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TEMPERATURE COEFFICIENTS OF AFFINITY CONSTANTS FOR THE BINDING OF ANTAGONISTS TO MUSCARINIC RECEPTORS IN THE RAT CEREBRAL CORTEX

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1 The temperature coefficients of binding of a series of muscarinic antagonists to their receptors in membrane preparations from the rat cerebral cortex has been examined.

2 At 37°C the affinity constants agree with those determined by antagonism of acetylcholine-induced contractions of the guinea-pig ileum.

3 The temperature-dependence of the affinity constants is low; for the antagonists examined, the affinity constants at 0°C differ by less than a factor of 3 from those measured at 37°C.

4 There are qualitative similarities between the estimates of the temperature coefficient of binding of the antagonists to receptors in ileum strips and in the cerebral cortex.

Introduction

The effects of temperature on the affinity of binding of muscarinic antagonists can be studied only over a very narrow range with intact preparations producing a mechanical response, such as the guinea-pig ileum, because the contractions of the tissue are very sluggish below 30°C (Barlow, Berry, Glenton, Nikolau & Soh, 1976; Barlow & Burston, 1979). There is therefore considerable uncertainty attached to these estimates of the temperature coefficient. It was decided to examine the binding of some of these antagonists to membrane-bound receptors obtained from rat cerebral cortex, as these measurements can be made over a wider range of temperature. Measurements were made at 0°, 30° and 37°C with the labelled ligands, (−)-[^{3}H]-methylscopolamine, [^{3}H]-methylatropine and N-[2′,3′-$^3H_2$-propyl]-N,N-dimethyl-2-aminoethylbenzilate bromide (propylbenzilylcholine). In addition the affinities of the (R)- and (S)-enantiomers of the phenylcyclohexylglycollic esters of choline and its N,N,N-triethyl analogue have been measured at 0°, 20° and 37°C.

Methods

Full details of the preparations of the labelled ligands, the synaptosome fragments from rat cortex and the binding assay have been given elsewhere (Hulme, Birdsall, Burgen & Mehta, 1978). The membranes were suspended in Krebs-Henseleit solution, the protein concentration being 0.2 mg/ml in the experiments with [^{3}H]-methylatropine and [^{3}H]-methylscopolamine and 0.5 mg/ml in those with [^{3}H]-propylbenzilylcholine. At 37°, 30° and 20°C, the incubation period was 15 min and at 0°C it was 1 h. The membranes were then centrifuged (14,000 *g*, 60 s) in a microcentrifuge (Quickfit, model 320) and the pellet superficially washed with Krebs-Henseleit solution and resuspended in scintillant. Non-specific binding was estimated from binding in the presence of 3-quinuclidinylbenzilate, 10^{-6} M. Binding of labelled methylscopolamine and methylatropine was assayed at concentrations ranging from 3×10^{-11} to 10^{-8} M and propylbenzilylcholine binding was estimated from the inhibition by unlabelled material (10^{-9} to 10^{-6} M) of the binding of labelled material (10^{-10} M). All concentrations are corrected for depletion of free ligand concentration by binding to the receptors. The results for methylatropine are expressed in terms of the (−)-enantiomer, (−)-hyoscyamine-methiodide, the (+)-form not binding at the concentrations used (Hulme *et al.*, 1978).

In the experiments with the enantiomeric forms of the phenylcyclohexylglycollic esters (Barlow, Franks & Pearson, 1973), the (R)-forms were tested in concentrations ranging from 3×10^{-10} to 10^{-6} M for their effects on the binding of labelled (−)-methylscopolamine, 3×10^{-9} M; the (S)-forms were tested in concentrations ranging from 10^{-9} to 10^{-5} M for their effects on the binding of labelled propylbenzilylcholine, 10^{-9} M. After allowance for non-specific binding, the percentage inhibition of the binding of the

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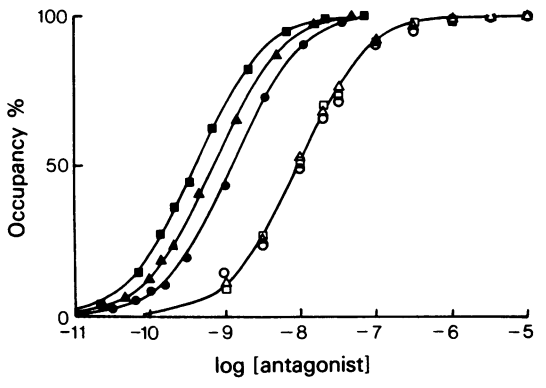

Figure 1 Occupancy concentration curves for the binding of the (S)-phenylcyclohexylglycollic ester of triethylcholine (open symbols) and the (R)-phenylcyclohexylglycollic ester of choline (closed symbols) to membranes from the rat cortex at 0°C (○, ●), 20° (△, ▲) and 37°C (□, ■). The curves are best fit simple mass action binding curves to the data. For clarity of presentation, only one curve for the (S)-compound is shown. The occupancy concentration curves for the (R)-analogue have been generated by correcting the inhibition-concentration curves by the appropriate factor $(1 + K.L)$ where K is the affinity constant of [^{3}H]-methylscopolamine at that temperature and L is the concentration of the radioligand in the competition experiment.

labelled ligand as a function of concentration of the antagonist was fitted to the mass-action curve by a non-linear least-squares procedure and the affinity constant of the antagonist calculated. The apparent affinity constants, measured by inhibition of [^{3}H]-(−)-methylscopolamine, were multiplied by the factor $(1 + K.L)$ to give the true affinity constant, K being the affinity constant for (−)-methylscopolamine and L its concentration in the binding experiment.

