Br. J. Pharmac. (1976), **58**, 613–620

A COMPARISON OF AFFINITY CONSTANTS FOR MUSCARINE-SENSITIVE ACETYLCHOLINE RECEPTORS IN GUINEA-PIG ATRIAL PACEMAKER CELLS AT 29°C AND IN ILEUM AT 29°C AND 37°C

R.B. BARLOW, K.J. BERRY, P.A.M. GLENTON, N.M. NIKOLAOU & K.S. SOH

Departments of Pharmacology, Universities of Edinburgh and Bristol

1 The affinity of 17 compounds for muscarine-sensitive acetylcholine receptors in atrial pacemaker cells and ileum of the guinea-pig has been measured at 29°C in Ringer-Locke solution. Measurements were also made at 37°C with 7 of them.

2 Some of the compounds had much higher affinity for the receptors in the ileum than for those in the atria. For the most selective compound, 4-diphenylacetoxy-*N*-methylpiperidine methiodide, the difference was approximately 20-fold. The receptors in the atria are therefore different in structure from those in the ileum.

3 The effects of temperature on affinity are not the same for all the compounds tested, indicating different enthalpies and entropies of adsorption and accounting for some of the difficulty experienced in predicting the affinity of new compounds.

Introduction

From measurements of the affinity constants of a number of compounds for muscarine-sensitive acetylcholine receptors in the ileum, bronchial muscle and iris of the guinea-pig, Barlow, Franks & Pearson (1972) concluded that the receptors were indistinguishable. Estimates of the affinity constant of a compound were not significantly different for the three types of tissue and, when allowance is made for the effects of different recording conditions on the estimates of affinity constants (Butt, 1972), any differences in receptor structure, which may exist but cannot be detected, must be small. Although Arunlakshana & Schild (1959) obtained results with mepyramine and diphenhydramine which suggested that histamine receptors in ileum and respiratory tract were similarly indistinguishable, the findings are surprising in view of the different time-course of the responses to the agonist; bronchial muscle contracts very much more slowly than the ileum. They are also surprising in view of the marked differences which are found in nicotine-sensitive receptors both in the specificity of blocking agents and in the stereospecificity of nicotine itself (Barlow & Hamilton, 1965).

The present work describes an attempt to see whether the muscarine-sensitive acetylcholine receptors of the guinea-pig atrial pacemaker cells are also indistinguishable from those of the ileum. There were three reasons for choosing this preparation. First, because the agonist causes hyperpolarization in this tissue, compared with depolarization in the other preparations. Second, because of the discovery that there are differences among types of histamine receptor and that the H_2-receptors occur in atria (Black, Duncan, Durant, Ganellin & Parsons, 1972). Third, because of the possible practical importance of drugs which would selectively block muscarine-sensitive acetylcholine receptors in the ileum more than those in the heart.

For testing the actions of drugs on isolated atria it has been customary to work at 29°C and to use Ringer-Locke solution (Burn, 1952). We attempted to work at 37°C but found the responses to carbachol became erratic after an hour or two, so we reverted to using 29°C. We therefore also made measurements on the ileum at this temperature and with the same physiological salt solution. The values on the ileum were different from those previously obtained at 37°C in Tyrode solution and the effect of temperature appeared to be different for different compounds. This suggested that it might be possible to obtain some idea of the enthalpy of adsorption (ΔH) of the antagonist because

$$\frac{\Delta \ln K}{\Delta \frac{1}{T}} = \frac{-\Delta H}{R}$$

and hence to assess entropies of adsorption, ΔS, because

$$\ln K = \frac{-\Delta G}{RT}$$

and

$$T\Delta S = \Delta H - \Delta G$$

We therefore made measurements with some of the compounds at 37°C in Ringer-Locke solution.

