures 2a and b).

Reasons for the variable effects of groups on affinity

The great variation in the effects which a group has on affinity already considered (Figs 1 and 2) is very striking. It is even bigger (over 3 log units) for the replacement of hydrogen by cyclohexyl in the open-chain compounds (Figure 3a). Even the

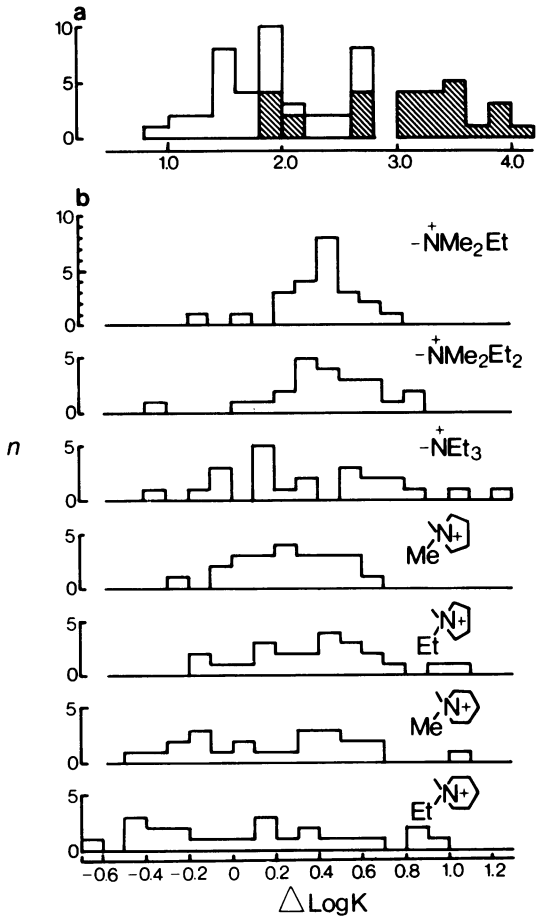


Fig. 3 Effects of changes in structure on log affinity constant of open-chain compounds. The histograms indicate the number of comparisons (*n*) for which the change in structure produced a change in log K lying within the range shown.

(a) Replacing hydrogen by cyclohexyl. Values calculated from the results of Abramson *et al.* (1969) are shown as open blocks and those of Barlow *et al.* (1973a) are shown as diagonal hatching.

(b) Replacing trimethylammonium by other onium groups. Values are calculated from the combined results of Abramson *et al.* (1969) and Barlow *et al.* (1973a). Note that there is a variation of more than 1 log unit in the effects of replacing only one methyl group by ethyl and that the variation is even bigger with large groups such as triethylammonium and ethylpiperidinium. (The range of the histograms in this section is 0.1 log units, compared with 0.2 log units in Figure 1).

effects of quite simple changes in the onium group of the open-chain compounds are variable (Fig. 3b) and bigger changes in the onium group appear to lead to more variable effects.

A possible explanation for the variation was given by Abramson *et al.* (1969) who concluded that the introduction of any substituent which could contribute to binding was likely to disturb existing binding to a variable, and often considerable extent. It is also possible that the substituent, in addition to effects on the adsorbed form, may alter affinity by altering the preferred conformation of the compounds in solution making it easier, or more difficult, for the molecule to be adsorbed.

With the open-chain compounds it is unlikely that the variation in the effects of the particular groups considered, introduced at the ends of the molecule, can be ascribed simply to effects on preferred conformation. If the variation in the effect of a substituent, X, were over a range of 2 log units it would be necessary to suppose that for the two extreme pairs of compounds, A_1H and A_1X, and A_nH and A_nX, the changes in conformation going from A_1H to A_nH, and from A_1X to A_nX lead to free energy increments which differ by 2.8 kcals/mole (11.8 kJ/mole). From n.m.r. spectroscopy, Partington, Feeney & Burgen (1972) have shown that the conformation of the O–C–C–$\overset{+}{N}$ bond in benziloylcholine is predominantly *gauche*, as it is in acetylcholine, and Pearson (1972) has found it to be the same in mandelylcholine and α-methyltropylcholine. The *n*-pentyl compounds are likely to be in an all-*trans* conformation, though their n.m.r. spectra are too complex for coupling constants to be elucidated, but it is not likely that the introduction of the substituents considered could lead to changes in conformation in one series which differed by as much as 2.8 kcals/mole from the change in the other series.