Results and Discussion

The results of the binding studies are shown in Figure 1 and Table 1. All the binding curves approximate very closely to simple mass action isotherms for binding to a uniform population of antagonist binding sites. This is clearly illustrated in Figure 1. These results confirm and extend our previous measurements of antagonist binding constants at 30°C which indicated no significant heterogeneity of antagonist binding sites (Hulme *et al.*, 1978).

At 37°C the affinity constants measured in the binding assay agree quite closely with those estimated in the guinea-pig ileum assay (Table 1), the mean difference being 0.11 ± 0.09 log unit. Independent estimates of log K for an antagonist generally agree with 0.1 log unit and 0.04 log unit in the case of ileum assay and the binding assay respectively.

The temperature coefficients of affinity for the antagonists are presented in Table 1. The notable feature of these data is that for all antagonists the temperature coefficients are low, Q_{10} values varying from 0.73 to 1.78. Arrhenius plots of the data appear to be linear, no data point differing by more than 0.04 log unit from the best fit straight line. This indicates that there is no abrupt thermal transition affecting the antagonist binding properties of the receptor, be it mediated by a direct effect on the protein or by an indirect effect caused by a gel-liquid crystalline phase transition or pre-transition phenomena known to occur in lipids (Hesketh, Smith, Houslay, McGill, Birdsall, Metcalfe & Warren, 1976).

The temperature coefficients of binding of these antagonists fall into two groups: esters of tropic acid or benzilic acid have a negative temperature coefficient, whereas esters of phenylcyclohexylglycollic acid have a positive or zero temperature coefficient. The affinity constant of another benzilate ester, 3-quinuclidinylbenzilate has also been reported to have a negative temperature coefficient (Sugiyama, Daniels & Nirenberg, 1977). However, it should be noted that the measurements were made under different ionic conditions and on a different tissue.

The temperature coefficients of binding are qualitatively similar in the ileum and cortex. In only one instance is there a difference in the sign (with S-(−)-phenylcyclohexylglycollylcholine). However, there are considerable quantitative differences with the coefficients in the ileum usually being larger than those on the cortex. These may possibly be due simply to the greater errors associated with measurements on the ileum but they appear to exaggerate the size, regardless of the sign, which is remarkable. They do not appear to be connected simply with the difficulties of making measurements with highly potent compounds on the ileum, because, whereas there is a marked difference with (−)-methylatropine, there is only a slight difference with the S-(−)-phenylcyclohexylglycollic ester of triethylcholine, which has comparable affinity.

The values of $T\Delta S$ for the results with the cortex are all positive. For the phenylcyclohexylglycollic esters of triethylcholine and for the (+)-ester of choline the values are, within the limits of error, similar to those with the ileum. With the (−)-ester of choline the values are greatly different (+18 compared with +4) and similarly with methylatropine (+10 compared with −16) and with methylscopolamine (+9 compared with −22) (Barlow *et al.*, 1976). In each of these instances binding to the receptors on the frag-

Table 1 Effects of temperature on the affinity constant of antagonists for muscarinic receptors in the rat cerebral cortex

	log K, *cortex*				*log* K, *ileum**		Δ *log* K/Δ*T*		TΔS (*kcal/mol*)
	0°	20°	30°	37°	37°		*Cortex*	*Ileum**	*Cortex*
(−)-S-methylscopolamine (methylhyoscine)									
	10.40 ±0.02	9.82†	9.78 ±0.03	9.64 ±0.01	9.55	9.67	−0.010	−0.060	+9
(−)-S-methylatropine (methylhyoscyamine)									
	9.94 ±0.02	9.73†	9.65 ±0.03	9.57 ±0.01	9.84	9.70	−0.010	−0.097	+10
Propylbenzilylcholine									
	8.20 ±0.03	8.01†	7.98 ±0.05	7.84 ±0.09	8.03		−0.008	—	+7
Phenylcyclohexylglycollic esters of (a) choline									
(S)-(−)	8.89 ±0.03	9.14 ±0.03		9.39 ±0.02	9.78	9.65	+0.015	−0.021	+18
(R)-(+)	7.28 ±0.03	7.44 ±0.04		7.59 ±0.05	7.23	7.26	+0.006	+0.007	+14
(b) triethylcholine									
(S)-(−)	9.30 ±0.04	9.34 ±0.03		9.45 ±0.02	9.68	9.60	+0.004	+0.014	+15
(R)-(+)	8.00 ±0.02	8.06 ±0.01		8.05 ±0.02	7.87	7.99	+0.001	+0.013	+12

* Values for the ileum are taken from Abramson, Barlow, Franks & Pearson, 1974; Barlow & Burston, 1979 and Hulme *et al.*, 1978.
† Values interpolated from measurements at 0°, 30°, 37°C.

ments of rat cortex appears to be associated with more (total) disorder than binding to receptors in intact ileum. It is possible, therefore, that there are differences between the receptors in the two preparations. These could be investigated by testing more of the enantiomeric pairs on the cortex or by binding studies with membrane fragments from the ileum.

The experiments with muscarinic receptors from rat cortex confirm that there are differences in the temperature coefficient of adsorption of antagonists dependent on chemical structure and are related to effects on the entropy of adsorption. The actual size of these effects, however, is still uncertain.

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