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TEMPERATURE COEFFICIENTS OF AFFINITY AND ENTROPIES OF ADSORPTION FOR ENANTIOMERIC PAIRS OF COMPOUNDS ACTING AT MUSCARINIC RECEPTORS IN THE GUINEA-PIG ILEUM

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1 The affinities of a series of enantiomeric pairs of esters of phenyl*cyclo*hexylglycollic acid, of a pair of esters of α-methyltropic acid and of hyoscyamine methiodide have been measured for the muscarinic receptors of the guinea-pig ileum at 30° and 37°C and estimates have been made of their free energies, enthalpies and entropies of adsorption.

2 With (−)-S-hyoscyamine methiodide the enthalpy of adsorption is negative whereas with the (+)-R-enantiomer it is positive.

3 With the esters of phenyl*cyclo*hexylglycollic acid the size of the onium group appears to be as important as the stereochemical configuration in determining entropy. Large onium groups appear to be associated with an increase in entropy though this can only be measured approximately because of the narrow range of temperature which can be used.

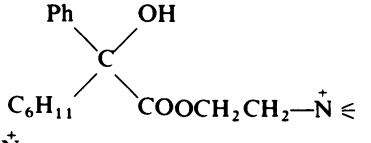
Introduction

In previous work Barlow, Berry, Glenton, Nikolaou & Soh (1976) found that the effects of temperature on affinity for the muscarinic acetylcholine receptors of the guinea-pig ileum differ appreciably from one compound to another. For many of the substances tested the affinity constant was higher at 29° than at 37°C, indicating an exothermic binding process, but there were others where the adsorption was apparently endothermic. Estimates of the entropy of adsorption were calculated from the affinity constant and its temperature coefficient

$$\left(\Delta G = -RT \ln K;\right.$$
$$\left.\Delta H = -R \frac{\Delta \ln K}{\Delta 1/T};\quad T\Delta S = \Delta H - \Delta G\right)$$

and also differed appreciably from one compound to another.

To find out more about the effects of chemical structure on the entropy of adsorption, we have now examined a series of enantiomeric pairs of esters of phenyl*cyclo*hexylglycollic acid:



where $-\overset{+}{N}$ $-\overset{+}{N}MeEt_2$, $-\overset{+}{N}Et_3$, methylpyrrolidinium, ethylpyrrolidinium, methylpiperidinium and ethylpiperidinium. Their affinity has already been measured at 37°C (Barlow, Franks & Pearson, 1973) and because of the high degree of stereospecificity it was thought that there might be consistent differences between the entropies of adsorption of the (+)-R- and (−)-S-enantiomers. Belleau & Lavoie (1968), for instance, found that the temperature coefficient for the binding of (+)-S-acetyl-β-methylcholine to acetylcholinesterase indicated that ΔH was positive and ΔS also positive, whereas the results for (−)-R-acetyl-β-methylcholine indicated that ΔH and ΔS were both negative.

We have also studied the enantiomeric forms of hyoscyamine methiodide, for comparison with hyoscine methiodide (Barlow *et al.*, 1976) and the enantiomeric forms of an ester of α-methyltropic acid, which has as high stereospecificity as the phenyl*cyclo*hexylglycollic esters but lower affinity.

Methods

The guinea-pig ileum was set up in aerated Tyrode solution containing hexamethonium, 2.75×10^{-4} M, with the responses recorded isotonically and a load of about 0.5 g. Carbachol was used as the agonist and the conditions were the same as in previous work (Barlow *et al.*, 1973; Barlow *et al.*, 1976), except that the contact time was extended to 40 s in the measurements at 30°C. In each experiment a solution of the

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antagonist, B, was tested simultaneously on one piece of ileum at 30.0 ± 0.2°C and on another piece at 37.0 ± 0.2°C and the concentrations of carbachol were increased so that the responses in the presence of the antagonist were similar to the controls (Edinburgh Staff, 1974). At 30°C it was necessary to wait 40 min or more before the responses to carbachol in the presence of the more active (−)-compounds became constant, compared with about 30 min at 37°C. The sizes of the responses to carbachol in the absence and in the presence of the antagonist were used to calculate the exact dose ratio.

The effect of temperature on log affinity constant (Δ log K) is indicated by the increment in log (dose-ratio − 1) because the compounds are competitive antagonists and the dose-ratio = 1 + BK (Gaddum, 1937; Schild, 1949) so

$$\Delta \log K = \log\left(\frac{\text{dose-ratio at } 30^{\circ}C - 1}{\text{dose-ratio at } 37^{\circ}C - 1}\right)$$

and is positive for an exothermic process. At least four estimates of this increment were obtained for each compound. There was no evidence of racemisation of the enantiomers during the experiments or on storage of the stock solutions (2 days, 4°C).