The work falls into two parts. In the first, measurements were made with 10 compounds on the ileum and atria at 29°C in Ringer-Locke solution and, in the second part, measurements were also made simultaneously with the ileum at 37°C. In the first part comparisons were made of values of log K, as in previous work (Barlow *et al.*, 1972), but it is possible that part of the error attached to them may be associated with the choice of the concentrations of antagonist tested, if the compounds are not behaving strictly competitively. Some antagonists are known which produce results consistent with competition at low concentrations but which are not competitive at higher concentrations (Abramson, Barlow, Mustafa & Stephenson, 1969; Lüllmann, Ohnesorge, Schauwecker & Wasserman, 1969; Mitchelson, 1975). Although the compounds were tested in concentrations in which they seemed to be acting competitively, because the results obtained with higher concentrations were reasonably in agreement with those obtained with lower concentrations, it seemed desirable to perform a second group of experiments in which any possible bias was eliminated by testing the compounds at exactly the same concentration on the two types of preparation and comparing the dose-ratios.

Methods

The guinea-pig ileum and atria were suspended in Ringer-Locke solution and aerated with O_2 (Edinburgh Staff, 1972). In the first group of experiments hexamethonium was present in a concentration of 3×10^{-4}M; in the second group the concentration was 2.76×10^{-4}M, except in one set in which the hexamethonium was omitted. The contractions of the ileum were recorded isotonically. Those of the atria were recorded in the first group of experiments with a strain-gauge (load about 0.2 g) and a Devices pen-recorder and in the second group with a very light spring-loaded transducer, made by Mr R.O. Morris, connected to a Vitatron potentiometric recorder.

Drug and wash solutions were applied automatically. Carbachol was the agonist except in a few experiments with acetyl-β-methylcholine. For the atria the agonist was given once every 16 min and allowed to act for 3 min in the first group of experiments and for 4 min in the second group, and then washed out. The preparation was washed again 5 min later. The time required for 50 beats was measured with a stopwatch at frequent intervals and the effect of the agonist was expressed as the percentage increase in time, calculated from the value at the end of the application of the agonist and the value just before its application. The size of the contractions was nearly always reduced much more than the rate and the dose-effect curves were usually steep; quite a small increase in concentration was sufficient to increase the effect from slight slowing to apparent arrest of the atria, with any contractions being too small to be counted. After a control period in which responses were obtained with low and high doses of agonist (often 2 and 4×10^{-7}M carbachol) the preparation was exposed to the concentration of antagonist and responses obtained with increased concentrations of agonist. Because of the long time interval necessary between doses of agonist, the calculation of the dose-ratio was usually based on two pairs of control responses (to low and high concentrations of agonist) and two pairs after the action of the antagonist.

The experiments on the ileum were performed exactly as described previously, with the agonist allowed to act for 30 s and given once every 90 s (Barlow, Franks & Pearson, 1973). The concentration of agonist needed to obtain suitable control responses (often 1 and 2×10^{-7}M) was similar to that used in the experiments with the atria.

With some compounds the responses of the ileum to the agonist in the presence of the antagonist became regular within a few minutes but with the more potent compounds tested in very dilute solution it was often necessary to wait 20 min and even up to 40 min in some instances. A similar time interval was allowed in the experiments on the atria. Usually it seemed to be sufficient to ignore the first response obtained in the presence of the antagonist (after about 12 min exposure) and to use subsequent responses (after about 28 min exposure) which seemed to be regular, but with the most potent compounds the interval was lengthened to 40 minutes.

Each antagonist was tested in the same concentration on the ileum as on the atria and usually the same dose-ratio was tried initially. With nearly all the compounds it was clear that the dose-ratios were different for the two preparations and the concentrations of agonist were altered in order to measure the dose-ratio as accurately as possible. The initial dose-ratio tested on the ileum at 37°C in the second group of experiments was calculated from the published values of log K (in Tyrode solution) and subsequently adjusted when necessary. In the first group of experiments each antagonist was tested in at least two concentrations and the dose-ratios were used

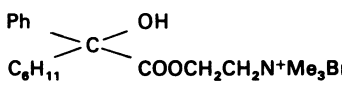
to calculate affinity constants. The result was expressed as the mean estimate of log K ± standard error based on the number of preparations used, as in previous work (Abramson *et al.*, 1969). In the second group of experiments only one concentration of each antagonist was tested. Mean estimates of the dose-ratio were calculated and the effect of changing temperature or changing tissue expressed as the ratio of the values of dose-ratio −1. The logarithm of this ratio should be the same as the difference in values of log K.

