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Selective enhancement of antagonist ligand binding at muscarinic M_2 receptors by heparin due to receptor uncoupling

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Abstract

The selectivity of heparin in inducing potentiation of binding of antagonist ligands to muscarinic receptors was investigated at the five known subtypes of muscarinic receptors. The effects of heparin on binding of [^{3}H]*N*-methylscopolamine at equilibrium was studied in Chinese hamster ovary (CHO) cells which express each of the individual muscarinic receptor subtypes and in membranes prepared from these cells. Heparin markedly increased equilibrium binding of subsaturating concentrations of the ligand only in membranes of CHO cells which express muscarinic M_2 receptors. These effects of heparin were qualitatively similar to those obtained in heart membranes. In contrast, heparin did not influence ligand binding to muscarinic M_2 receptors in intact cells. The positive cooperative effects of heparin at muscarinic receptors were abolished following treatment of cells with pertussis toxin. The latter treatment by itself resulted in a significant increase in [^{3}H]*N*-methylscopolamine binding. Taken together with previous reports of heparin-induced uncoupling of receptors and G-proteins, these data suggest that the effects of heparin on ligand binding to muscarinic M_2 receptors might be due to disruption of receptor-G-protein interactions which results in enhancement of binding of antagonist ligands to the receptor.

Keywords: Muscarinic receptor; Heparin; Receptor subtype; Transfected cell; Allosteric interaction; Receptor coupling

1. Introduction

There is a large series of compounds which influence the interaction of ligands at muscarinic acetylcholine receptors in an allosteric manner (Lee and El-Fakahany, 1991a). In a mechanistic sense, these compounds bind to the muscarinic receptor at a binding domain which is different from or partially overlapping with the binding domain of agonists and competitive antagonists (Lee and El-Fakahany, 1991a). There is evidence that these allosteric compounds and competitive agents simultaneously bind to the receptor to form a ternary complex, in which each of the two ligands influences the affinity of binding of the other to the receptor (Ehlert, 1987).

In most cases, such interaction results in decreased binding affinity of a competitive agent which interacts with the primary binding domain of the receptor (Lee and El-Fakahany, 1988; Ellis et al., 1991). In a few cases, however, binding of certain allosteric agents increases the affinity of binding of a competitive ligand to muscarinic receptors. The most well known example of this latter class of allosteric compounds is the neuromuscular blocker alcuronium (Tucek et al., 1990; Proska and Tucek, 1994; Jakubik and Tucek, 1994). The positive allosteric effects of alcuronium are more apparent in tissues which contain high concentrations of muscarinic M_2 receptors (Tucek et al., 1990). Another example of positive allosteric agents which has not been characterized as well is heparin. It has been shown by Ehlert and co-workers that heparin increases the affinity of binding of [^{3}H]*N*-methylscopolamine at cardiac muscarinic receptors (Gerstin et al., 1992). In this work, we investigated whether heparin exhibits selectivity in its interactions at the five known subtypes of muscarinic receptors expressed in Chinese hamster ovary (CHO) cells. Our results indicate marked selectivity of heparin at muscarinic M_2 receptors and that the binding site of heparin might not be on the cell surface. Furthermore, we provide evidence that the effects of heparin in potentiating ligand binding to the receptor might be the result of disruption of receptor-G-protein interactions.

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2. Materials and methods

2.1. Cell culture

CHO cell lines which individually express human M_1-M_5 muscarinic receptors in a stable fashion were grown in Dulbecco's modified Eagle medium containing 10% bovine calf serum and 0.005% geneticin as described in detail previously (Wang and El-Fakahany, 1993).

2.2. Preparation of cell membranes

CHO cells were detached using D1 solution (Hu and El-Fakahany, 1993) containing 0.05% trypsin. Cells were homogenized by Polytron (twice at 23 000 rpm for 15 s each) in a buffer composed of 30 mM Hepes, 0.5 mM EGTA and 100 mM NaCl (pH 7.4). The homogenate was centrifuged at $1000 \times g$ for 10 min, followed by centrifugation of the resulting supernatant at $30\,000 \times g$ for 30 min. The pellet was suspended in 30 mM Hepes/0.5 mM EGTA (pH 7.5).

