Short Communication

Determination of Thermodynamic Constants for Agonist Binding to Muscarinic Receptors from Rat Cerebral Cortex

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Binding of ligands to muscarinic receptors has been studied in great detail using several radiolabelled antagonists.[1,2,3] The temperature coefficients for the binding of antagonists to muscarinic receptors have also been investigated.[4,5,6] The data indicate that the binding of muscarinic antagonists is mainly entropy driven. The thermodynamics of muscarinic agonist binding have, however, not been studied. Data for agonist binding to other receptors, such as the beta-adrenergic receptor,[7] are, however, available.

In the study reported here, thermodynamic constants for some muscarinic cholinergic agonists have been calculated from competition curves obtained with the muscarinic antagonist [^{3}H]-*N*-methyl-4-piperidinyl benzilate (^{3}H-4-NMPB).[8] The results indicate that the binding of agonists is driven by changes in free energy and enthalpy, unlike that of antagonists which is largely entropy driven.[4,9]

Experimental

Ligand binding studies using homogenates of cerebral cortex from male Sprague Dawley rats (695 rpm, 15 up and down strokes in a glass/Teflon homogenizer) were carried out as described earlier.[10] In experiments with carbamylcholine, *d*-tubocurarine (10 μM) was present, and in experiments with acetylcholine, eserine (10 μM) and *d*-tubocurarine (10 μM) were present. Protein was incubated together with appropriate additions for 15 min at 37 °C, 30 min at 22 °C, 60 min at 15 °C and 90 min at 0 °C, these periods being sufficient for equilibrium to be attained (data not shown). IC_{50} for agonists was defined as the concentration of agonist giving 50 % inhibition of ^{3}H-4-NMPB binding at zero antagonist concentration. This value of "K_d" was calculated from the dependence of K_d on inhibitor concentration[11] [eqn. (1)]

$$K_d' = K_d\left(1 + \frac{I}{K_I}\right) \quad (1)$$

where K_d is the dissociation constant at zero antagonist concentration, K_d' (or IC_{50}) is the value determined for the agonist in the presence of I nM ^{3}H-4-NMPB and K_I is the dissociation constant for the 3H-4-NMPB-receptor complex at the appropriate temperature. Specific binding was defined as the difference in binding in the absence and the presence of 10 μM atropine. This was in all cases identical to the binding in the presence of 10 mM agonist.

Results and discussion

To determine whether the specific binding of ^{3}H-4-NMPB was affected by changes in assay, binding curves for ^{3}H-4-NMPB were determined for temperatures ranging from 0 °C to 37 °C. The affinity for ^{3}H-4-NMPB was approximately constant (K_d = 1 nM), while the binding capacity increased from 0.4 pmol at 0 °C to 0.6 pmol at 37 °C

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(data not shown). It can thus be concluded that any change in the affinity of muscarinic agonists with temperature, measured in competition with ^{3}H-4-NMPB, does not reflect the change in affinity for the ^{3}H-antagonist. To determine the effect of temperature on muscarinic agonist binding, curves for the competition between ^{3}H-4-NMPB and acetylcholine, carbamylcholine ("carbachol"), oxotremorine and pilocarpine, respectively, were determined for 0, 15, 22 and 37 °C. Although it is known that agonist binding is more complex than antagonist binding,[12] K_d was defined as the IC_{50} value for inhibition of ^{3}H-4-NMPB binding, since this is an estimate of the ability of the ligand to interact with muscarinic receptors in competition with a known muscarinic antagonist.[9] The K_d values for inhibition of ^{3}H-4-NMPB binding for the agonists are shown as a function of temperature (Fig. 1). The K_d values for all agonists increased with temperature for temperatures higher than 15–20 °C. These findings are in agreement with reports showing that muscarinic receptor-mediated responses (measured as increase in cGMP levels) are de-sensitized only at temperatures above 15–20 °C,[13] indicating increased conformational mobility of the membrane proteins.

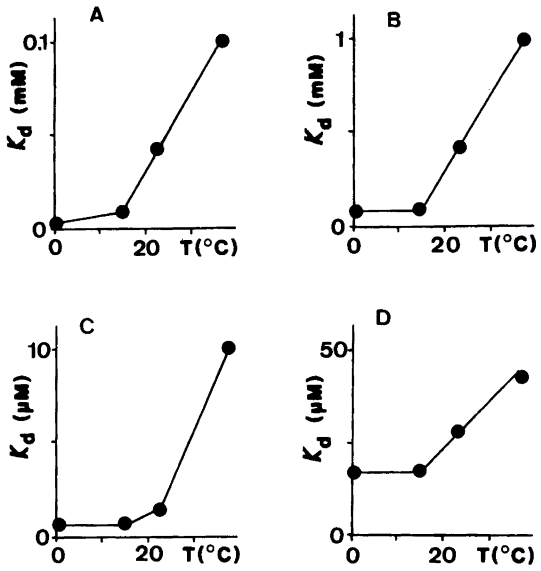

Fig. 1. IC_{50} (K_d) values for (A) acetylcholine, (B) carbamylcholine, (C) oxotremorine and (D) pilocarpine as a function of temperature.