The temperatures at which the experiments were carried out, 29°C and 37°C, are subject to errors in measurement and control which may be as big as ±0.5°C.

Compounds

Carbachol, hexamethonium bromide and acetyl-β-methylcholine chloride were obtained from Koch-Light Ltd; the latter was recrystallized before use. Carbachol and hexamethonium bromide were also obtained from Sigma Chemical Co. Ltd. Atropine sulphate and hyoscine hydrobromide were obtained from BDH Ltd. The other antagonists were samples for which analyses have already been published

Table 1 Values of the mean estimate of log K for guinea-pig atria and ileum are shown with the standard error and number of estimates. The agonist was carbachol, except where indicated and the Ringer-Locke solution contained hexamethonium (3×10^{-4}M). Results for the ileum at 37°C are from previous work in Tyrode solution. Differences between values of log K for the ileum and atria are indicated by Δ_1 and between values at 29° and 37°C by Δ_2

	Atria 29°C	*Ileum* 29°C	Δ_1	*Ileum* 37°C	Δ_2
4-Phenylacetoxy-*N*-methyl-piperidine HCl	5.355 ±0.019 (4)	5.572 ±0.008 (7)	0.217	5.586	−0.014
$Ph_2CHCH_2OCH_2CH_2N^+Me_3Br^-$	6.087 ±0.040 (4)	6.194 ±0.010 (5)	0.107	6.413	−0.219
$Ph_2CHCH_2OCH_2CH_2N^+Et_3Br^-$	6.529 ±0.016 (4)	6.376 ±0.009 (7)	−0.153	6.374	0.002
Diphenylacetyltropine HCl	7.405 ±0.013 (4)	7.956 ±0.017 (6)	0.551	8.110	−0.154
4-Diphenylacetoxy-*N*-methyl-piperidine HCl	7.571 ±0.019 (4)	8.362 ±0.027 (7)	0.791	8.361	0.001
Diphenylacetyltropine-methiodide	7.948 ±0.027 (4)	8.665 ±0.032 (6)	0.717	8.669	−0.004
$Ph_2C(OH)COOCH_2CH_2N^+Me_3Br^-$	8.120 ±0.048 (5)	8.932 ±0.023 (5)	0.812	8.511	0.421
with acetyl-β-methyl-choline as agonist	8.158 ±0.045 (3)	8.936 ±0.031 (4)			
Atropine	9.126 ±0.044 (3)	9.259 ±0.051 (4)	0.133	9.007	0.252
Atropine methiodide	8.776 ±0.023 (5)	9.531 ±0.066 (6)	0.755	9.454	0.077
	9.034 ±0.010 (3)	9.576 ±0.042 (9)	0.542	9.482	0.094

(Abramson *et al.*, 1969; Barlow *et al.*, 1973; Barlow, Franks & Pearson, 1974).

Results

The results of the first group of experiments are summarized in Table 1 which shows the mean estimate of log K, with the standard error and number of preparations, for the atria and ileum at 29°C in Ringer-Locke solution. Published values of log K at 37°C in Tyrode solution are also included for comparison. It would be expected that there would be a bigger error attached to the estimates on the atria because the dose-ratios are calculated from much

Table 2 Values of the mean estimate of the dose-ratio are shown with the standard error and number of estimates in experiments where the concentration of the antagonist was tested on guinea-pig atria at 29° and on ileum at 29° and 37°C; weighted means (see text) and values derived from them are shown in italics. The agonist was carbachol and the Ringer-Locke solution contained hexamethonium (2.76×10^{-4}M) except where indicated. The column headed Δ_1 shows the log of the ratio (dose-ratio − 1) on the ileum to (dose-ratio − 1) on the atria and Δ_2 shows the log of the corresponding ratio for the ileum at 29° and 37°C