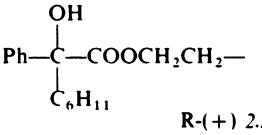
Compounds

Carbachol chloride and hexamethonium bromide were obtained from Sigma. The synthesis and analytical details of the pairs of enantiomers have been described by Barlow *et al.* (1973).

Results

The results are shown in Table 1. The estimates of log K at 37°C calculated from the dose-ratios are usually within 0.1 log units of the values obtained in previous work, except with the (+)-ethylpyrrolidinium compound, where there is a difference of 0.4 log units for which we have no explanation. Although there is considerable uncertainty attached to the estimates of the temperature increment in log K, it is clear that it is not the same for all compounds. In only 2 of the more active (−)-enantiomers out of the 11 tested is the sign negative, indicating an endothermic process, whereas this occurs with 6 of the (+)-enantiomers. The two (−)-enantiomers with negative temperature increments are the methyldiethyl and triethylammonium compounds and the results reinforce the suggestion, made from results

Table 1 Effects of temperature on dose-ratio and log affinity constant



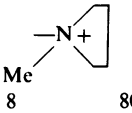
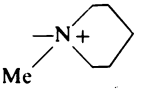
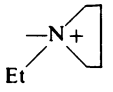
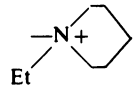
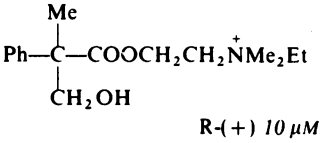
S-(−) 25 nM				R-(+) 2.5 μM			
30°C	37°C	Δ	n	30°C	37°C	Δ	n
			$-\overset{+}{N}Me_2H$				
16.9	15.0	0.05	6	15.3	16.2	−0.03	5
±1.3	±0.8	±0.03		±0.5	±0.9	±0.03	
8.80	*8.75*			*6.76*	*6.78*		
	(8.87)				*(6.98)*		
			$-\overset{+}{N}Me_3$				
215.6	151.4	0.15	5	39.0	43.4	−0.05	5
±11.1	±4.2	±0.02		±1.5	±2.5	±0.02	
9.93	*9.78*			*7.18*	*7.23*		
	(9.65)				*(7.26)*		
			$-\overset{+}{N}Me_2Et$				
288.5	267.8	0.04	14	139.7	123.1	0.06	7
±22.7	±23.1	±0.04		±6.3	±10.7	±0.03	
10.06	*10.03*			*7.74*	*7.69*		
	(10.04)				*(7.88)*		
							
230.3	219.4	0.02	8	80.8	88.3	−0.04	4
±15.8	±16.9	±0.02		±5.8	±5.7	±0.03	
9.96	*9.94*			*7.50*	*7.54*		
	(9.63)				*(7.49)*		

Table 1 Continued

			—$\overset{+}{N}$MeEt$_2$				
181.2 ±10.7 *9.86*	205.3 ±20.0 *9.91* (*10.00*)	−0.04 ±0.03	7	275.6 ±10.4 *8.04*	290.8 †15.2 *8.06* (*8.15*)	−0.02 ±0.03	5
							
102.3 ±5.7 *9.61*	94.1 ±5.9 *9.57* (*9.47*)	0.04 ±0.03	6	82.4 ±5.4 *7.51*	83.1 ±6.4 *7.52* (*7.33*)	0.00 ±0.01	5
							
237.5 ±12.9 *9.98*	173.6 ±14.2 *9.84* (*9.72*)	0.14 ±0.04	12	287.9 ±10.7 *8.06*	341.1 ±21.1 *8.13* (*7.76*)	−0.07 ±0.02	9
			—$\overset{+}{N}$Et$_3$				
96.0 ±7.1 *9.58*	119.4 ±7.5 *9.67* (*9.60*)	−0.10 ±0.04	7	152.0 ±8.1 *7.78*	186.2 ±10.7 *7.87* (*7.99*)	−0.09 ±0.02	7
							
55.4 ±3.3 *9.34*	51.7 ±2.6 *9.31* (*9.25*)	0.03 ±0.04	9	120.0 ±14.1 *7.68*	111.7 ±14.9 *7.65* (*7.41*)	0.03 ±0.02	8
							
	S-(−) *100 nM*				R-(+) *10 μM*		
73.6 ±2.2 *8.86*	50.4 ±2.8 *8.69* (*8.84*)	0.18 ±0.03	5	64.9 ±5.6 *6.81*	39.3 ±3.2 *6.58* (*6.65*)	0.20 ±0.04	5
			Hyoscyamine methiodide				
	S-(−) *10 nm*				R-(+) *1 μM*		
105.7 ±11.7 *10.02*	36.3 ±5.1 *9.55* (*9.67*)	0.48 ±0.06	4	58.1 ±3.1 *7.76*	45.8 ±3.5 *7.65* (*7.72*)	0.11 ±0.01	4