2.3. Radioligand binding assay

Membranes were incubated with 0.1 nM [^{3}H]*N*-methylscopolamine in 1 ml of 30 mM Hepes, 0.5 mM EGTA and 5 mM $MgCl_2$ (pH 7.5). Incubations were for 1 h at 25°C in the absence and in the presence of different concentrations of heparin. Binding in intact cells was determined by incubating cells with the radioligand for 60 min at 37°C in Krebs-Henseleit buffer (see Wang and El-Fakahany, 1993 for composition). Nonspecific binding was defined in the presence of 2 μM atropine. Bound ligand was separated by filtration as described previously (Wang and El-Fakahany, 1993) and radioactivity was quantitated by liquid scintillation counting.

2.4. Data analysis

Dose-response curves were fitted according to a logistic four-parameter model using the computer program GraphPad (GraphPad, San Diego, CA). Statistical comparisons were performed using Student's *t*-test.

3. Results

3.1. Effects of heparin on ligand binding to different subtypes of muscarinic receptors expressed in CHO cells

The effects of heparin in increasing the specific binding of [^{3}H]*N*-methylscopolamine at various subtypes of muscarinic receptors was studied in membranes prepared from CHO cells which express the individual M_1-M_5 receptor subtypes. Interestingly, heparin-induced potentiation of [^{3}H]*N*-methylscopolamine binding was observed only at muscarinic M_2 receptors (Fig. 1). [^{3}H]*N*-Methylscopolamine binding at muscarinic M_2 receptors was increased to $173 \pm 7\%$ of control, with an average EC_{50} (concentration of heparin which resulted in 50% maximal increase in binding of [^{3}H]*N*-methylscopolamine) of 0.13 ± 0.02 μM (Fig. 1). These effects of heparin in membranes of CHO-M_2 cells were comparable in magnitude to those observed in cardiac membranes where heparin increases [^{3}H]*N*-methylscopolamine binding to a maximal value of $171 \pm 13\%$ of control (Fig. 1). However, heparin was slightly more potent in CHO-M_2 cells as compared to heart, since it exhibited an EC_{50} value of 0.4 ± 0.06 μM in the latter tissue. This value is slightly, but significantly, higher than that observed in CHO-M_2 cells ($P < 0.05$).

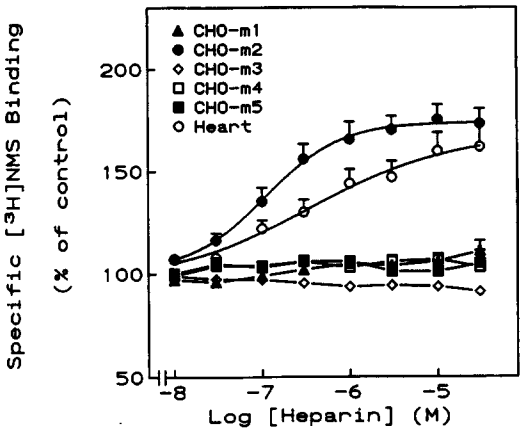

Fig. 1. Effects of heparin on [^{3}H]*N*-methylscopolamine binding at various subtypes of muscarinic receptors. Membranes of CHO cells which express each of the individual M_1-M_5 muscarinic receptor subtypes or cardiac membranes were incubated with 0.1 nM [^{3}H]*N*-methylscopolamine in the absence and in the presence of increasing concentrations of heparin as described under Methods. Data presented are the means + S.E.M. of three to eight independent experiments. [^{3}H]NMS, [^{3}H]*N*-methylscoploamine.

3.2. Comparison of the effects of heparin at muscarinic M_2 receptors in cell membranes and in intact cells

We compared the effects of heparin in enhancing the specific binding of [^{3}H]*N*-methylscopolamine at muscarinic M_2 receptors in intact CHO cells which express these receptors and in membranes of these cells. Surprisingly, the positive allosteric effects of heparin previously observed in CHO cell membranes were absent in intact cells (Fig. 2).