	Atria *29°C*	*Ileum* *29°C*	Δ_1	*Ileum* *37°C*	Δ_2
(−)-Hyoscine 10^{-7}	95.5	364	0.584	174	0.322
	±19.5	±42.9		±26.9	
	(6)	(8)		(6)	
	93.0	*372*	*0.606*	*198*	*0.275*
(−)-Hyoscine methiodide	77.8	660	0.933	138	0.682
2×10^{-8}M	±9.00	±78.5		±25.2	
	(5)	(7)		(6)	
	76.5	*634*	*0.923*	*123*	*0.715*
$Ph_2CHCH_2OCH_2CH_2N^+Me_3Br^-$	14.6	14.7	0.003	14.6	0.003
5×10^{-6}M	±0.74	±2.06		±0.95	
No hexamethonium	(5)	(6)		(5)	
	14.6	*13.4*	*−0.040*	*14.6*	*0.040*
with hexamethonium		12.0		9.41	0.117
		±1.54		±0.27	
		(3)		(3)	
		11.5		*9.44*	*0.095*
$Ph_2CHCOOCH_2CH_2N^+Me_3Br^-$	25.6	47.9	0.280	44.1	0.037
5×10^{-7}M	±4.52	±3.00		±4.05	
	(5)	(5)		(5)	
	23.2	*48.2*	*0.328*	*44.1*	*0.039*
4-Diphenylacetoxy-*N*-methyl-	7.28	132	1.319	63.3	0.323
piperidine methiodide	±1.10	±5.44		±7.22	
10^{-7}M	(4)	(6)		(7)	
	7.52	*132*	*1.303*	*62.1*	*0.331*
repeated	5.02	72.8	1.252	49.9	0.167
	±0.12	±2.07		±3.35	
	(3)	(4)		(4)	
	4.98	*73.2*	*1.259*	*49.8*	*0.170*
4-Benziloyloxy-*N*-methyl-	10.0	36.4	0.595	50.8	−0.148
piperidine methiodide	±1.29	±5.26		±8.67	
10^{-8}M	(4)	(6)		(5)	
	10.0	*36.8*	*0.600*	*48.2*	*−0.120*
3-Diphenylacetoxy-	43.8	89.4	0.315	61.1	0.168
quinuclidine methiodide	±7.69	±10.4		±4.55	
10^{-6}M	(4)	(5)		(6)	
	42.6	*90.6*	*0.333*	*61.8*	*0.168*
Diphenylacetyl-pseudo-	27.6	91.1	0.530	65.4	0.146
tropine methiodide	±1.65	±6.20		±3.77	
5×10^{-7}M	(4)	(7)		(8)	
	27.6	*90.9*	*0.529*	*66.6*	*0.137*

smaller numbers of responses than in the experiments with the ileum. There are systematic errors which exceed the statistical errors in this type of experiment (Abramson *et al.*, 1969) but it seems unlikely that the error attached to the mean estimates of log K on the atria is greater than 0.15 log units, compared with 0.1 log units on the ileum. In separate experiments the mean estimate of log K for diphenylethoxyethyltrimethylammonium bromide was calculated to be 6.421 (3), compared with 6.529 in Table 1 and mean estimates for methylatropinium were 8.872 (2) and 8.592 (4) compared with 8.776.

