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Short communication

Pharmacological analysis of the mode of interaction of McN-A-343 at atrial muscarinic M_2 receptors

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Abstract

The mode of interaction of the muscarinic M_2 receptor partial agonist, McN-A-343 (4-(*m*-chlorophenylcarbamoyloxy)-2-butynyltrimethylammonium), was investigated in the electrically-driven guinea-pig left atrium. Comparison of the negative inotropic responses to this agent with those of the full agonist, carbachol, followed by operational model-fitting, yielded estimates of the dissociation constant (K_A) and efficacy (τ) of McN-A-343. Following partial receptor inactivation with propylbenzylcholine mustard, the properties of McN-A-343 as an inhibitor of the carbachol-mediated responses were assessed. Analysis of the data, according to both competitive and allosteric models of interaction, favored the former model. The log K_B of McN-A-343 as an antagonist was -4.65 ± 0.06, and was not significantly different from the log K_A of this agent as an agonist. © 1997 Elsevier Science B.V.

Keywords: McN-A-343; Muscarinic receptors; Operational model; Allosteric interaction

1. Introduction

The ability of a diverse range of compounds to allosterically modulate the binding of classical, or orthosteric, ligands at all five subtypes of the muscarinic acetylcholine receptors has been acknowledged for some time (see Lee and El-Fakahany, 1991; Tucek and Proska, 1995). To date, the majority of ligands identified as allosteric modulators have been found to act as inhibitors of functional responses mediated by muscarinic receptors. However, evidence has also been presented for a possible allosteric mode of interaction of the agonist, (4-(*m*-chlorophenylcarbamoyloxy)-2-butynyltrimethylammonium) (McN-A-343). Birdsall et al. (1983) employed a radioligand binding experimental paradigm in rat cerebral cortex and myocardium to explore the interaction between McN-A-343 and [^{3}H]*N*-methylscopolamine. In these experiments, where increasing concentrations of [^{3}H]*N*-methylscopolamine were utilized, McN-A-343 demonstrated a progressive inability to maximally inhibit radioligand binding, a hallmark of negative allosteric modulation (see Lee and El-Fakahany, 1991). This phenomenon was most marked in rat myocardium. In contrast, a more recent binding study by Waelbroeck (1994) found no evidence for McN-A-343 recognizing an accessory site on rat cardiac muscarinic receptors.

Although radioligand binding studies have been employed more frequently than traditional isolated tissue experiments in the delineation and quantification of allosteric phenomena, functional verification of allosteric interactions lies at the heart of the potential therapeutic exploitation of modulating agents. Furthermore, very few studies have addressed the consequences of allosteric perturbation on muscarinic receptor-G protein coupling (but see Jakubík et al., 1996), yet the possibility of receptor activation via the allosteric binding site would represent a novel and significant therapeutic target in disorders of impaired signalling via the orthosteric binding site.

Thus, the aim of the current study was to employ a functional experimental paradigm to further evaluate the mode of interaction of McN-A-343 at guinea-pig atrial muscarinic M_2 receptors.

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2. Materials and methods

Guinea-pig atria were prepared as described previously (Christopoulos and Mitchelson, 1994) and suspended between two platinum wire electrodes in a 20 ml organ bath containing Krebs–Henseleit buffer (Lanzafame et al., 1996). Inotropic responses to electrical stimulation were measured as described previously (Lanzafame et al., 1996).

Cumulative concentration–response curves for the negative inotropic effects of carbachol (Sigma, St. Louis) and McN-A-343 (Research Biochemicals, Natick) were established, with washing and a 15 min rest period between establishment of each curve.

After washout and a further 15 min rest period, 3 μM propylbenzylcholine mustard (DuPont, USA) was added to the bath for 30 min, in order to irreversibly alkylate a portion of the muscarinic receptors in the tissue. Subsequently, the preparation was washed regularly over a 60 min period after which no responses to McN-A-343 were observed. This compound was then employed as an inhibitor of responses to carbachol. Each concentration of McN-A-343 (30–500 μM) was allowed to equilibrate with the tissue for 60 min including washing and replacement every 20 min.

The properties of McN-A-343 as a partial agonist of guinea-pig atrial muscarinic receptors were assessed by simultaneously fitting each control pair of McN-A-343 and carbachol concentration–response curves generated per tissue to the operational model of agonism (Black and Leff, 1983), utilizing Eqs. (1) and (2), respectively:

$$E = \frac{E_m \tau^n [A]^n}{(K_A + [A])^n + \tau^n [A]^n}, \quad (1)$$

$$E = \frac{E_m [A]^n}{EC_{50}^n + [A]^n}, \quad (2)$$

where E is the effect, expressed as percentage inhibition, [A] is the concentration of agonist, EC_{50} is the concentration of the full agonist resulting in half the maximum effect, E_m is the maximum possible response of the tissue, K_A is the agonist–receptor equilibrium dissociation constant, n is the slope of the transducer function linking occupancy to response, and τ is the operational definition of efficacy.