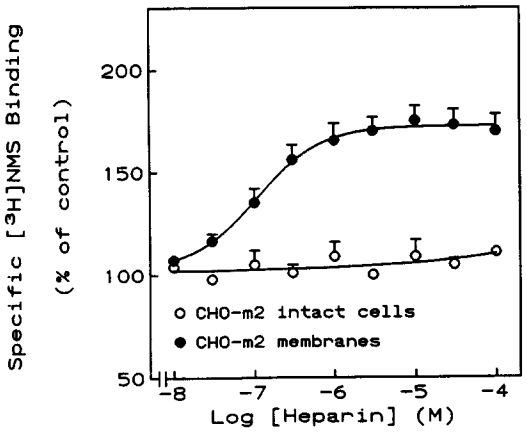

Fig. 2. Differential effects of heparin on [^{3}H]*N*-methylscopolamine binding in intact CHO-m2 cells and in cell membranes. CHO cells which express M_2 muscarinic receptors or membranes prepared from these cells were incubated with 0.1 nM [^{3}H]*N*-methylscopolamine in the absence or in the presence of increasing concentrations of heparin as described under Methods. Data are shown as the means + S.E.M. of three to eight independent experiments. [^{3}H]NMS, [^{3}H]*N*-methylscopolamine.

3.3. Requirement of intact receptor-G-protein coupling for the effects of heparin

One possible interpretation for the effectiveness of heparin in membranes but not in intact cells is that heparin might influence the conformation of the receptor by interacting with cytoplasmic receptor domains. Alternatively, heparin might interact with other intracellular proteins which in turn interact with muscarinic receptors, e.g. G-proteins. This would result in altering receptor conformation in an indirect manner. Two experimental designs were employed to study the latter possibility. The first was to test the effects of heparin in cells pretreated with pertussis toxin (100 ng/ml for 16–18 h) which causes uncoupling of muscarinic M_2 receptors from G-proteins (Eglen et al., 1988). In this case, pretreatment of cells with pertussis toxin under conditions which resulted in complete uncoupling of muscarinic M_2 receptors to inhibition of adenylate cyclase (data not shown) totally abolished the response to a maximally effective concentration of heparin (Fig. 3). The second experimental protocol was to compare the effects of heparin at wild-type muscarinic M_2 receptors and at a receptor mutant which exhibits dampened coupling to inhibition of adenylate cyclase (Zhu et al., 1994). The specific mutation studied in this case is a change of arginine 121 in the muscarinic M_2 receptor sequence to asparagine (Zhu et al., 1994). This residue is located at the beginning of the second intracellular loop of muscarinic M_2 receptors, and its mutation results in significant attenuation of receptor-mediated inhibition of cyclic AMP formation (Zhu et al., 1994). This mutation, however, did not result in

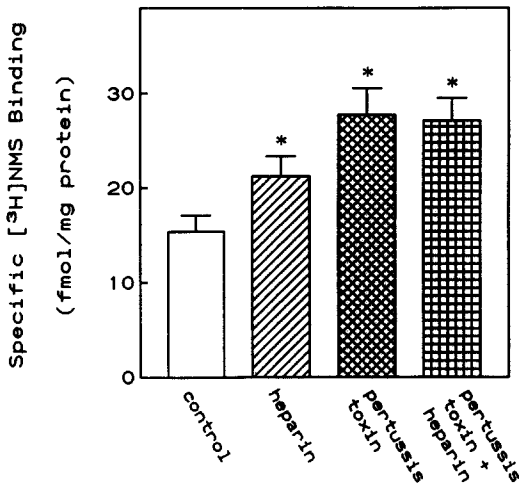

Fig. 3. Absence of heparin-induced enhancement of [^{3}H]*N*-methylscopolamine binding in membranes of CHO-m2 cells treated with pertussis toxin. CHO-m2 cells were incubated for 16–18 h with or without pertussis toxin (100 ng/ml). Cell membranes were prepared and incubated with 0.1 nM [^{3}H]*N*-methylscopolamine in the absence and in the presence of 100 μM heparin. Data are presented as the means + S.E.M. of six to ten independent experiments. * Significantly higher than the values obtained in control cells, $P < 0.05$. Heparin did not induce a statistically significant increase in [^{3}H]*N*-methylscopolamine binding in membranes of cells pretreated with pertussis toxin. [^{3}H]NMS, [^{3}H]*N*-methylscopolamine.