The enthalpy change for the binding of the muscarinic agonists was determined using the integrated Van't Hoff equation [eqn. (2)]

$$\ln(1/K_d) = -\Delta H^\circ/(RT) + \Delta S^\circ/R \quad (2)$$

where K_d is the value for the agonist, ΔH° is the change in enthalpy, ΔS° the change in entropy, R is the gas constant (1.99 cal mol^{-1}) and T is the temperature in Kelvin. The Gibbs free energy of binding (ΔG°) can be calculated from eqn. (3). From the values of ΔH° and ΔG° the change in entropy, ΔS°,

$$\Delta G^\circ = -RT \ln K_d \quad (3)$$

upon binding of the agonist can be calculated using eqn. (4):

$$\Delta G^\circ = \Delta H^\circ - T\,\Delta S^\circ \quad (4)$$

The calculated values of ΔH°, ΔS° and ΔG° for acetylcholine, carbachol, oxotremorine and pilocarpine, respectively, are listed in Table 1. Binding of acetylcholine entails the largest enthalpy change, −25.1 kcal mol^{-1}, and there is a gradual decrease in the above order of agonists. Furthermore, the binding of each of these agonists involves a relatively large change in free energy (ΔG°), viz. from −5.6 kcal mol^{-1} to −7.9 kcal mol^{-1}.

Binding reactions in general are presumed to involve a large positive entropy change.[7] It is therefore reasonable to assume that binding of muscarinic agonists should also entail an increase in entropy. However, the opposite, a decrease in entropy, has been observed.[7] A proposed explanation for these findings[7] is that the agonist induces conformational changes in the receptor protein or in surrounding protein.[7] Binding of the muscarinic agonists involves an entropy change which is strongly negative for acetylcholine (−62.8 entropy units) and carbachol (−39.6 e.u.), slightly negative for oxotremorine (−4.8 e.u.) and positive for pilocarpine (+4.7 e.u.) (Table 1). Muscarinic agonists will most likely produce conformational changes in the receptor which are most pronounced for potent agonists such as acetylcholine and carbamylcholine. Earlier studies have also indicated that interconversions between different conformational states of the muscarinic receptor can take place.[14,15,16] It is

Table 1. Thermodynamic parameters for agonist binding to muscarinic cholinergic receptors from rat cerebral cortex. The parameter values were calculated for 37 °C.

Ligand	$\Delta G°$/ kcal mol^{-1}	$\Delta H°$/ kcal mol^{-1}	$\Delta S°$/ J mol^{-1} K^{-1}
Acetylcholine	−5.6	−25.1	−62.8
Carbamylcholine	−5.1	−17.4	−39.8
Oxotremorine	−7.9	−9.4	−4.8
Pilocarpine	−7.1	−5.6	+4.7

also interesting to note that the range of values for the entropy change associated with binding of muscarinic agonists is greater than for binding of beta-adrenergic agonists, for which only strongly negative values were found.[7] One possible explanation for this is that muscarinic agonists are less dependent on a conformational change of the receptor or of surrounding proteins to induce a cellular response. Conformational changes of the muscarinic receptor may be a mechanism for inactivation. An example of this is muscarinic antagonist-induced conformational changes of the receptor.[6] The observations presented here indicate that muscarinic agonists, like beta-adrenergic agonists, bind in such a manner that much of the associated free energy change can be explained by enthalpy changes. These enthalpy changes seem to be correlated to the efficacy of the agonist, the binding of a strong agonist such as acetylcholine producing a much larger enthalpy change (−25.1 kcal mol^{-1}) than that of a weak agonist such as pilocarpine (−5.6 kcal mol^{-1}). The binding of muscarinic antagonists, like beta-adrenergic antagonists,[7] appears to be entropy driven.[4,5,6]

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References

1. Birdsall, N. J. M., Burgen, A. S. V. and Hulme, E. C. *Mol. Pharmacol. 14* (1978) 723.
2. Heilbronn, E. and Bartfai, T. *Progr. Neurobiol. 11* (1978) 171.
3. Hulme, E. C., Birdsall, N. J. M., Burgen, A. S. V. and Metha, P. *Mol. Pharmacol. 14* (1980) 737.
4. Barlow, R. B., Birdsall, N. J. M. and Hulme, E. C. *Br. J. Pharmacol. 66* (1979) 587.
5. Almon, R. R. and Martner, J. E. *Mol. Pharmacol. 17* (1979) 141.
6. Järv, J. and Bartfai, T. *Acta Chem. Scand., Ser. B36* (1982) 487.
7. Weiland, G. A., Minneman, P. and Molinoff, P. B. *Nature (London) 281* (1979) 114.
8. Kloog, Y. and Sokolovsky, M. *Brain Res. 134* (1978) 167.
9. Kloog, Y. and Sokolovsky, M. *Brain Res. 144* (1978) 31.
10. Hedlund, B. and Bartfai, T. *Naunyn-Schmiedeberg's Arch. Pharmacol. 317* (1981) 126.
11. Wong, J. T.-F. *Kinetics of Enzyme Mechanisms,* Academic Press, New York 1975.
12. Hedlund, B., Grynfarb, M. and Bartfai, T. *Naunyn-Schmiedeberg's Arch. Pharmacol. 320* (1982) 3.
13. El-Fakahany, E. and Richelson, E. *J. Neurochem. 34* (1980) 1288.
14. Sokolovsky, M., Gurwitz, D, and Galron, R. *Biochem. Biophys. Res. Commun. 94* (1980) 487.
15. Gurwitz, D. and Sokolovsky, M. *Biochem. Biophys. Res. Commun. 94* (1980) 493.
16. Gurwitz, D. and Sokolovsky, M. *Biochem. Biophys. Res. Commun. 96* (1980) 1296.

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