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Short Communications

Agonist – Antagonist – Muscarinic Receptor Ternary Complex *

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Binding to the muscarinic actylcholine receptor [1,2] from rat brain has been studied using radioactively labeled antagonists: [^{3}H]-3-quinuclidinyl benzilic acid ([^{3}H]-3-ONB),[3] *N*-[^{3}H]-methyl-4-piperidinyl benzilic acid ([^{3}H]-4-NMPB).[4] Our studies on the kinetics of the association of these ligands with the muscarinic receptor indicated that the binding process involves two consecutive equilibria.[5] We have expanded these studies to involve experiments with unlabeled agonists and labeled antagonists.[6] It was found that though agonists are able to fully block antagonist binding in equilibrium binding experiments they did not appear to influence the equilibrium constant of the first binding step of an antagonist to the receptor (R+A: Scheme 1). A tentative explanation of these results could be found by assuming that the agonist (B) could bind to the free receptor (R) and to the receptor antagonist complex (RA) with the same affinity. Such a scheme (Scheme 1) involves a ternary complex (RAB) of antagonist (A), agonist (B) and receptor (R).

We here report experiments that indicate that the structural prerequisite of a ternary complex, the presence of two binding sites, comes to expression also in equilibrium binding experiments using either [^{3}H]-antagonists ([^{3}H]-3-QNB or [^{3}H]-4-NMPB) or a mixture of [^{3}H]-agonist ([^{3}H]-pilocarpine) and [^{3}H]-antagonist ([^{3}H]-4-NMPB).

Table 1. Equilibrium binding constants of muscarinic antagonists to the muscarinic receptor from rat cerebral cortex.*

Ligand	Dissociation constants (nM) K_1	K_2
[^{3}H]-3-Quinuclidinyl benzilic acid	0.4	13.0
[^{3}H]-*N*-Methyl-4-piperidinyl benzilic acid	1.0	17.0

* The results of Fig. 1 were fitted to eqn. (1), where v stands for specifically bound ligand or determined graphically by means of double reciprocal plots.

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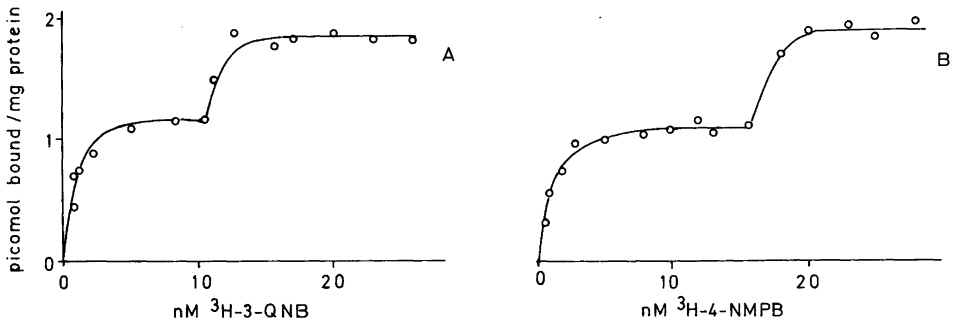

Fig. 1. Binding of A, [^{3}H]-3-QNB and B, [^{3}H]-4-NMPB to preparations of membranes from rat cerebral cortex.

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Table 2. Binding of [^{3}H]-pilocarpine and [^{3}H]-4-NMPB to muscarinic receptors in homogenates from rat cerebral cortex.*

Ligand	Specific binding/mol mg^{-1} (mean ± s.d.)
Experiment 1	
[^{3}H]-4-NMPB (10 nM)	0.43 (0.01)
[^{3}H]-4-NMPB (10 nM) + pilocarpine (100 nM; unlabeled)	0.38 (0.007)
[^{3}H]-4-NMPB (10 nM) + [^{3}H]-pilocarpine (100 nM)	1.08 (0.06)
[^{3}H]-pilocarpine (100 nM)	1.10 (0.2)
Experiment 2	
[^{3}H]-3-QNB (4 nM)	0.45 (0.01)
[^{3}H]-3-QNB (4 nM) + oxotremorine (100 μM)	0.02 (0.02)
[^{3}H]-3-QNB (4 nM) + carbamylcholine (1 mM)	0.03 (0.02)
[^{3}H]-3-QNB (16 nM)	0.85 (0.05)
[^{3}H]-3-QNB (16 nM) + oxotremorine (100 μM)	0.03 (0.2)

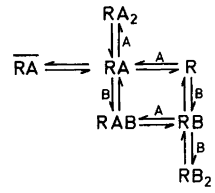
* Note that the specific binding capacity is lower than in Fig. 1 because here homogenates were used while there membranes were used.

$$v = \frac{V_1 L}{K_1 + L} + \frac{V_2 L}{K_2 + L} \quad (1)$$

Table 1 and Fig. 1 show the results of equilibrium binding studies with [^{3}H]-antagonists and the muscarinic receptor from rat cerebral cortex. Two plateaus were observed in the specific binding *versus* concentration of free [^{3}H]-antagonist curve when [^{3}H]-3-QNB, [^{3}H]-4-NMPB were present in concentration 0–50 nM. It appears from the data of Table 1 that at sufficiently high antagonist concentrations binding of a second antagonist molecule takes place to the receptor antagonist complex (RA) or to another protein. (Acetylcholinesterase is known to bind muscarinic ligands such as atropine, however, the dissociation constant for the enzyme-atropine complex is about 10^{-5} M[7], thus much larger than that found for the second binding site). Binding of [^{3}H]-3-QNB to both the high and low affinity sites could be fully blocked by muscarinic agonists such as oxotremorine (100 μM) in a competitive manner (*cf.* Table 2).

Experiments with [^{3}H]-labeled agonist and [^{3}H]-antagonists seem to provide more direct evidence for a ternary complex (Table 2). At [^{3}H]-4-NMPB concentrations (10–12 nM) that saturate the first binding site for this antagonist but not the second one (*cf.* Fig. 1 and Table 1) additional [^{3}H] binding was observed when [^{3}H]-pilocarpine was added to the [^{3}H]-4-NMPB receptor mixture.

We would like to suggest Scheme 1 as an explanation of the results of experiments on association rates [6] on equilibrium binding of [^{3}H]-antagonists to a lower affinity (second) site and on equilibrium binding of mixtures of [^{3}H]-labeled agonist and antagonist. It is suggested that agonists, like antagonists, may also bind to two sites on the receptor (RB_2).



Scheme 1. R, free receptor; A, antagonist; B, agonist; RA*, isomerized form of RA[5].

If Scheme 1 applies, the equation which relates the amount of bound [^{3}H]-antagonist (v) to the concentrations of receptor (R), agonist (B) and [^{3}H]-antagonist (A) at equilibrium for binding of both A and B is the following

$$v = \frac{K_1 A + K_2 AB + K_3 A^2}{K_4 + K_5 A + K_6 B + K_7 AB + K_8 A^2 + K_9 B^2}$$

where K_i are constants.

Experimental. Binding experiments and preparation of membranes from cerebral cortex of male Sprague-Dawley rats were carried out as described previously.[5]

[^{3}H]-4-NMPB (1.22 TBq/mmol) was generously supplied by Dr. Mordechai Sokolovsky, Tel Aviv University. [^{3}H]-Pilocarpine (370 GBq/mmol) and [^{3}H]-3-QNB (794 GBq/mmol) were purchased from New England Nuclear Co., Boston.

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