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ANTIBODIES TO MUSCARINIC ACETYLCHOLINE RECEPTORS IN MYASTHENIA GRAVIS

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IgG obtained from patients with myasthenia gravis block the specific binding of the muscarinic antagonists (^{3}H)-N-methyl-4-piperidyl benzilate (4NMPB) and (^{3}H)-Quinuclidinyl benzilate to rat brain muscarinic acetylcholine receptors. IgG obtained from healthy controls have a much smaller effect. The inhibitory effect of the myasthenic IgG increases linearly with immunoglobulin concentration and has no effect on the affinity of the muscarinic receptors towards (^{3}H)-4NMPB (K_D = 0.7 ± 0.1 nM). This implies that the inhibition is probably due to the blocking of some of the muscarinic receptors by the myasthenic IgG, and not due to alteration in affinity of all the receptors. It remains to be established whether the presence of anti-muscarinic receptor activity in the serum of myasthenic patients is of importance in the pathophysiology and diagnosis of myasthenia gravis.

Introduction

The pathogenesis of myasthenia gravis (MG) is thought to involve the production of antibodies directed against postsynaptic nicotinic acetylcholine (ACh) receptors (1-4). However, certain phenomena associated with MG may be due to presynaptic disorders. For example, Cull-Candy et al.(5) have reported that ACh release from myasthenic nerve terminals is enhanced relative to healthy controls. Presynaptic muscarinic ACh receptors are believed to play a regulatory role in some cholinergic and noncholinergic synapses (for review see 6). We therefore decided to examine the possibility that myasthenic patients contain antibodies against muscarinic ACh receptors, in addition to the previously characterized, antibodies against nicotinic ACh receptors and other hu-

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man tissues and components (7). In this communication we report that the serum of MG patients contain antibodies having antimuscarinic ACh receptor activity, and that these antibodies block the specific binding of muscarinic ligands.

Materials and Methods

(^{3}H)-N-methyl-4-piperidyl benzilate ((^{3}H)-4NMPB), 33 Ci/mmole, (^{3}H)-quinuclidinyl benzilate ((^{3}H)-QNB), 29 Ci/mmole and unlabeled muscarinic ligands are those described and used previously (8).

IgG was separated from the serum of both myasthenic patients and controls by ammonium sulfate (40%) precipitation. It was resuspended, at the original volume in 100 mM Tris-HCl pH 7.4, and dialyzed extensively against modified Krebs-Ringer buffer. Protein was determined according to Lowry et al. (9).

The effect of IgG from myasthenic patients and from controls on the specific binding of the muscarinic antagonists (^{3}H)-4NMPB (10) and (^{3}H)-QNB (11) to rat brain homogenate was assayed by filtration (12) and by centrifugation (8)

The reaction mixture of the filtration assay (200 µl) contained modified Krebs Ringer buffer (12), 0.3 pmole (^{3}H)-4NMPB binding sites of rat brain homogenate, 100 µl IgG, and tritium labeled antagonist (0.25-10 nM). The reaction mixture was equilibrated (30 min, 25°C) and the specific binding of the radiolabeled ligands to the receptors was assayed by filtration as previously described (12). Binding of (^{3}H)-4NMPB and (^{3}H)-QNB that was inhibited by 1 µM of atropine was considered to be specific. In the centrifugation assay, the reaction mixture (250 µl) contained modified Krebs Ringer buffer, 0.05 pmole (^{3}H)-4NMPB binding sites of rat brain homogenate, 200 µl IgG, and 0.25-10 nM of either (^{3}H)-4NMPB or (^{3}H)-QNB. The reaction mixture was equilibrated as described above, and the material was pelleted by centrifugation in a microcentrifuge. The extent of specific antagonist binding to the receptors was then assayed as previously described (8).