After partial receptor alkylation, the properties of McN-A-343 as a competitive antagonist or as an allosteric inhibitor were assessed by simultaneously analyzing each family of concentration–response curves generated per tissue according to Eqs. (3) and (4), respectively (Lazareno and Birdsall, 1993, 1995):

$$E = \text{Basal} + \frac{E_{max} - \text{Basal}}{1 + \left\{[EC_{50}]\left([B]^s / K_B + 1\right)/[A]\right\}^n}. \quad (3)$$

$$E = \text{Basal} + \frac{E_{max} - \text{Basal}}{1 + \{[EC_{50}]\{([Z] + K_Z)/([Z]/\alpha + K_Z)\}/[A]\}^n}. \quad (4)$$

where E_{max} and Basal refer to the maximum and threshold (if any) response values in the tissue, [A] is the concentration of agonist, n is the slope of the concentration–response curve, [B] is the concentration of competitive antagonist, [Z] is the concentration of allosteric inhibitor, K_B is the competitive antagonist–receptor equilibrium dissociation constant and K_Z is the allosteric inhibitor–receptor equilibrium dissociation constant. In Eq. (3), s is equivalent to the slope of a Schild plot. In Eq. (4), the symbol α, denotes the co-operativity factor (Ehlert, 1988) for the interaction between an allosteric and orthosteric ligand, and is a quantitative estimate of the maximum reciprocal alteration of affinity that each ligand exerts on the other.

Constrained, simultaneous curve-fitting was performed using the program Microsoft Excel. Data are given as mean ± SEM. The standard errors derived from the fitting procedure, corresponding to internal fitting error, were utilized in the assessment of goodness-of-fit (Leff et al., 1990). Comparisons of the dissociation constants obtained from operational model fitting and logistic/Schild (Eq. (3)) curve-fitting were made by Student's t-test (two-tailed) with the level of significance set as $P < 0.05$.

3. Results

In the electrically-driven guinea-pig atrium, McN-A-343 behaved as a partial agonist, causing a maximal inhibitory effect corresponding to approximately 30% of the carbachol maximum (Fig. 1). Also shown in Fig. 1 are the results of operational model-fitting of the data, for the mean of eight different experiments.

After partial receptor alkylation with propylbenzyl-

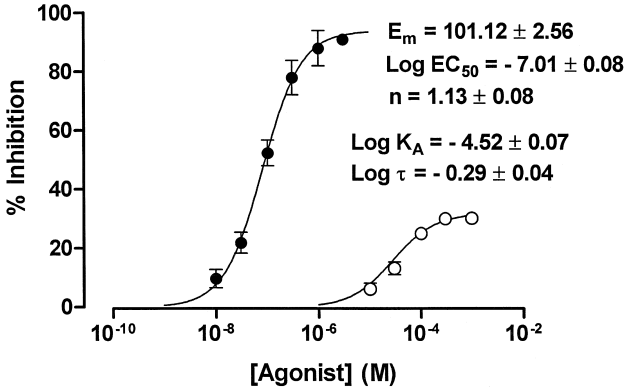

Fig. 1. Constrained, simultaneous analysis of the concentration–response curves to the agonists McN-A-343 (○) and carbachol (●) in the electrically-driven guinea-pig left atrium, according to the operational model of agonism (Eqs. (1) and (2), respectively in Section 2). The values of the parameters (mean ± SEM; $n = 8$) are shown on the graph. Where error bars are not shown, they lie within the dimensions of the symbol.

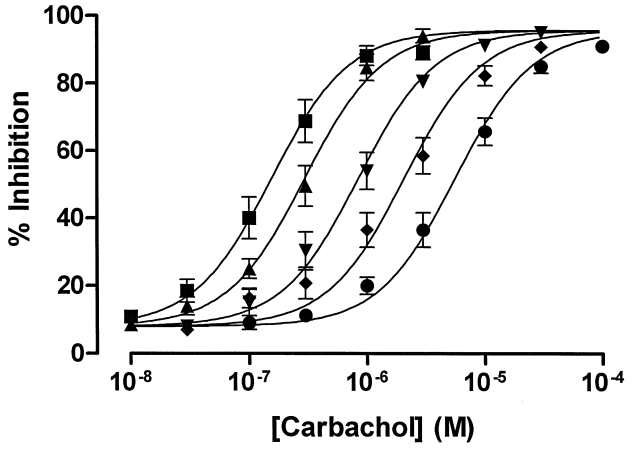

Fig. 2. Constrained, simultaneous analysis of the carbachol concentration–response curves, in the electrically-driven guinea-pig left atrium, in the absence (■) and presence of McN-A-343, 30 (▲), 100 (▼), 300 (◆), and 500 (●) μM, according to Eq. (3) in Section 2. The Schild slope factor was estimated as 1.14 ± 0.14 ($n = 8$), and was not significantly different from unity ($P > 0.05$). The log K_B of McN-A-343 was estimated as -4.65 ± 0.06. Where error bars are not shown, they lie within the dimensions of the symbol.

