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GUANINE NUCLEOTIDES MODULATE MUSCARINIC RECEPTOR BINDING IN THE HEART

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SUMMARY

The binding properties of muscarinic receptors in homogenates of the rat myocardium are altered in the presence of guanine nucleotides. There is a small increase in the affinity constant of the antagonist, [^{3}H]-N-methylscopolamine, and a large decrease in agonist binding. The most potent nucleotide is Gpp(NH)p, with GTP and GDP both being about ten times less active. The effect of GTP on agonist binding is transient whereas that of Gpp(NH)p is more persistent.

INTRODUCTION

Guanine nucleotides have been shown to modulate the binding of agonists to several neurotransmitter and hormonal receptors such as the β-adrenergic receptor in cultured cell lines, amphibian erythrocytes and canine myocardium (1-4) and α-adrenergic, dopamine, and opiate receptors in brain (5-9). In all these cases the affinity for agonists is reduced with minimal change in the affinity for antagonists. In this paper we describe a similar effect of guanine nucleotides on the binding of agonists and antagonists to muscarinic receptors in homogenates of rat myocardium.

MATERIALS AND METHODS

Rat hearts were perfused in situ with ice-cold isotonic saline, excised, and homogenised in 9 volumes of 0.32M sucrose buffered with 20 mM Na-HEPES pH 7.0 at 0°. If required, atria and ventricles were processed separately. Preliminary homogenisation of ventricular tissue was carried out in a

Abbreviations: NMS - N-methylscopolamine

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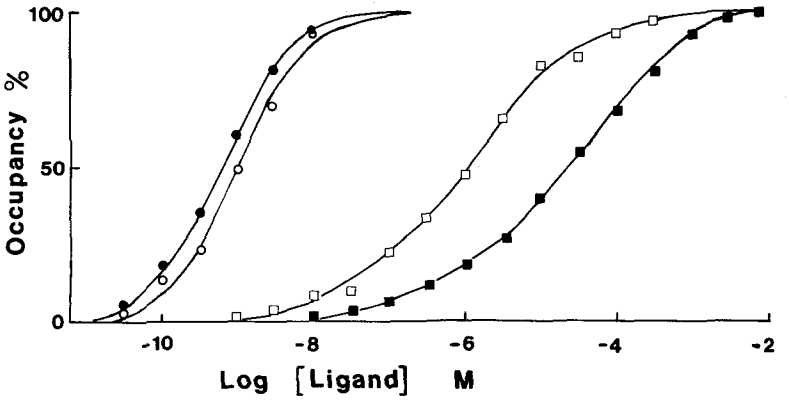

FIGURE 1. Occupancy-concentration curves for the binding of N-methylscopolamine (circles) and carbachol (squares) to muscarinic receptors in a homogenate of whole heart in the absence (open symbols) or presence (closed symbols) of GTP (10^{-4}M).

The NMS curves are the best fit simple mass action binding curves with affinity constants 1.1 ± 0.2 x 10^{9} M^{-1} (○) and 1.8 ± 0.2 x 10^{9} M^{-1} (●), calculated by non-linear least squares regression analysis. GTP produced no significant change in the concentration of antagonist sites (60 pmoles/g.protein).

Sorvall Omnimixer (setting 3, 15s) and was followed by 20 up and down strokes in a Potter Elvehjem homogeniser (520 rpm). Atria were homogenised directly in the Potter homogeniser and whole hearts treated as for ventricular tissue. Homogenates were filtered through cheese cloth before use.

For the binding assays, homogenates were diluted 1:4 with 100 mM NaCl/10 mM $MgCl_2$/20mM Na-HEPES, pH 7.0, and preincubated for 10 min at 30° before addition to microcentrifuge tubes containing a subsaturating concentration of [^{3}H]-N-methylscopolamine ([^{3}H]-NMS, 10 Ci/mmole, 3 x 10^{-10} M final concentration), together with unlabelled competing ligands and nucleotides as indicated in the text or figure legends. Incubation was carried out for 10 min at 30° and was terminated by centrifugation as previously described (10). Assays were carried out in quadruplicate and the non-specific binding defined as the radioactivity bound or entrapped in the pellet when the incubation medium contained 10^{-6}M 3-quinuclidinyl-benzilate.

Protein was estimated by the Lowry method using bovine serum albumin as standard. The nucleotides were purchased from Sigma London or Boehringer London.