The results suggested that there were differences between the affinity for the muscarine-sensitive receptors in the atria and ileum, greater than could be accounted for by possible experimental errors. They also suggested that the effects of temperature on affinity were different for different compounds and that it would be desirable to make measurements at the two different temperatures with compounds tested simultaneously in the same physiological salt solution. The dose-ratios obtained in the second group of experiments are summarized in Table 2. In calculating the mean it seemed that the error might be reduced by weighting the results depending on the correction which had been made to calculate the matching dose-ratio from that actually tested. The values were therefore weighted according to the square of this correction factor (expressed as a number less than 1): for a perfect match between responses in the presence of the antagonist and responses in its absence the weight was one. For a poor match in which the high concentration in the presence of the antagonist matched the low concentration in its absence (so the calculated dose-ratio is exactly twice that actually tested), or with the low concentration in the presence of the antagonist matching the high concentration in its absence (so the calculated dose-ratio is exactly half that actually tested), the weight was only 0.25. The weighted means so obtained, however, did not differ greatly from the unweighted ones, nor did the derived values, Δ_1 and Δ_2 (see Table 2).

The individual dose-ratios were used to calculate log K as in the previous work and the differences between the mean values of log K also gave values of Δ_1 and Δ_2 which were very close to those calculated from the log of the ratios of (dose-ratio -1). For example the values of Δ_1 for hyoscine were 0.584 from values of mean dose-ratios, 0.606 from values of weighted means dose-ratios, and 0.587 from the difference between mean values of log K.

From the repetition of some of the tests, however, it is clear that there remain large experimental errors but the differences between the affinity of many of the compounds for the receptors in the atria and the ileum at 29°C are much greater than these. Although the compounds selected nearly all had higher affinity for the ileum than for the atria (Figure 1), the difference

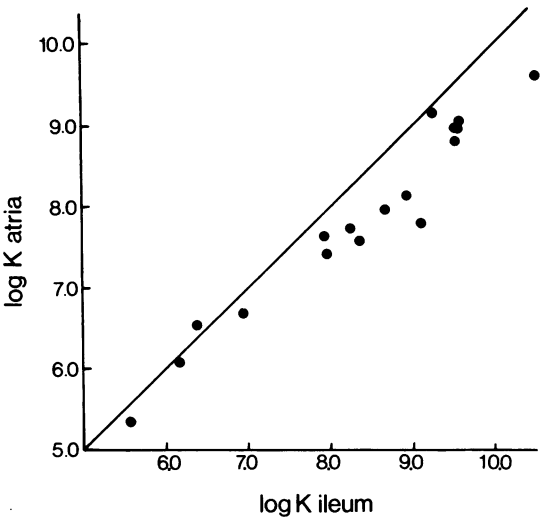


Figure 1 Mean values of log affinity constant for the muscarine-sensitive receptors in the guinea-pig atria plotted against those for the receptors in the ileum measured at the same temperature (29°C) and in the same physiological salt solution (Ringer-Locke solution). If the affinity were the same for both receptors, the results should lie along the straight line.

does not appear to depend only on affinity for the ileum so could not be explained as arising simply from some bias associated with measuring high affinity. The most selective compound, 4-diphenylacetoxy-methylpiperidine methiodide, which has only about 1/20th the affinity for the receptors in the atria that it has for those in the ileum, is actually slightly weaker than atropine on the ileum.

There is much less certainy about differences in the effects of temperature on affinity. Estimates of log K at 29°C are probably subject to greater errors than those at 37°C, partly because of the slower response of the muscle and partly because of poorer control of temperature. Values of Δ_2 listed in Table 1 are of very doubtful significance because they were not obtained from experiments made in the same physiological salt solution. The mean value of Δ_2 for the results in Table 2 is 0.20 ± 0.076 (s.e.; 9 results) so it seems probable that the values for hyoscine methiodide and for 4-benziloyloxy-*N*-methylpiperidine methiodide are significantly different from the others. There does not seem to be any reason for believing that the compounds all have the same temperature coefficient.