choline mustard, which resulted in the abolition of the agonistic properties of McN-A-343, the latter agent was utilized as an inhibitor of the negative inotropic responses to carbachol, producing parallel, dextral shifts of the carbachol concentration–response curves. The results of constrained, simultaneous analysis of the data from each tissue, according to Eq. (3), are shown in Fig. 2. The slope parameter, s, was $1.14 + 0.14$ ($n = 8$) and was not significantly different from unity ($P > 0.05$). Constraining this value to unity allowed for estimation of the log K_B (-4.65 ± 0.06), which was not significantly different from the log K_A estimated from operational model-fitting ($P > 0.05$).

The same data were also analyzed according to a model of allosteric interaction (Eq. (4)). Although the data were able to be fitted to this model, where log $K_Z = -4.72 \pm 0.08$, large degeneracies were noted in the estimation of the negative co-operativity factor, α. The values estimated after each fit were > 1000, and were highly dependent on the initial estimate. Standard error estimation was not attempted, as it was concluded that the α parameter was redundant. Under these circumstances, Eq. (4) becomes indistinguishable from Eq. (3), itself based on the simpler (competitive) model of interaction.

4. Discussion

Simultaneous operational model-fitting of the carbachol and McN-A-343 concentration–response curves in guinea-pig atria confirmed the weak partial agonist activity of the latter agent. The finding of a mean log K_A of -4.52 is in general agreement with previous affinity estimates for McN-A-343 at M_2 receptors (Elnatan and Mitchelson, 1993), obtained using a technique whereby partial agonists modify responses to full agonists. As both agonists were tested in the same preparation, and as carbachol has been shown to possess a large effective receptor reserve in this tissue (Furchgott, 1978), the low τ value (0.51) derived from the present analysis is indicative of poor intrinsic efficacy of McN-A-343 at the M_2 subtype. This finding is consistent with previous reports (Elnatan and Mitchelson, 1993; Wang and El-Fakahany, 1993).

The comparative method (Barlow et al., 1967) for estimating agonist affinity and relative efficacy, as utilized in this study, assumes that both the full and partial agonist activate the same receptor, via a shared binding site, in the generation of their associated responses. A most intriguing finding, however, was made by Birdsall et al. (1983) from radioligand binding studies in rat cerebral cortex and, particularly, rat myocardium. In the latter tissue, analysis of the McN-A-343/[^{3}H]*N*-methylscopolamine inhibition binding isotherms, according to an allosteric ternary complex model, adequately fitted the data with an α value of approximately 40. However, more recent binding experiments by Waelbroeck (1994) were contradictory.

The possibility that muscarinic receptors may be activated via their allosteric sites represents an alternative means of exploiting receptor signalling and subtype selectivity, in a therapeutic sense. In the current study, however, simultaneous analysis of the data sets generated for the inhibition of the carbachol-mediated negative inotropic responses by increasing concentrations of McN-A-343, found no evidence for deviation from simple competitive behavior. The estimation of the affinity of McN-A-343, firstly as a partial agonist and, subsequently, as an antagonist in the same preparation further served as an internal check of the two modes of analysis. Application of an allosteric model of interaction yielded insufficient convergence with regards to the estimate of the cooperativity factor, α, as the generated values were highly dependent on the initial estimate. Obviously, the regression algorithm could not apportion a value between the parameters α and K_Z. This type of parameter redundancy should be taken as grounds for rejecting the model for a simpler one (Motulsky and Ransnas, 1987).

It is possible that the range of McN-A-343 concentrations employed in the present study were not sufficient for detecting high degrees of negative co-operativity. However, if the estimate of $\alpha = 40$ from Birdsall et al. (1983) is used, smaller shifts than those observed would have been expected. For example, a concentration of 500 μM McN-A-343, which yielded a shift of the control carbachol concentration–response curve of approximately 36-fold should have yielded a shift of approximately 20-fold, based on Eq. (4) above. It is also possible, however, that the interaction between McN-A-343 and carbachol may be characterized by a higher degree of negative co-operativity than the interaction between McN-A-343 and [^{3}H]*N*-meth-

ylscopolamine. Additionally, as the detection of allosteric phenomena is more evident in low ionic strength media (Waelbroeck, 1994), the physiological buffer utilized in the current study may have predisposed the interacting ligands towards higher degrees of negative co-operativity, and thus to behave in a manner indistinguishable from orthosteric competition.

In conclusion, although previous work has been presented to suggest an allosteric mode of interaction for McN-A-343 with cardiac muscarinic receptors, the present pharmacological study, employing McN-A-343 as both partial agonist and antagonist, found no deviation from simple, competitive behavior.

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