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The effect of sulphydryl block on the binding of H_1-antagonists to the muscarinic receptor

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The treatment of brain muscarinic receptors by *p*-chlormercuribenzoate is known to change structure-activity relationships for antagonists. The changes in binding affinity of H_1-antagonists for this receptor have been measured; the changes are similar to those for intrinsic muscarinic antagonists.

Introduction It was shown by Birdsall, Burgen, Hulme & Wong (1983a,b) that when muscarinic receptors in cerebral cortex membrane fragments are reacted with *p*-chlormercuribenzoate (PCMB) at a concentration and for a time sufficient to remove detectable free sulphydryl groups, marked changes result in the structure-binding characteristics of both agonists and antagonists. The activity of all agonists is greatly reduced, their rank order of potency is changed and the heterogeneity of binding lost.

The effect on antagonist binding is less dramatic, some are increased in activity, others decreased and the rank order changed. Evidence was presented that these changes are due to conformational changes in the receptor.

When such radical changes are produced in the characteristics of a receptor, the question naturally arises as to whether these changes can be classified as a perturbation of a receptor whose overall character is unchanged, or whether the receptor has totally novel characteristics, i.e. is the PCMB treated receptor a variant muscarinic receptor or is it now receptor X. Since receptors in broken cell preparations are normally classified by their structure-binding profiles, this approach alone may be circular. Note that even if our classification were based on an amino acid sequence we should not escape the dilemma when as in the present case the changes are due to conformational change. We really have no alternative to examining the receptor properties on a broad canvas to see if radically new properties have been induced. This is much more difficult than it might appear at first sight since we have no clues as to where to look.

An approach that might produce leads is to examine drugs whose primary classification is based on another receptor but which 'cross-react' with the muscarinic receptor.

It is well known that histamine H_1-antagonists invariably are also muscarinic antagonists and that the selectivity even in very potent H_1-antagonists is not great. This suggests that there is probably a strong homology between muscarinic and H_1-receptors at least in the 'ground state' to which antagonists are thought to bind (Burgen, Birdsall & Hulme, 1979).

The agonist selectivity between histamine and acetylcholine can be accounted for by distinctive activated states.

It was for these reasons that we have undertaken the examination of the effects of PCMB on the binding of H_1-antagonists to muscarinic receptors.

Methods The methods are essentially those described by Birdsall *et al.* (1983a). P_2 fractions were prepared from homogenates of rat cerebral cortex. The binding of test substances was measured in competition with 2×10^{-10} M [^{3}H]-propylbenzylcholine ([^{3}H]-PrBCh) in a medium of 100 mM NaCl, 50 mM HEPES HCl, pH 7.2. The treatment with PCMB was exposure to 1 mM for 15 min at 30°C, i.e. the conditions for PCMB-2 in Birdsall *et al.* (1983a).

[^{3}H]-PrBCh was kindly given by Dr Birdsall. The diphenhydramine analogue, BS 8978 ((±)-4-methyl benzhydryl ether of choline; Harris, Hespe, Nauta, Rekker, Timmerman & de Vries, 1975) was a gift from Dr J.M. Young. The other antagonists were from commercial sources.

Results The results on six H_1-antagonists are shown in Table 1 as mean ± s.d. It can be seen that in all cases the affinity of the H_1-antagonists was reduced by PCMB, the reduction ranging from a factor of 2.4 for mepyramine to 15.8 for diphenhydramine. These changes fall within the range found for conventional antimuscarinic drugs (Birdsall *et al.*, 1983b). The log mean reduction in affinity they found was 0.72 ± 0.84 (s.d.), whereas the anti-H_1 drugs had a mean reduction of 0.86 ± 0.32 (s.d.).

There was no significant correlation between the affinity for the muscarinic receptor, and the change produced by PCMB, nor with the affinity for the H_1-receptor.

Table 1 Binding of H_1-antagonists to muscarinic receptors – effect of *p*-chlormercuribenzoate (PCMB)

H_1-antagonist	*Control*	*+PCMB*	Δ	*Affinity for H_1**
	($\log M^{-1}$)			
Doxepin	7.05(±0.015)	6.09(±0.04)	0.96	9.15
BS 8978	6.92(±0.005)	6.37(±0.08)	0.55	9.00**
Promethazine	8.18(±0.007)	7.05(±0.035)	1.13	8.53
Diphenhydramine	6.92(±0.08)	5.72(±0.04)	1.20	8.49
Mepyramine	4.98(±0.01)	4.60(±0.06)	0.38	8.34
Chlorpromazine	6.70(±0.04)	5.79(±0.04)	0.91	7.45

* Data from Chang, Tran & Snyder (1979)
** Harris *et al.*, 1975

There appeared to be a negative correlation between the selectivity of the drug for H_1 over muscarinic receptor and the change produced by PCMB.

Discussion The purpose of this study was to see if ligands whose specificity was optimised with respect to another receptor showed greater or smaller changes in binding affinity when the muscarinic receptor was modified than was found for ligands that were optimised for the muscarinic receptor.

One might suppose that either possibility would occur depending on whether the specificity of the receptor was increased or decreased.

The result in this study does not seem to indicate that either of these possibilities is the case and that the H_1-antagonists are treated just as though they were primary anti-muscarinic drugs. This does not support the possibility that the change in the receptor produced by PCMB is radical as far as antagonist specificity is concerned – the receptor has become a variant muscarinic receptor and nothing more.

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