significant changes in the concentration-response curve of heparin in enhancing the binding of [^{3}H]*N*-methylscopolamine, either in terms of its potency or maximal effects (Fig. 4). Thus, heparin increased [^{3}H]*N*-methylscopolamine binding to a maximal of 153 ± 8% and

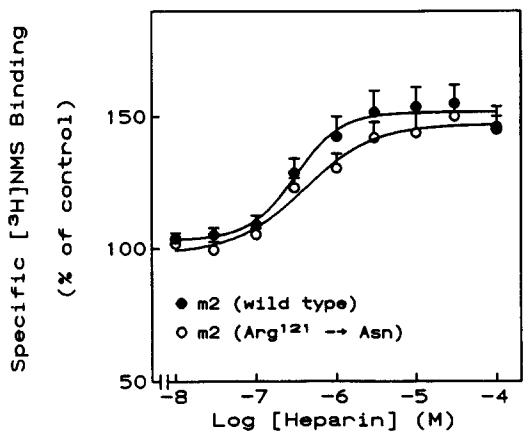

Fig. 4. Comparison of the effects of heparin on [^{3}H]*N*-methylscopolamine binding to wild-type and mutant muscarinic receptors. Membranes were prepared from CHO cells which express either wild-type M_2 muscarinic receptors or M_2 receptors in which the arginine (Arg) residue located at position 121 was mutated to asparagine (Asn), then incubated with 0.1 nM [^{3}H]*N*-methylscopolamine in the absence or in the presence of increasing concentrations of heparin. Data are presented as the means + S.E.M. of six or seven independent experiments. [^{3}H]NMS, [^{3}H]*N*-methylscopolamine.

$152 \pm 2\%$ of control at wild-type and mutant muscarinic M_2 receptors, respectively, with corresponding EC_{50} values of 0.4 ± 0.05 and 0.6 ± 0.2 μM (Fig. 4).

4. Discussion

The main findings of these studies are summarized as follows. First, heparin is selective for muscarinic M_2 receptors in terms of eliciting positive allosteric effects on ligand binding. Second, these effects of heparin are only apparent in broken cell preparations. Third, the effects of heparin on the binding of antagonist ligands to muscarinic M_2 receptors might be due to its ability to dissociate the receptor-G-protein complex.

The selectivity of heparin for muscarinic M_2 receptors shown in this study is in accord with the general higher sensitivity of this subtype of muscarinic receptors to allosteric modulation (Ellis et al., 1991; Lee and El-Fakahany, 1991b). The effects of heparin in enhancing binding of [^{3}H]*N*-methylscopolamine to muscarinic M_2 receptors was evident both in membranes of CHO cells which express muscarinic M_2 receptors and in heart which has been shown to contain only the M_2 subtype of muscarinic receptors (Waelbroeck et al., 1987). The results in heart are similar to those reported by Gerstin et al. (1992) who also demonstrated that heparin-induced increase in [^{3}H]*N*-methylscopolamine binding is due to an increase in ligand binding affinity without a change in maximal binding. The slight discrepancy in the potency of heparin in modulating [^{3}H]*N*-methylscopolamine binding in different tissues which express the same subtype of muscarinic receptors is reminiscent of similar observations in the case of other allosteric modulators of muscarinic receptors (Hu et al., 1992).

Huang et al. (1990) studied the effects of heparin and related compounds on ligand binding to α- and β-adrenoceptors. The main conclusion of their study was that heparin is able to cause uncoupling of receptors and G-proteins. This is supported by other reports that members of this class of polyanionic compounds attenuate agonist-mediated activation of G-proteins at α_2-adrenoceptors (Willuweit and Aktories, 1988) and opiate receptors (Butler et al., 1988). Gerstin et al. (1992) have also demonstrated that heparin, dextran and trypan blue uncouple cardiac muscarinic M_2 receptors from inhibition of adenylate cyclase. Huang et al. (1990) speculated that heparin might interact with the receptor at a domain which is involved in receptor coupling to G-proteins, or with the *C*-terminus of G-proteins which is believed to be the site of interaction with receptors. Both sites have been predicted to form an amphipathic α-helix, within which heparin and other polyanionic compounds would intercalate (Huang et al., 1990).