In some types of compound the effect of a substituent on preferred conformation could undoubtedly be large but if it is not important in the particular compounds under consideration, and if results have been obtained with enough pairs of compounds, the biggest effect produced by a substituent may be somewhere near its 'true' effect, that is its contribution to binding when its introduction does not disturb existing binding.

Maximum effects

The maximum effect on affinity of an additional methylene group attached to the onium nitrogen atom seems to be about 0.9 log units (Fig. 1) indicating that it can contribute about 1.3 kcal/mole (5.3 kJ/mole) to the free energy of adsorption. Higher effects have been observed for the methylation of some tertiary bases (0.97 for the methylation of benziloylpseudotropine and 1.4 and 1.1 for the methylation of (+)-α-methyl-

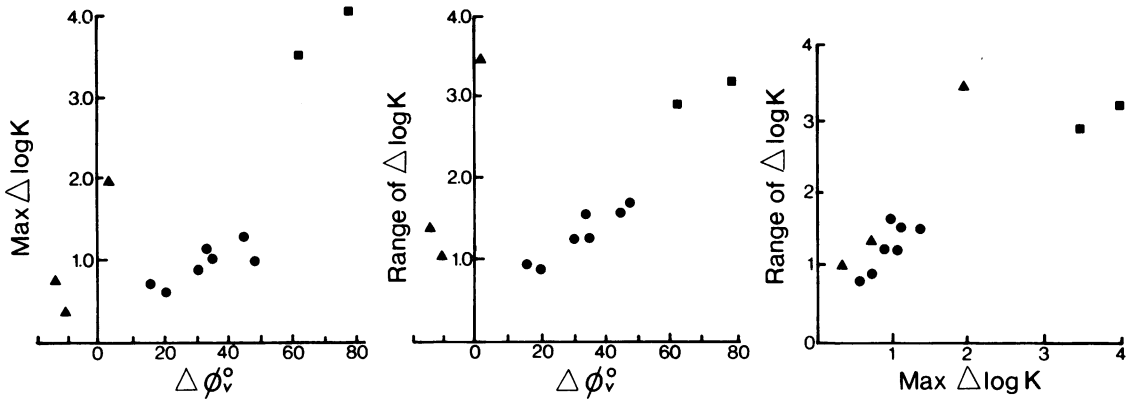


Fig. 4(a) The maximum effects of groups on affinity compared with their effects on size. (b) The variation in the effects of groups on affinity compared with their effects on size. (c) The variation in the effects of groups on affinity compared with their maximum effects.

Effects on size are assessed from the increment in apparent molal volume at infinite dilution ($\Delta\phi_V^o$); (•) indicates changes in the onium group; (■) indicates replacement of hydrogen by phenyl or cyclohexyl; (▲) indicates changes involving groups containing oxygen (Table 4). Note that for groups containing only carbon and hydrogen the maximum effect (a) and the range of effect (b) appear to be related to size and that groups which can make big contributions to binding are associated with big variations in their effects (c).

tropylethylpyrrolidine and piperidine, respectively) but are probably due to incomplete ionization of the base. At 25 ± 0.05° C, the pK_a of racemic α-methyltropylethylpyrrolidine, determined by electrometric titration of the hydrochloride (1.2×10^{-2} M) with sodium hydroxide (10^{-1} M), was estimated to be 8.5; at 37° C the pK_a will be about 8.2 and the compound may be only 75% ionized. The estimates for the analogous diethylamine and piperidine at 25° C were 8.7 and 8.6, respectively, and the value for atropine was 9.6 (at these concentrations all the bases stayed in solution during the titration).

The maximum effect of replacing hydrogen by phenyl so far observed was an increase in log K of 3.5 units (Fig. 2a), for the replacement of hydrogen by hydroxyl it was 1.9 units (Fig. 2b), and for the replacement of hydrogen by cyclohexyl it was 4.1 units (Fig. 3a).