RESULTS AND DISCUSSION

Figure 1 shows the effect of GTP (10^{-4}M) on the binding of the antagonist N-methylscopolamine (NMS) and the agonist

carbachol. It can be seen that GTP produces a modest increase in the affinity of the antagonist from 1.1 ± 0.2 x 10^9 M^{-1} to 1.8 ± 0.2 x 10^9 M^{-1} without any change in form of the curve, which in both cases is well fitted by a simple mass action curve. It also produces a large decrease in agonist binding, the I_{50} changing by a factor of 20 from 1.1 x 10^{-6} M to 2.3 x 10^{-5} M. In accord with the findings in other tissues (11-14) the agonist binding curve is not a simple mass action one, but is lower in slope. We have provided evidence that this is due to the presence of more than one class of receptor (11). The shift of the agonist binding curve in the presence of GTP could be due to a change in the binding affinities of these classes of receptor or to a shift in their proportions. The results shown in Figure 1 do not at present allow a decision to be made between these alternatives.

The effects of other nucleotides on agonist binding are shown in Table 1. It can be seen that GDP is about equi-active with GTP but that Gpp(NH)p is about ten times as active. ATP, ADP, AMP, Ad, App(NH)p, cAMP and cGMP were essentially inactive. The effects of GTP on agonist binding are relatively short-lived (Figure 2), no doubt due to hydrolysis to inactive products, whereas those of Gpp(NH)p are more persistent. It is evident that the effects of GTP are rapidly reversible and that those shown in Figure 1 are not the maximum attainable. The GTP effect is seen in homogenates of both atria and ventricles, a matter of some interest since the ventricles do not have a cholinergic innervation whereas the auricles do. Preliminary experiments on the effects of ions suggest that Mg^{++} is important for the effect and cannot be replaced by Ca^{++}. A similar effect to that found here in the heart has also been

TABLE 1

EFFECT OF NUCLEOTIDES ON BINDING OF CARBACHOL (10^{-5} M)

	Nucleotide (M)			
	10^{-6}	10^{-5}	10^{-4}	10^{-3}
GTP	86	79	51	28
GDP	-	-	52	32
Gpp(NH)p	70	49	45	-
cGMP	-	-	98	100
ATP	-	-	95	91
ADP	-	-	99	94
App(NH)p	-	89	90	-
AMP	-	-	96	96
cAMP	-	-	93	96
Adenosine	-	-	98	100

Carbachol binding was assessed by the inhibition of specific [^{3}H]-NMS binding. Values in the Table are expressed relative to a control value (no nucleotide) which was set at 100. At 10^{-5}M carbachol, there was 78-83% inhibition of specific ^{3}H-PrBCh binding.

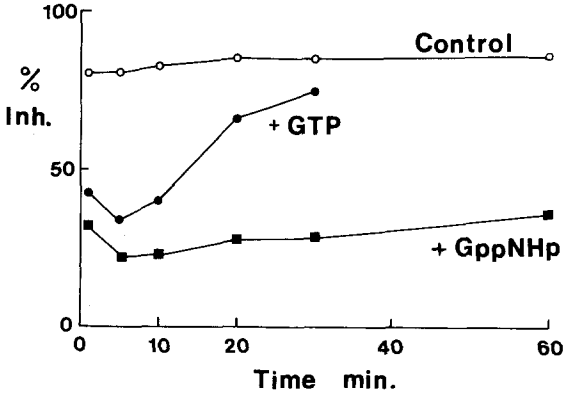

FIGURE 2. Time course of carbachol (10^{-5}M) inhibition of specific ^{3}H -NMS binding in the absence (O) and presence of GTP (10^{-4}M, ●) or Gpp(NH)p (10^{-5}M, ■).

observed in brain homogenates although it is smaller in extent (Berrie, C.P., Birdsall, N.J.M., Burgen, A.S.V. and Hulme, E.C., unpublished results).

The results reported here are in substantial agreement with the results reported for the nucleotide effects on the binding of other neurotransmitters to their receptors (1-9). Several of these receptors have been demonstrated to be coupled to adenylate cyclase systems. Our results suggest that the activation of muscarinic receptors should affect a cyclase system even in a broken cell preparation. Indeed it has been found that carbachol decreases GTP stimulated cAMP production in heart homogenates (Berrie, C.P., Birdsall, N.J.M., Burgen, A.S.V. and Hulme, E.C., unpublished results). This extends the well-documented effect of muscarinic agonists on cAMP levels in whole cell preparations (15-18) to a direct coupled effect in the myocardial cell membrane.

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