There is evidence that muscarinic M_2 receptors exist in a state where they are significantly precoupled to G-proteins in the absence of their activation by receptor agonists (Matesic et al., 1991). The precoupled state is favored under conditions similar to those required for the demonstration of heparin-induced increase in ligand binding at muscarinic M_2 receptors (Gerstin et al., 1992), i.e. low ionic strength and presence of Mg^{2+} (Hulme et al., 1981). It is likely that the observed enhancement of [^{3}H]*N*-methylscopolamine binding by heparin at muscarinic M_2 receptors is the result of uncoupling of receptor-G-protein interactions. This speculation is supported by the reported similar effects of uncoupling of receptors and G-proteins by GTP in enhancing binding of antagonist ligands to muscarinic receptors (Martin et al., 1984). Similarly, decreased receptor coupling to G-proteins in the presence of physiological sodium concentrations also results in enhanced binding of antagonist ligands at muscarinic receptors (Hulme et al., 1981). Further effects of heparin on ligand binding to muscarinic receptors are not observed under the latter conditions (Gerstin et al., 1992). Thus, the existence of significant receptor-G-protein interactions is a prerequisite for the demonstration of the effects of heparin. This might explain the complete disappearance of the effects of heparin at muscarinic M_2 receptors expressed in CHO cells following uncoupling of receptors from G-proteins by pertussis toxin treatment. In fact, this treatment by itself resulted in a significant increase in binding of [^{3}H]*N*-methylscopolamine to muscarinic receptors (Fig. 3). On the other hand, the effects of heparin in enhancing the binding of [^{3}H]*N*-methylscopolamine were not altered at mutant muscarinic M_2 receptors which are partially uncoupled from inhibition of adenylate cyclase (Zhu et al., 1994). This might be due to the existence of significant residual receptor-G-protein interactions at these receptor mutants. Other alternative interpretations, however, should be considered to explain the discrepancy in the results obtained using pertussis toxin or the receptor mutant. First, heparin might indirectly alter the conformation of the muscarinic receptor by binding to G-proteins coupled to this receptor. In this case, pertussis toxin-induced ADP-ribosylation of $G_{\alpha i}$ subunits coupled to muscarinic M_2 receptors might inhibit the binding of heparin to the G-protein. In contrast, heparin binding to G-proteins remains unaltered in the case of mutant receptors. Second, although this particular receptor mutation dampens receptor coupling to G-proteins upon receptor activation by agonists, the mutant receptor might still be precoupled to G-proteins in the absence of agonists. Thus, heparin-induced dissociation of this receptor-G-protein interaction under basal conditions would result in increased [^{3}H]*N*-methylscopolamine binding. A support for this interpretation

is that this particular mutation does not result in an enhancement of the affinity of binding of [^{3}H]*N*-methylscopolamine to the receptor (Zhu et al., 1994). This is in contrast to the increase in ligand binding observed following dissociation of the receptor-G-protein complex by pertussis toxin treatment (this work). The existence of a precoupled state of this mutant muscarinic M_2 receptor is suggested by the ability of pertussis toxin treatment to enhance the binding of 0.1 nM [^{3}H]*N*-methylscopolamine in cell membranes to 132% in comparison to the untreated group (not shown).

It is obvious, however, that the mechanism of modulation of receptor binding by heparin is different from that of other known agents that influence the conformation of muscarinic receptors in a positive cooperative fashion. For example, alcuronium is able to alter ligand binding at the primary binding site of the receptor both in intact cells and in broken cell preparations (unpublished observations). The absence of effects of heparin in intact cells suggests that heparin does not interact directly with the conventional ligand binding site on muscarinic receptors, but might instead influence ligand binding by interacting with an intracellular domain of the receptor. Alternatively, heparin might indirectly alter ligand binding by interacting with a cellular component which is coupled to the receptor, e.g. G-proteins. This might indirectly lead to changing the conformation of the receptor to make it more favorable for ligand binding.

