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The effect of McN-A-343 on muscarinic receptors in the cerebral cortex and heart

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McN-A-343 behaves as a competitive agonist in binding to muscarinic receptors in the cerebral cortex. In its interaction with myocardial muscarinic receptors it is not competitive but it retains features of agonist binding.

Introduction McN-A-343 (3-(*m*-chlorophenylcarbamoyloxy)-2-butynyltrimethylammonium) chloride increases the blood pressure and heart rate, both responses being readily blocked by atropine (Roszkowski, 1961; Fozard & Muscholl, 1972). This unusual combination of effects of a muscarinic agonist has been explained as a selective stimulatory muscarinic effect on sympathetic ganglia which overcomes the much weaker direct muscarinic effects on the heart and vasculature. In this paper we describe a preliminary investigation of the effect of McN-A-343 on muscarinic receptors in membrane preparations from the cerebral cortex and myocardium of the rat.

Methods All manipulations and assays were the same as described previously (Hammer, Berrie, Birdsall, Burgen & Hulme, 1980). The buffer used was 100 mM NaCl, 10 mM $MgCl_2$, 20 mM HEPES (pH 7.0) and the radioligand, [^{3}H]-N-methylscopolamine ([^{3}H]-NMS) specific activity 53 Ci/mmol. Incubation was carried out for 2 h at 30°C and terminated by centrifugation as described previously (Hulme, Birdsall, Burgen & Mehta, 1978). Data were analysed by non-linear least squares regression analysis using appropriate models.

Results In Figure 1a, the effect of McN-A-343 in inhibiting the binding of [^{3}H]-NMS to muscarinic receptors in the cerebral cortex is shown. The specific binding of [^{3}H]-NMS is completely suppressed by a high concentration of McN-A-343. However, the curve on the left does not fit a simple mass action relationship (Hill coefficient 0.8) but can be fitted adequately by assuming the presence of low and high affinity sites for McN-A-343, as has been described for other muscarinic agonists (Birdsall, Burgen & Hulme, 1978; Birdsall, Hulme & Burgen, 1980). The non-linear least squares fit is given by $45 \pm 12\%$ of the sites being of low affinity ($6.0 \pm 1.4 \times 10^4$ M^{-1}), and the remainder having high affinity ($4.0 \pm 0.8 \times 10^5$ M^{-1}). There are a small proportion of sites in the cerebral cortex which have super-high affinities for agonists (Birdsall *et al.*, 1978) and these can be assessed by competition of McN-A-343 with a low-concentration of the potent agonist [^{3}H]-oxotremorine-M ($< 10^{-9}$ M). The affinity so found is $2.2 \pm 0.2 \times 10^6$ M^{-1}. McN-A-343 thus appears to behave like other poor and partial agonists in that it has a rather small overall range of affinities (37 fold) for the sub-populations of agonist binding sites (Birdsall *et al.*, 1980). The curve to the left in Figure 1a was determined at a low concentration of [^{3}H]-NMS (9×10^{-11} M) and low receptor occupancy (0.26). The curve on the right was determined at a [^{3}H]-NMS concentration of 7.9×10^{-9} M at which the occupancy by [^{3}H]-NMS was 0.97. If the NMS and McN-A-343 interaction is competitive, one would predict a parallel shift of 24 fold between the curves; the measured shift of 26 fold is in satisfactory agreement.

Similar experiments were carried out on a homogenate of rat heart (Figure 1b). The binding curve on the left of the figure was determined in the presence of 9×10^{-11} M [^{3}H]-NMS. It can be seen that the specific binding of [^{3}H]-NMS is not completely suppressed but a plateau is reached at 96% inhibition. The right-hand inhibition curve was obtained with 3.4×10^{-8} M [^{3}H]-NMS and in this case the maximum inhibition was 57%. At an intermediate [^{3}H]-NMS concentration (2.8×10^{-9} M), the plateau was at 92%. It is evident, therefore, that McN-A-343 does not behave as a simple competitive ligand in its interactions with NMS and muscarinic receptors in the myocardium.