Results

The possibility that myasthenic patients contain antibodies directed against the ligand binding sites of muscarinic ACh receptors was examined by determining the effect of IgG from myasthenic patients on the binding capacity of rat brain homogenates towards the specific muscarinic antagonists (^{3}H)-N-methyl-4-piperidyl benzilate (10) and (^{3}H)-Quinuclidinyl benzilate (11). As depicted in Figure 1, IgG from MG patients blocked the specific binding of (^{3}H)-4NMPB. The effect of IgG obtained from 16 myasthenic patients (22.5 $\pm$ 5% reduction in binding capacity under the conditions described for Fig. 1) was fourfold greater than that caused by IgG obtained from 13 apparently healthy controls matched for age and sex (5.3 $\pm$ 4% reduction in binding capacity) ($p < 0.001$, student's t-test). Similar results were obtained when muscarinic binding capacity was assayed using the antagonist (^{3}H)-QNB (not shown). The affinity of the receptors towards (^{3}H)-4NMPB (K_D = 0.7 $\pm$ 0.1 nM) was unaltered by the presence of the antibodies (Fig. 2), implying that the decreased binding brought about by the IgG from myasthenic patients was due not to alteration in affinity of all the muscarinic ACh receptors towards (^{3}H)-4NMPB, but

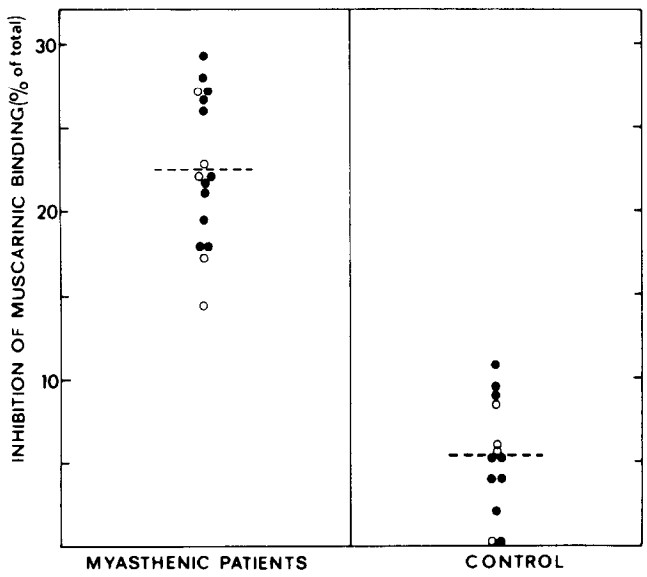

Fig. 1. Inhibition of binding of the muscarinic antagonist (^{3}H)-4NMPB to rat brain muscarinic receptors by IgG from myasthenic patients and from controls. The binding of (^{3}H)-4NMPB was assayed by filtration (•) and by centrifugation (o) as described in Materials and Methods.

rather due to blocking of some of these receptors. The inhibitory effect of myasthenic IgG increased linearly with immunoglobulin concentration within the range investigated (0 - 0.4 ml IgG in a reaction volume of 0.5 ml containing 0.05 pmole of (^{3}H)-4NMPB binding sites) (Fig. 3). It should be

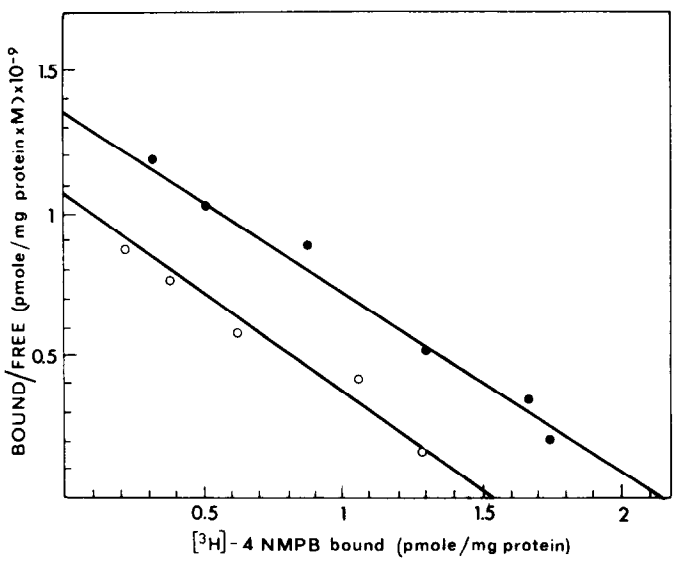

Fig. 2. Scatchard plot (13) of the binding of the muscarinic antagonist (^{3}H)-4NMPB to rat brain homogenate in the presence of IgG obtained from a control (•) and from a myasthenic patient (o). (^{3}H)-4NMPB binding was assayed by centrifugation as described in the Materials and Methods and the range of (^{3}H)-4NMPB concentrations examined was 0.2-10 nM.