For changes in structure involving groups which contain only carbon and hydrogen it might be expected that their contribution to binding would be related to their size. The maximum effects have therefore been compared with estimates of the effect of the group on size, obtained from measurements of apparent molal volumes at infinite dilution (Table 4, Figure 4a). The results appear to support this idea provided changes in the onium group are not too big. With the larger groups, such as triethylammonium, and with those that are less flexible, such as pyrrolidinium and piperidinium, the maximum effect so far observed falls short of what might be expected, suggesting that there is only limited access to this part of the receptor.

With the hydroxyl group, however, the maximum effect on binding is far bigger than would be expected from its remarkably small size in solution. Other changes involving groups containing oxygen atoms (Table 4) also bear no obvious relation to the change in size and indicate that the binding is of a different nature from that involved in groups containing only carbon and hydrogen.

For hydrocarbon groups it might also be expected that the size would determine the disturbance which could be caused to existing binding. The range of effects of groups on affinity has therefore been plotted against the increment in apparent molal volume at infinite dilution (Fig. 4b) and the results support the idea. The values for the hydroxyl group and for other changes involving oxygen atoms, however, show that small size is not necessarily associated with small variation in effects on affinity. Groups containing polar atoms may cause big disturbances to existing binding, as well as big contributions to binding, even though they are small in size.

Prediction of affinity

To predict the effect of a group on affinity it is necessary to know the contribution which the group could make, the disturbance it causes to

Table 4 Effects of changes in structure on log K. This is based on estimates of log K obtained by Abramson *et al.* (1969) and Barlow *et al.* (1973a) as well as those obtained in the present work

(a) The extreme values of the change in log K are shown together with the number of comparisons made (n) and the change in apparent molal volume at infinite dilution ($\Delta\phi_V^o$), calculated from results of Barlow, Lowe, Pearson, Rendall & Thompson (1971) or from results shown below.

	log K				
	max.	*min.*	*range*	n	$\Delta\phi_V^o$
Replacement of $-\overset{+}{N}Me_3$ by:					
$\overset{+}{N}Me_2Et$	0.722	−0.187	0.909	23	15.6
$\overset{+}{N}MeEt_2$	0.893	−0.339	1.232	23	30.0
$\overset{+}{N}Et_3$	1.252	−0.303	1.555	23	44.7
methylpyrrolidinium	0.614	−0.227	0.841	23	19.9
ethylpyrrolidinium	1.035	−0.191	1.226	23	34.6
methylpiperidinium	1.082	−0.477	1.559	23	33.6
ethylpiperidinium	0.992	−0.654	1.646	23	48.0
Replacement of −H by:					
Ph	3.507	0.624	2.883	77	63
C_6H_{11}	4.041	0.875	3.166	60	79
OH	1.938	−1.492	3.430	59	1
Replacement of $-CH_2CH_2-$ by:					
−CO−O−	0.700	−0.647	1.347	24	−13.8
$-CH_2-O-$	0.341	−0.664	1.005	32	−10.9

(b) Values of apparent molal volume at infinite dilution (ϕ_V^o) at 25° C for quaternary ammonium bromides measured by the method of Lowe, Macgilp & Pritchard (1973). A single asterisk indicates that measurements were made with the iodide and were converted to estimates for bromide by subtracting 11.5 cm^3/mole. Results taken from the work of Barlow *et al.* (1971) are indicated by † and of Barlow & Franks (1973) by ††

