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Short Communications

The Importance of Hydrophobic Interactions in the Antagonist Binding to the Muscarinic Acetylcholine Receptor

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The list of the muscarinic antagonists has been largely extended since Dale defined muscarinic receptors on the basis of stimulation by muscarine and blockade by atropine.[1] Similarly to acetylcholine or atropine all of these compounds possess quaternary ammonium group, hydrophobic substituents and often also polar fragments like ether, ester or alcohol groups.[2,3] Despite extensive studies it is not known which physico-chemical and structural factors govern the binding effectiveness of the muscarinic antagonists to the receptor site and are responsible for the more than millionfold variation in affinity of these ligands.[2,3] In the present communication the role of hydrophobic interaction in the ligand – receptor complex formation is characterized and two principally different mechanisms of hydrophobic binding are identified.

The hydrophobic properties of antagonists were characterized proceeding from the octanol – water partition system and the effective hydrophobicity constants π' for the whole ligand molecule were calculated from the fragmental constants, making use of the tabulated f-parameters,[4] as described by Rekker *et al.*[4,5] As all compounds used in the following analysis involve a quaternary nitrogen atom, its contribution was not taken into account and thus all calculated π-constants are equally shifted relative to the actual log P values for antagonists.

It is well established that binding of the muscarinic antagonists to the receptor follows the mass-action law for simple equilibrium (eqn. 1),[2,3]

$$R + A \overset{K_d}{\rightleftharpoons} RA \qquad (1)$$

and thus the constants pK_d refer to the free energy of the binding reaction and can be used in LFE relationships. The experimental K_d values for muscarinic receptor from rat brain were selected from literature[2,3,6,7] to compile systematic series of antagonists in which hydrophobicity can be considered as a single variable structural factor. Altogether, the following series were constructed: *a*, alkyl- or arylsubstituted ammonium ions; *b*, carboxylic esters containing ammonium group in the alcohol portion; *c*, esters of hydroxyacids, containing ammonium group in their alcohol portion.

The compounds included in these three series are shown in Table 1.

Within each of these series the binding affinity (K_d) of antagonists is governed by hydrophobicity of the drug molecule and the linear relationships

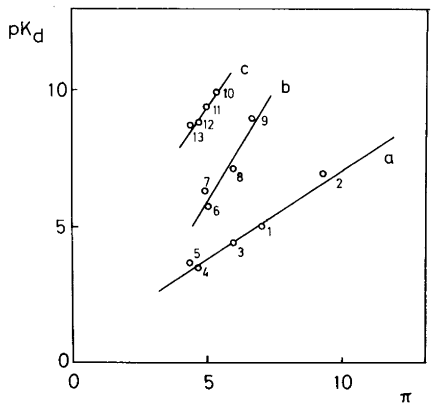

Fig. 1. Linear relationship between pK_d values, derived from ligand binding experiments and the hydrophobicity constants π'. Three series, *a*, *b*, and *c*, were constructed.

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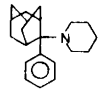
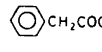
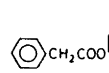
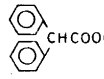
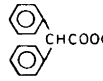
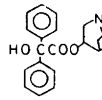
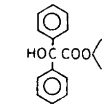
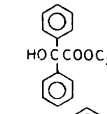
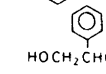
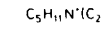
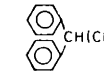
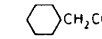
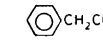
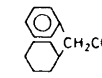
Table 1. The structural formulae of the compounds included in the study and the available pK_d and pK'_d values derived from binding and bioassay experiments, respectively. Compounds *1* – *5* constitute series *a*, *6* – *9* series *b*, *1* – *5* and *14* – *17* series *d*, *6* – *9* and *18* – *20* series *e*.

	Formula	pK_d	pK'_d	π'
1		5 04	4.64	7 2
2		7 03	7 02	9 2
3		4 45	4 77	5 9
4		3 53	2 70	4 6
5		3 77	3 49	4 3
6		6 34	6 20	4 9
7		5 80	5 50	5.0
8		7 20	7 16	5 9
9		9 00	9 06	6 6
10		9 85	9 8	5 25
11		9 46		4 9
12		8 85		4 6
13		8 80		4 35
14			4 59	6 4
15			5 18	6 5
16			5 39	7 6
17			7 02	8 2
18			5 07	5 3
19			4 53	4 25
20			8 44	7 0

between pK_d and π'-values can be described by a single-parameter, eqn. (2),[8] where ϕ is the intensity

$$pK_d = C + \phi_{\pi'} \qquad (2)$$

factor of the hydrophobic effect (Fig. 1). Thus it is assumed that all parts of the antagonist molecules are involved in hydrophobic interaction with the receptor site which must be large enough to accommodate the bulky substituents. (The present data do not allow more detailed analysis of the binding site "topography".) The following ϕ-values were obtained for series $\phi_a = 0.7 \pm 0.2$; $\phi_b = 1.4 \pm 0.3$; $\phi_c = 1.4 \pm 0.4$, respectively.

Proceeding from these values of the intensity factor of the hydrophobic effect the muscarinic antagonists can be clearly divided into two groups; with $\phi > 1$ and $\phi < 1$.