It is to be noted, however, that the effects of heparin on ligand binding in intact cells and in membranes were studied in high and low ionic strength buffers, respectively. It is known that high ionic strength diminishes the effects of heparin on ligand binding to cardiac muscarinic receptors (Gerstin et al., 1992). We therefore compared the magnitude of heparin-induced enhancement of [^{3}H]*N*-methylscopolamine binding in membranes of CHO cells which express muscarinic M_2 receptors in the two buffers. These additional experiments showed that the effects of heparin were significantly dampened in Krebs buffer at 25°C and were completely abolished in this buffer at 37°C (data not shown). The latter incubation conditions are similar to those used in the case of intact cells. Thus, the apparent lack of effect of heparin on ligand binding in intact cells might be, at least in part, due to different incubation conditions. This hypothesis would have been substantiated by studying the effects of heparin in intact cells incubated in a low ionic strength buffer. This experiment, however, is not feasible, since cells will rupture under these conditions. Without this experiment, the diminished response to heparin in intact cells could be due to additional factors other than the use of a high ionic strength buffer. For example, muscarinic M_2 receptors might not be as tightly coupled to G-proteins in intact cells as compared to membranes, due to the presence of high intracellular GTP concentrations. This is supported by the observation that pertussis toxin treatment results in only a very small increase in [^{3}H]*N*-methylscopolamine binding to intact cells (not shown). Taken together, these observations complicate the interpretation of the lack of effects of heparin in intact cells in relation to its cellular site of action.

The question remains as to why heparin is so selective for the M_2 subtype of muscarinic receptors. One possible interpretation is that heparin recognizes intracellular sequences on the muscarinic receptor which are unique for the M_2 receptor subtype. Alternatively, heparin might selectively interact with a subset of G-proteins which are specifically recognized by muscarinic M_2 receptors, and this interaction indirectly results in alteration of receptor conformation. The latter interpretation is less likely due to the lack of effects of heparin on ligand binding to M_4 receptors. It is currently believed that both M_2 and M_4 muscarinic receptors are coupled to similar signal transduction pathways (Wang and El-Fakahany, 1993). A third possibility is that coupling of muscarinic M_2 receptors to G-proteins under resting conditions exhibits a higher affinity than that of muscarinic M_4 receptors. An argument against this possibility is that uncoupling of muscarinic M_4 receptors from G-proteins by pertussis toxin resulted in enhancing the binding of 0.1 nM [^{3}H]*N*-methylscopolamine to 152% in comparison with the untreated group (not shown). This is comparable to the effects of pertussis toxin on [^{3}H]*N*-methylscopolamine binding at muscarinic M_2 receptors. Thus, these results suggest the existence of significant interaction of muscarinic M_4 receptors and G-proteins, similar to that of M_2 receptors, under basal conditions.

In summary, our data demonstrate a high selectivity for heparin at the M_2 subtype of muscarinic receptors in terms of its ability to enhance binding of antagonist ligands to the receptor. There is evidence that the effects of heparin on ligand binding might be the result of its ability to dissociate receptor-G-protein interactions in a receptor subtype-specific manner.

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References

Butler, S.J., E. Kelly, F. McKenzie, S. Guild, M. Wakelam and G. Milligan, 1988, Differential effects of suramin on the coupling of receptors to individual species of pertussis toxin sensitive guanine