	ϕ_V^o *(Br−)*	$\Delta\phi_V^o$ *for H → Ph*
$PhCH_2\overset{+}{N}Me_3$ †	176.0	
$Me_4\overset{+}{N}$*†	114.0	61.5
$PhCH_2CH_2\overset{+}{N}Me_3$ ††	191.9	
$CH_3CH_2\overset{+}{N}Me_3$ *†	128.8	63.1
$PhOCH_2CH_2\overset{+}{N}Me_3$ ††	196.8	
$HOCH_2CH_2\overset{+}{N}Me_3$ *	130.2	66.6
$Ph(CH_2)_5\overset{+}{N}Me_3$ ††	240.1	
$CH_3(CH_2)_4\overset{+}{N}Me_3$ *†	177.7	62.4
$Ph_2CHCOOCH_2CH_2\overset{+}{N}Me_3$	291.0	
$CH_3COOCH_2CH_2\overset{+}{N}Me_3$ *†	163.9	63.6
		H → C_6H_{11}
$C_6H_{11}(CH_2)_5\overset{+}{N}Me_3$	256.8	
$CH_3(CH_2)_4\overset{+}{N}Me_3$ *†	177.7	79.1
		Ph → C_6H_{11}
$C_6H_{11}(CH_2)_5\overset{+}{N}Me_3$	256.8	
$Ph(CH_2)_5\overset{+}{N}Me_3$ ††	240.1	16.7
$PhC_6H_{11}CHCOOCH_2CH_2\overset{+}{N}Me_3$	308.2	
$Ph_2CHCOOCH_2CH_2\overset{+}{N}Me_3$	291.0	17.2
$PhC_6H_{11}C(OH)COOCH_2CH_2\overset{+}{N}Me_3$ *	307.1	
$PhC_6H_{11}CHCOOCH_2CH_2\overset{+}{N}Me_3$	291.8	15.3
		H → OH
$HOCH_2CH_2\overset{+}{N}Me_3$ *	130.2	
$CH_3CH_2\overset{+}{N}Me_3$ *†	128.8	1.4
$Ph_2C(OH)COOCH_2CH_2\overset{+}{N}Me_3$	291.8	
$Ph_2CHCOOCH_2CH_2\overset{+}{N}Me_3$	291.0	0.8
$PhC_6H_{11}C(OH)COOCH_2CH_2\overset{+}{N}Me_3$ *	307.1	
$PhC_6H_{11}CHCOOCH_2CH_2\overset{+}{N}Me_3$	308.2	−1.1

existing binding, and its effects on preferred conformation. The affinity constant of the new compound must be calculable if the necessary free energy increments are known but these are complex and involve adsorbed drug as well as drug in solution. It should be possible to set an upper limit to the affinity of a new compound, if the effects of the groups on adsorbed drug are large compared with their effects on drug in solution, by adding together the maximum contributions which they could make. However, this is likely to be a considerable overestimate, because it is improbable that all the groups can be introduced in such a way that they produce their maximum effects. For example, starting with the value of 3.7 for log K for *n*-pentyltrimethylammonium, the effect of changing $-CH_2CH_2-$ to $-CO-O-$ and replacing hydrogen by phenyl should set an upper limit to the affinity of phenylacetylcholine of 3.7 + 0.7 + 3.5 = 7.9, compared with the experimental value of 4.5. For cyclohexylphenylglycolloylcholine the value would be 3.7 + 0.7 + 3.5 + 4.0 + 1.9 = 13.8, compared with the experimental value of 9.6 for the more active (R) isomer. Even though some of the difference might be due to the substituents forcing the molecules into conformations unfavourable for adsorption (though they might also force them into favourable conformations), it is likely to be due largely to disturbances of binding at the receptor.

To predict the affinity of new compounds, then, the real problem is to predict the extent to which the effects of the various substituents will fall short of the maximum. Because this is so very variable it is only reasonable to attempt to predict affinity if results have already been obtained with very similar compounds. Predictions of this kind, however, are of very limited value. To be of any real use, predictions should indicate what substituents would increase affinity markedly but it is precisely these groups which produce the most variable effects (Figure 4c).

In view of the obvious difficulties in the prediction of the affinities of antagonists for postganglionic acetylcholine receptors of the guinea-pig ileum, it is remarkable that attempts to predict biological activity have been claimed to be successful, for example by the methods of Hansch (Hansch *et al.*, 1962; review by Tute, 1971). These are all the more striking because some of them involve agonist activity which will depend on two unrelated biological properties (affinity and efficacy). It seems reasonable to expect that the maximum contributions of groups to affinity may be correlated with chemical parameters so the successful prediction of activity might be possible when the disturbing effects of substituents on affinity are either absent or constant. In some tests the biological activity does not involve actions at receptors and in others the conditions of the test are such that activity is limited by physicochemical properties (such as rates of access to sites of action). In other tests the compounds examined have been very similar in structure, corresponding to a situation where the disturbing effects of substituents may be roughly constant. With a relatively simply constructed receptor the disturbing effects of substituents might be low but in this situation affinity is likely to be low also. From the results obtained in this work the accurate prediction of the activity of potent compounds acting at receptors seems unlikely. In spite of claims for some excellent over-all correlations, the situation is not yet such that manufacturers can afford to risk omitting to test compounds because they are predicted to be inactive.

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