Discussion

In experiments on the effects of antagonists on the inotropic actions of carbachol on guinea-pig atria in

Tyrode solution at 30°C, Lüllman *et al.* (1969) obtained dose-ratios of 127 and 135 with 10^{-7}M atropine (log K = 9.10, 9.13) and 180 with 8×10^{-8}M methylatropinium (log K = 9.35). Although the value for atropine is similar to that obtained in this work (9.126), the value for methylatropinium is appreciably higher than ours (8.776) and closer to that expected for the ileum (9.531). This suggests that the receptors at which carbachol acts to affect the rate of beating are different from those at which it acts to decrease the force of contraction. Alternatively it must be supposed that we have in some way underestimated the affinity of particular compounds for the receptors in the atrial pacemaker cells, in spite of all our attempts to avoid any bias, which include using a longer time-cycle and agonist contact time than Lüllman *et al.* (1969; details given by Mitchelson, 1975, indicate a 7 min cycle with 3 min contact with agonist compared with our 16 min cycle). As the affinity of the enantiomers dexetimide and levetimide for the inotropic carbachol receptor has been measured by Gray, Lüllman, Mitchelson & Reil (1976) and there is very marked stereospecificity it would seem well worthwhile testing these compounds against the chronotropic actions of carbachol.

It would be expected that the specificity of compounds for different forms of the muscarine-sensitive acetylcholine receptors would be related to their chemical structure. As can be seen from Table 3, in the four instances studied the quaternary salts all had relatively higher affinity for the receptors in the ileum, but the extent is variable, being greater in atropine than in diphenylacetyltropine. Specificity for the ileum seems also to be increased by changes involving the introduction of oxygen. For example, the diphenylacetyl ester of choline has a higher value (0.30) than the analogous ether (0.11; not included in Table 3); the benzilic ester has a higher value still (0.81). Likewise the scopines have higher values than their tropine analogues. The replacement of choline by 4-hydroxy-*N*-methylpiperidine greatly increases specificity but this is reduced with the more complex ring structures, tropine, pseudo-tropine and 3-hydroxyquinuclidine. The effects are not additive; 4-benziloyloxy-*N*-methylpiperidine methiodide has a lower specificity than its diphenylacetyl analogue even though it combines three features apparently favourable to specificity, a quaternary group, a benzilic group and the 4-hydroxy-*N*-methylpiperidine ring. It has higher affinity for both types of receptor but the replacement of diphenyl by benziloyl increases affinity for the receptors in the atria over 10-fold whereas affinity for those in the ileum is only increased just over 2-fold, hence the decline in specificity.

It is almost certain that hydrophobic interactions make a major contribution to the binding of these compounds to the muscarine-sensitive acetylcholine receptor but the results suggest that at the receptors in the ileum there may be an appreciable contribution from polar interactions in suitable circumstances. Although it is possible to find compounds with reasonable specificity for receptors in the ileum, it seems that it is much more difficult to obtain compounds with specificity for the atria. This may indicate that the receptors differ only in some extra feature present in the receptors in the ileum and absent from those in the atria, or it may merely indicate that a sufficient variety of compounds has not yet been tested.

The effects of temperature on affinity have been

Table 3 Chemical structure and degree of specificity for muscarine-sensitive receptors in guinea-pig ileum compared with those in atria (29°C)

Acid	*Base*								
	Choline	N-*methylpip-eridin-4-ol*		*Tropine*		*Pseudo tropine*	*Scopine*		*3-Hydroxy-quin uclidine*
		Base	*MeI*	*Base*	*MeI*	*MeI*	*Base*	*MeI*	*MeI*
$PhCH_2COOH$		0.22							
$Ph_2CHCOOH$	0.30	0.79	1.32	0.55	0.72	0.53			0.32
$Ph_2COHCOOH$	0.81		0.58						
(±) Ph, OH, C, C_6H_{11}, COOH	0.54								
$PhCHCOOH$ (OH on CH_2)				0.13	0.75		0.58	0.93	

The values shown, Δ_1 in Tables 1 and 2, indicate the log of the ratio of the affinity constants; a value of 1.0 indicates that the affinity for the receptors in the ileum is 10 times that for the receptors in the atria. The tropic acid was racemic for the esters of tropine (atropine) but optically active for the esters of scopine ((—)-hyoscine).