The number of ligands involved in the analysis can be increased if not only binding data are used but also affinity constants determined by measurement of the contractile response of the guinea pig ileum are used. Appropriate data can be found in the work of Abramson *et al.*[9] on a large number of antagonists. It can be shown that there is a linear interrelationship with slope of approximately one between the pK_d and pK'_d values, obtained from direct binding experiments

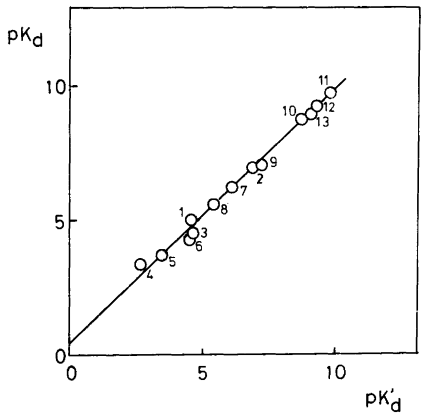


Fig. 2. The linear relationship between pK_d and pK'_d values derived from binding experiments or from bioassay on guinea-pig ileum, respectively.

to the receptor from rat brain and by measurements at the guinea pig ileum, respectively (Fig. 2). Thus the latter constants (pK'_d) also characterize the free energy of the ligand binding to the muscarinic receptor and may be used for structure-activity analysis.

From the pK'_d values and the π' values two series of compounds *d* and *e* were compiled. Fig. 3 shows that the same distinction of the intensity of the hydrophobic effect can be observed as in Fig. 1. Different ϕ-values are obtained for the two series *d* and *e* also in the case of the ileum receptor: $\phi_d = 0.8 \pm 0.3$ and $\phi_e = 1.5 \pm 0.2$. These differences seem to

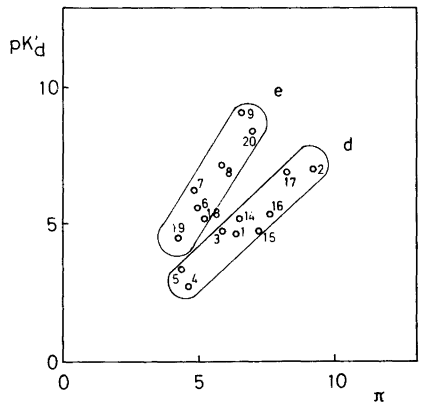


Fig. 3. The linear relationship between the pK'_d values and the hydrophobicity constants for the two series *d* and *e* of the compounds: Series *e* and *d* include series *a* and *b'*, respectively. Formulae of the compounds are given in Table 1.

be connected with the presence of the ester group in the antagonist molecule as this seems to be the main difference between the reaction series *a* and *d*, and series *b*, *c*, and *e*, respectively.

The intensity factor of the LFER's characterizes the mechanism of the appropriate interaction.[10,11] Therefore, proceeding from the ϕ-values obtained above, two different binding mechanisms for the muscarinic antagonists can be postulated.

The first binding mechanism, characterized by $\phi < 1$, corresponds to the simple extraction model of the hydrophobic interaction and is characteristic for many ligand binding processes to proteins with hydrophobic sites.[10]

The extra high affinity, characterized by $\phi > 1$, cannot be explained using this simple model, although such ϕ-values have been observed previously in the case of some enzyme-catalyzed reactions.[10,11] In these instances, multi-step processes were assumed and thus the observed ϕ-value for the overall process is an apparent constant consisting of several ϕ-increments; $\phi_{obs} = \sum_i \phi_i$. For all elementary steps $\phi_i < 1$ and thus the simple extraction model is valid for the individual steps.

In the light of these data the high intensity factor ϕ for some ester bond containing antagonists can be explained by a two-step binding mechanism, eqn. (3), where R, RA and RA* stand for the

$$R + A \overset{K_A}{\rightleftharpoons} RA \overset{K_i}{\rightleftharpoons} RA^* \qquad (3)$$

receptor, receptor–antagonist complex and its isomerized form. Indeed for some of the ester-type antagonists such as 3-quinuclidinyl benzilate and 4-*N*-methylpiperidinyl benzilate such a two-step binding mechanism has been established from kinetic studies.[12] One may hypothesize that both constants K_A and K_i could depend on hydrophobicity of the ligand to give $\phi_{obs} > 1$ when the observed dissociation constant is $K_d \approx K_A K_i$. Therefore further discussion of this binding mechanism calls first of all for thorough kinetic analysis of the antagonist binding reaction to obtain separately the constants K_A and K_i for the whole reaction series. It is possible that for the less active antagonists of the *a*-series of Fig. 1 the binding process involves only one step or there are other reasons why the isomerization constant K_i may be independent of the hydrophobicity of the ligand.

It should be noted furthermore that there is precisely twofold difference between the ϕ-values for the series *a* and *b*. A possible mechanism for such "double effects" of hydrophobic interaction in

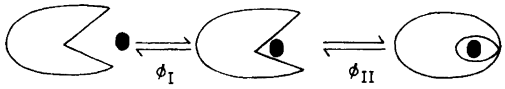


Fig. 4. The schematic picture of a ligand binding model involving isomerization of the receptor-ligand complex. The two reaction steps: binding and isomerization are characterized by intensity factors ϕ_I and ϕ_{II}, respectively.

enzyme catalyzed reactions has been offered by Aaviksaar *et al.*[11] and further discussed by Kljosov and Berezin.[10] This mechanism explains the "two-fold binding" of a ligand molecule in a hydrophobic site by a conformational change consisting of the "shutting" of the hydrophobic slit of the binding site on the protein (according to Fig. 4).

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