Discussion We have recently reported that gallamine interacts with the muscarinic receptor by a non-competitive mechanism which can be accounted for by a model in which gallamine can form a ternary complex with the receptor and a conventional antagonist or agonist (Birdsall, Burgen, Hulme &

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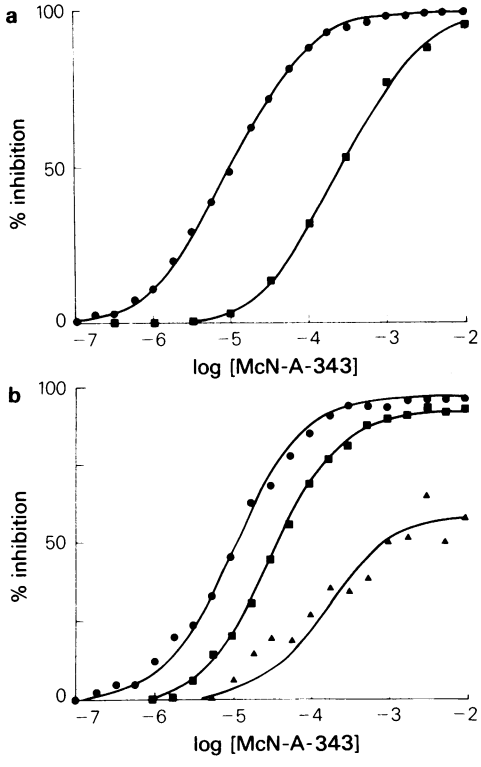

Figure 1 Inhibition by McN-A-343 of the binding of [^{3}H]-N-methylscopolamine to muscarinic receptors on membrane preparations from (a) rat cerebral cortex and (b) rat myocardium. (a) The concentrations of [^{3}H]-NMS were 9×10^{-11} M (●) and 7.9×10^{-9} M (■). The curve through the data points at the lower concentration of [^{3}H]-NMS is a non-linear least squares fit to a 2-site model with $45 \pm 12\%$ of the sites of low affinity ($6.0 \pm 1.8 \times 10^4 M^{-1}$), the remaining sites having a higher affinity ($4.0 \pm 0.8 \times 10^5 M^{-1}$). The curve through the data points at 7.9 nM [^{3}H]-NMS is the same curve shifted by a factor of 26. In this experiment, the affinity constant for [^{3}H]-NMS was $4.0 \times 10^9 M^{-1}$ (determined independently). (b) The concentrations of [^{3}H]-NMS were 1.0×10^{-10} M (●), 2.8×10^{-9} M (■) and 3.4×10^{-8} M (▲). The curves drawn through the data points are derived from the best fit values to the allosteric model, with the affinity of McN-A-343 ($9.9 \times 10^4 M^{-1}$) in the absence of NMS, ($2.4 \times 10^3 M^{-1}$) in the presence of a saturating concentration of NMS, the affinity of NMS being $8.7 \times 10^8 M^{-1}$.

Stockton, 1981). Through an allosteric mechanism, the affinities of both gallamine and the second ligand are reduced in this complex. The inhibition of binding of a ligand (e.g. [^{3}H]-NMS) is therefore due to a negative co-operative interaction rather than to competition. This mechanism of interaction of gallamine has been found to be qualitatively similar in the cortex and heart. Applying this approach to the data in Figure 1b gives the curves drawn in Figure 1b which are derived from the best fit values for the binding of McN-A-343 of $9.9 \times 10^4 M^{-1}$ in the absence of NMS and 2.4×10^3 in the presence of a saturating concentration of NMS, the negative co-operativity being ca. 40.

The affinity of ordinary muscarinic agonists (but not antagonists) in the heart is substantially reduced by guanine nucleotides (Berrie, Birdsall, Burgen & Hulme, 1979; Rosenberger, Roeske & Yamamura, 1979; Wei & Sulakhe, 1979) and this applies to McN-A-343 whose affinity is reduced by a factor of 4–6 by 10^{-4} M 5′-guanylylimidodiphosphate (GMPPNHP). However, it still does not develop a competitive type of binding interaction. There is little effect of GMPPNHP on the binding of McN-A-343 in the cortex, but this is also the case with other muscarinic agonists.

There is thus the interesting paradox that McN-A-343 is apparently binding to different types of site in these two tissues. It is important, however, to bear in mind that when negative co-operativity is very great, it becomes indistinguishable from competition except at very high ligand concentrations which may not be attainable under experimental conditions. If the findings in the cortex are due to the allosteric mechanism, the negative co-operativity would have to be in excess of 1500. Further work will be needed to settle this mechanism.

Nevertheless, in the heart McN-A-343 is an agonist and hence on binding must lead to the generation of the active state of the muscarinic receptor. Since it binds to a site distinct from that occupied by NMS on the receptor, this suggests that binding to this site can activate the receptor and that gallamine and McN-A-343 may behave as an antagonist-agonist pair.

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