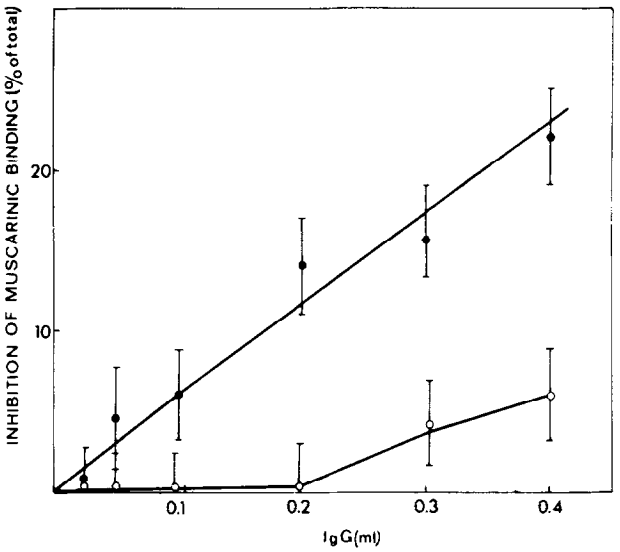

Fig. 3. The effects of varying concentrations of myasthenic (●) and control (o) IgG on the capacity of (^{3}H)-4NMPB binding to rat brain homogenates. (^{3}H)-4NMPB binding was assayed by centrifugation as described in the Materials and Methods, except that the volume of the reaction mixture was 500 µl and it contained 0.05 pmole of (^{3}H)-4NMPB binding sites as well as the indicated volume of control or myasthenic IgG. Results presented are average ± S.E. of two myasthenic patients and two controls.

noted that on account of technical limitations inherent in the receptor filtration assay technique, at high IgG concentrations (> 0.2 ml) the method of choice was the centrifugation assay (8). The myasthenic IgG fractions were obtained from patients who had received no drugs with antimuscarinic effects for at least several months, thus excluding a possible drug mediated effect. All IgG fractions were dialysed extensively prior to use.

The myasthenic IgG, as indicated above, was active against the muscarinic ligand binding site. It is known that MG patients contain an array of antibodies which recognize the nicotinic ligand binding site and other distinct sites on the nicotinic ACh receptor (14). In accordance, the myasthenic sera employed in this study contained antinicotinic antibodies; when assayed according to Brenner et al. (15), they revealed a titre over twofold higher than that of the controls. It is not yet known if the antimuscarinic antibodies are distinct from the antinicotinic IgG present in the serum of myasthenic patients, and whether they distinguish between pre- and postsynaptic muscarinic receptors. It should be noted that the specific myasthenic inhibition of the binding of muscarinic antagonists (i.e. the difference between the inhibition caused by myasthenic and control IgG shown in Fig. 3) seems to level off at IgG concentrations above 0.2 ml. This suggests that the myasthenic IgG may be affecting only a subpopulation of the (^{3}H)-4NMPB binding site.

The present finding that myasthenic sera contain antibodies against muscarinic ACh receptors is consistent with the supposition that the enhanced ACh release observed in myasthenic nerve terminals may be due to antibody mediated blocking of inhibitory presynaptic muscarinic ACh receptors (6), although other mechanisms cannot be ruled out. The pathological effects of these antibodies on postsynaptic muscarinic receptors and on muscarinic function in other organs remain to be established. It is of interest to note that Bannister & Hayes (16) have recently described a generalized smooth muscle disease resembling myasthenia gravis in which there is a defect of the muscarinic receptors. Clinical data indicate only a partial correlation between the titre of antinicotinic ACh receptor antibodies and the degree of weakness of myasthenic patients. One could speculate that antibodies against muscarinic and nicotinic ACh receptors together with antibodies against various muscle components may have a cumulative effect which results in producing the clinical state.

In conclusion, our results demonstrate that the sera of myasthenic patients contain antimuscarinic antibodies. The implications of this finding for the pathophysiology and diagnosis of myasthenia gravis, as well as for the isolation and biochemical characterization of antimuscarinic antibodies, remain to be established.

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