nucleotide binding proteins, Biochem. J. 251, 201.
Eglen, E.M., W.W. Montgomery and R.L. Whiting, 1988, Negative and positive inotropic responses to muscarinic agonists in guinea pig and rat atria in vitro, J. Pharmacol. Exp. Ther. 247, 911.
Ehlert, F.J., 1987, Estimation of the affinities of allosteric ligands using radioligand binding and pharmacological null methods, Mol. Pharmacol. 33, 187.
Ellis, J., J. Huyler and M.R. Brann, 1991, Allosteric regulation of cloned m1-m5 muscarinic receptor subtypes, Biochem. Pharmacol. 42, 1927.
Gerstin, E.H., T. Luong and F.J. Ehlert, 1992, Heparin, dextran and trypan blue allosterically modulate M_2 muscarinic receptor binding properties and interfere with receptor-mediated inhibition of adenylate cyclase, J. Pharmacol. Exp. Ther. 263, 910.
Hu, J. and E.E. El-Fakahany, 1993, Role of intercellular and intracellular communication by nitric oxide in coupling of muscarinic receptors to activation of guanylate cyclase in neuronal cells, J. Neurochem. 61, 578.
Hu, J., S.Z. Wang, C. Forray and E.E. El-Fakahany, 1992, Complex allosteric modulation of cardiac muscarinic receptors by protamine: potential model for putative endogenous ligands, Mol. Pharmacol. 42, 311.
Huang, R.R.C., R.N. Dehaven, A.H. Cheung, R.E. Diehl, R.A.F. Dixon and C.D. Strader, 1990, Identification of allosteric antagonists of receptor-guanine nucleotide-binding protein interactions, Mol. Pharmacol. 37, 304.
Hulme, E.C., C.P. Berrie, N.J.M. Birdsall and A.S.V. Burgen, 1981, Two populations of binding sites for muscarinic antagonists in the rat heart, Eur. J. Pharmacol. 73, 137.
Jakubik, J. and S. Tucek, 1994, Protection by alcuronium of muscarinic receptors against chemical inactivation and location of the allosteric binding site for alcuronium, J. Neurochem. 63, 1932.
Lee, N.H. and E.E. El-Fakahany, 1988, Influence of ligand choice on the apparent binding profile of gallamine to cardiac muscarinic receptors; Identification of three main types of gallamine-muscarinic receptor interactions, J. Pharmacol. Exp. Ther. 246, 829.
Lee, N.H. and E.E. El-Fakahany, 1991a, Allosteric antagonists of the muscarinic acetylcholine receptor, Biochem. Pharmacol. 42, 199.
Lee, N.H. and E.E. El-Fakahany, 1991b, Allosteric interactions at the m1, m2 and m3 muscarinic receptor subtypes, J. Pharmacol. Exp. Ther. 256, 468.
Martin, M.W., M.M. Smith and T.K. Harden, 1984, Modulation of muscarinic cholinergic receptor affinity for antagonists in rat heart, J. Pharmacol. Exp. Ther. 230, 424.
Matesic, D.F., D.R. Manning, G.R. Luthin, 1991, Tissue-dependent association of muscarinic acetylcholine receptors with guanine nucleotide-binding regulatory proteins, Mol. Pharmacol. 40, 347.
Proska, J. and S. Tucek, 1994, Mechanisms of steric and cooperative actions of alcuronium on cardiac muscarinic acetylcholine receptors, Mol. Pharmacol. 45, 709.
Tucek, S., J. Musilkova, J. Nedoma, J. Proska, S. Shelkovnikov and J. Vorlicek, 1990, Positive cooperativity in the binding of alcuronium and *N*-methylscopolamine to muscarinic acetylcholine receptors, Mol. Pharmacol. 38, 674.
Waelbroeck, M., M. Gillard, P. Robberecht and J. Christophe, 1987, Muscarinic receptor heterogeneity in rat central nervous system. I. Binding of four selective antagonists to three muscarinic receptor subclasses: a comparison with M2 cardiac muscarinic receptors of the C type, Mol. Pharmacol. 32, 91.
Wang, S.Z. and E.E. El-Fakahany, 1993, Application of transfected cell lines in studies of functional receptor subtype selectivity of muscarinic agonists, J. Pharmacol. Exp. Ther. 266, 237.
Willuweit, B. and K. Aktories, 1988, Heparin uncouples α_2-adrenoceptors from the G_i-protein in membranes of human platelets, Biochem. J. 249, 857.
Zhu, S.Z., S.Z. Wang, J. Hu and E.E. El-Fakahany, 1994, An arginine residue conserved in most G protein-coupled receptors is essential for the function of the m1 muscarinic receptor, Mol. Pharmacol. 45, 517.