examined by Paton & Rang (1966) who found that the affinity of lachesine for the muscarine-sensitive receptors in guinea-pig ileum was increased 1.3-fold by lowering the temperature from 37.5° to 30.5°C. Mustafa (1967) reported that log K for phenylacetoxyethyl-*N*- methylpiperidinium iodide was increased by 0.18 when the temperature was lowered from 37° to 27°C. These indicate very similar enthalpies of adsorption (ΔH), approximately −7 kcal (−29 kJ)/mol for lachesine and −7.8 kcal (−33kJ)/mol for the second compound. They differ greatly in their free energy of adsorption, however; for lachesine ΔG=−12.6 kcal (−53kJ)/mol whereas for the much weaker phenylacetoxyethyl compound ΔG=−7.4 kcal (−31 kJ)/mol. The corresponding changes in TΔS are +5.6 and −0.4 kcal/mol.

If compounds have similar enthalpies of adsorption, changes in free energy of adsorption must be due to changes in the entropy of adsorption. Though there may be situations in which binding is likely to be associated with increased disorder, there are other situations where the binding process might be expected to increase order in the system. It therefore seems unlikely that the enthalpy of adsorption will be the same for all compounds. It is certainly known that it is different for the binding of inhibitors to acetylcholinesterase (Belleau, Tani & Lie, 1965; Belleau & Lavoie, 1968) for which the effects of temperature on affinity can be measured more accurately and over a wider range than in experiments with guinea-pig ileum.

It would then be expected that values of Δ_2 in Table 2 should not all be the same and that it would be worth calculating the values of ΔH and TΔS (Table 4). Although ΔG is known reasonably accurately, the values of ΔH are not accurate because the errors in Δ_2 are large and must be multiplied by a large number. A difference in log K in these experiments of 0.1 corresponds to a value of ΔH=5.3 kcal/mol: estimates of TΔS could well be incorrect by this amount. The calculations do suggest, however, that the entropy changes associated with binding are different for different compounds. In some instances there appears to be a moderate increase in entropy but with hyoscine methiodide it appears that the order in the system increases. Further results are clearly desirable, particularly as the present experiments were made in Ringer-Locke solution (which does not contain Mg^{2+}) instead of the more usual Tyrode or Krebs solution.

The independence of the enthalpy and entropy terms accounts for the difficulty of predicting affinity accurately. As Abramson *et al.* (1974) have remarked 'To predict the affinity of new compounds, then, the real problem is to predict the extent to which the effects of the various substituents fall short of the maximum' and changes in structure which lead to increases in overall order, so that TΔS is negative, will make ΔG correspondingly more positive and tend to reduce affinity. To predict affinity successfully it will therefore be necessary to have some idea of the effects of substituents on either the enthalpy or entropy of binding and this information cannot be obtained from measurements at only one temperature.

We thank the Wellcome Trust for support. K.S.S. was on leave from the Department of Pharmacology, University of Malaysia, Kuala Lumpur.

Table 4 Estimates of the enthalpies and entropies of adsorption for compounds to muscarine-sensitive acetylcholine receptors in the guinea-pig ileum calculated from the results shown in Table 2

	ΔG	*ΔH*	*TΔS*
(−)-Hyoscine	−13.1	−17.1	−4.0
(−)-Hyoscine methiodide	−14.0	−36.4	−22.4
$Ph_2CHCH_2OCH_2CH_2N^+Me_3Br^-$	−8.7	−6.4	+2.3
Diphenylacetylcholine bromide	−9.7	−2.1	+7.6
4-Diphenylacetoxy-*N*-methyl-piperidine methiodide	−12.5	−17.1 −9.1*	−4.6 +3.4*
4-Benziloyloxy-*N*-methyl-piperidine methiodide	−13.5	+8.0	+21.5
3-Diphenylacetoxy-quinuclidine methiodide	−10.9	−9.1	+1.8
Diphenylacetyl-pseudo-tropine methiodide	−11.4	−8.0	+3.4

Values are in kcal/mol (multiply by 4.18 to convert to kJ/mol). The asterisk indicates the second set of results obtained with this compound. Note that the errors attached to the estimates of ΔH and TΔS may be as big as ± 5 kcal/mol.