Dose-ratios (±s.e. of n results) are shown for pairs of experiments at 30 and 37 C; Δ indicates the mean value of

$$\log\left(\frac{\text{dose-ratio at } 30° - 1}{\text{dose-ratio at } 37° - 1}\right).$$

The concentrations of antagonist used are indicated and the numbers in italics are the values of log K calculated from the mean dose-ratio and the concentrations. Results of previous estimates at 37 C are shown in parentheses (Barlow, Franks & Pearson, 1973).

Table 2 Estimates of free energies, enthalpies and entropies of adsorption

		ΔG	ΔH	$T\Delta S$	$\Delta\phi_v^0$
$\overset{+}{N}Me_2H$	(−)	−12.4	−3.1 ± 1.9	9	−14
	(+)	−9.6	+1.6 ± 1.8	11	
$\overset{+}{N}Me_3$	(−)	−13.8	−9.4 ± 1.4	4	0
	(+)	−10.2	+2.8 ± 1.5	13	
$\overset{+}{N}Me_2Et$	(−)	−14.2	−2.5 ± 2.5	12	15
	(+)	−10.9	−3.8 ± 1.8	7	
Methylpyrrolidinium	(−)	−14.1	−1.4 ± 1.5	13	19
	(+)	−10.7	+2.5 ± 1.7	13	
$\overset{+}{N}MeEt_2$	(−)	−14.0	+2.8 ± 1.6	17	30
	(+)	−11.4	+1.4 ± 1.6	13	
Methylpiperidinium	(−)	−13.6	−2.3 ± 2.0	11	33
	(+)	−10.6	+0.1 ± 0.7	11	
Ethylpyrrolidinium	(−)	−13.9	−8.9 ± 2.4	5	34
	(+)	−11.5	+4.2 ± 1.2	16	
$\overset{+}{N}Et_3$	(−)	−13.7	+6.0 ± 2.3	20	44.5
	(+)	−11.1	+5.4 ± 1.0	17	
Ethylpiperidinium	(−)	−13.2	−1.8 ± 2.2	11	48
	(+)	−10.8	−2.1 ± 1.0	9	
α-Methyltropic esters	(−)	−12.3	−11.0 ± 2.0	1	
	(+)	−9.3	−12.0 ± 2.6	−3	
Hyoscyamine methiodide	(−)	−13.5	−29.2 ± 3.9	−16	
	(+)	−10.8	−6.7 ± 0.7	4	

Estimates of ΔG are correct to ±0.2 Kcal/mol or less. The errors attached to ΔH are calculated from the standard error of the temperature increment in log K (Δ in Table 1). The errors attached to TΔS will be at least as big and are probably between 3 and 5 Kcal/mole. The size of the onium group is indicated by the increment in apparent moal volume at infinite dilution ($\Delta\phi_v^0$), taken from results obtained with analogous alkylonium salts (Barlow, Lowe, Pearson, Rendall & Thompson, 1971).

with a similar series of amides of diphenylacetic acid (Barlow, Bremner & Soh, 1978), that large onium groups are associated with an endothermic binding process.

Discussion

The estimates of ΔH and TΔS calculated from log K and the temperature increment of log K are shown in Table 2. Although there is considerable uncertainty about the values, it is remarkable that the result for (−)-hyoscyamine methiodide (TΔS = −16 Kcal/mol) is similar to that already obtained (Barlow *et al.*, 1976) for (−)-hyoscine methiodide (TΔS = −22 Kcal/mol). The (+)-enantiomer, however, causes an increase in entropy. In the phenyl*cyclo*hexylglycollic esters it appears that the size of the onium group is at least as important as the arrangement at the asymmetric carbon atom in determining the effect on entropy, though the shape may also be involved. The values of TΔS increase in the series $-\overset{+}{N}Me_2Et < -\overset{+}{N}MeEt_2 < -\overset{+}{N}Et_3$, with little difference between the (−)- and (+)-enantiomers, but the values for the methylpiperidinium and ethylpiperidinium compounds are all about the same.

The results indicate the likely limits to estimates of TΔS which can be obtained by measuring dose-ratios derived from muscular responses to an agonist. They are not good enough to justify any speculation about the nature of the adsorption process and an attempt has therefore been made to see whether they can be confirmed in experiments with isolated receptor preparations obtained from rat brain, with which the binding can be studied over a wider range of temperature. This work is described in the next paper.

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