References

ABRAMSON, F.B., BARLOW, R.B., MUSTAFA, M.G. & STEPHENSON, R.P. (1969). Relationships between chemical structure and affinity for acetylcholine receptors. *Br. J. Pharmac.*, **37**, 207–233.

ABRAMSON, F.B., BARLOW, R.B., FRANKS, FIONA M. & PEARSON, J.D.M. (1974). Relationships between chemical structure and affinity for prostganglionic acetylcholine receptors of the guinea-pig ileum. *Br. J. Pharmac.*, **51**, 81–93.

ARUNLAKSHANA, O. & SCHILD, H.O. (1959). Some quantitative uses of drug antagonists. *Br. J. Pharmac. Chemother.*, **14**, 48–58.

BARLOW, R.B., FRANKS, FIONA M. & PEARSON, J.D.M. (1972). A comparison of the affinities of antagonists for acetylcholine receptors in the ileum, bronchial muscle and iris of the guinea-pig. *Br. J. Pharmac.*, **46**, 300–314.

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

BARLOW, R.B. & HAMILTON, J.T. (1965). The stereospecificity of nicotine. *Br. J. Pharmac. Chemother.*, **25**, 206–212.

BELLEAU, B. & LAVOIE, J.L. (1968). A biophysical basis of ligand-induced activation of excitable membranes and associated enzymes. A thermodynamic study using acetylcholinesterase as a model receptor. *Canad. J. Biochem.*, **46**, 1397–1409.

BELLEAU, B., TANI, H. & LIE, F. (1965). A correlation between the biological activity of alkyltrimethylammonium ions and their mode of interaction with acetylcholinesterase. *J. Amer. Chem. Soc.*, **87**, 2283–2285.

BLACK, J.W., DUNCAN, W.A.M., DURANT, C.J., GANELLIN, C.R. & PARSONS, E.M. (1972). Definition and antagonism of histamine H_2-receptors. *Nature, Lond.*, **236**, 385–390.

BURN, J.H. (1952). *Practical Pharmacology*, p. 23, Oxford: Blackwell.

BUTT, ALISON A. (1972). The effects of different recording conditions on the estimates of affinity constants of antagonists for acetylcholine receptors in the guinea-pig ileum. *Br. J. Pharmac.*, **46**, 312–314.

EDINBURGH STAFF (1972). *Pharmacological Experiments on Isolated Preparations*. 2nd edition, pages 2, 58, 112. Edinburgh: Churchill Livingstone.

GRAY, J.A., LÜLLMANN, H., MITCHELSON, F. & REIL, G.-H. (1976). Stereoselective binding in cardiac tissue of the enantiomers of benzetimide, an antimuscarinic drug. *Br. J. Pharmac.*, **56**, 485–490.

LÜLLMANN, H., OHNESORGE, F.K., SCHAUWECKER, G.-C. & WASSERMANN, O. (1969). Inhibition of the actions of carbachol and DFP on guinea-pig ileum isolated atria by alkane-bis-ammonium compounds. *Eur. J. Pharmac.*, **6**, 241–247.

MITCHELSON, F. (1975). Antimuscarinic action of an alkane-bis-ammonium compound alone and in combination with (+)-benzetimide. *Eur. J. Pharmac.*, **33**, 237–246.

MUSTAFA, M.G. (1967). Ph.D. Thesis, University of Edinburgh, pp. 40–41.

PATON, W.D.M. & RANG, H.P. (1966). A kinetic approach to the mechanism of drug action. In *Advances in drug research* vol 3, eds., Harper, N.J. & Simmonds, Alma B., p. 65. London: Academic Press.

(Received May 5, 1976. Revised June 4, 1976.)