Differential Properties of α-Bungarotoxin and Myasthenic IgG Bound to Cloned α-Subunit of Nicotinic Acetylcholine Receptor

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Nicotinic acetylcholine receptor (nAChR) is a pentameric receptor composed of four subunit types (α_2, β, γ, δ) (Conti-Tronconi et al. 1982; Hucho 1986). Several studies have shown that the acetylcholine binding sites are localized on the α-subunit (Carlin et al. 1986; Dunn et al. 1983; Hamilton 1985; Marks et al. 1986, Lindstrom 1986) and they can be inhibited by snake venom α-neurotoxin. Experimental results obtained with synthetic peptides and antibodies against α-subunit suggest that the α-toxin binding site(s) may be distributed at least in five areas (Atassi et al. 1986; Kordossi et al. 1987). It remains unknown whether these five toxin-binding regions are distinct binding sites or whether they are faces of one binding site (Mulac-Jeričevič et al. 1987). Recombinant DNA technology has enabled the expression of complete α-subunit, in particular the α-bungarotoxin binding site in *Escherichia coli* transformants as could be monitored simply by toxin overlays of colony blots (Gershoni 1987).

The aim of our work was to investigate whether IgG isolated from myasthenic patients binds (Drachman 1987) to the cloned α-subunit of nAChR. This protein is in *Escherichia coli* expressed as unprocessed and represents the precursor form of α-subunit.

cDNA library from denervated rat muscle was prepared in λgt11, amplified in *Escherichia coli* strain Y1088. An universal oligonucleotide probe for acetylcholine receptor genes as described by Buonanno et al. (1986) was used for the first screening of the library. Y1090 cells were infected with positive charons containing appropriate inserts (about 2 kilobases) and the expression of receptor proteins was monitored by binding of polyclonal antibodies against nAChR (of own provenance) and second antibody – ^{125}I protein A. The same protocol as with the polyclonal antibody against nAChR was used for the myasthenic IgG binding studies (Huynh et al. 1985).

Figure 1 shows the ligand overlay of colony blots with (^{125}I) α-bungarotoxin giving a very strong and more selective signal (A) as compared to that of the polyclonal antibody (B). A much weaker signal was obtained from myasthenic IgG bound to the same replica (Fig. 2). The question has arisen whether myasthenic IgG has the same binding properties as do polyclonal antibodies and

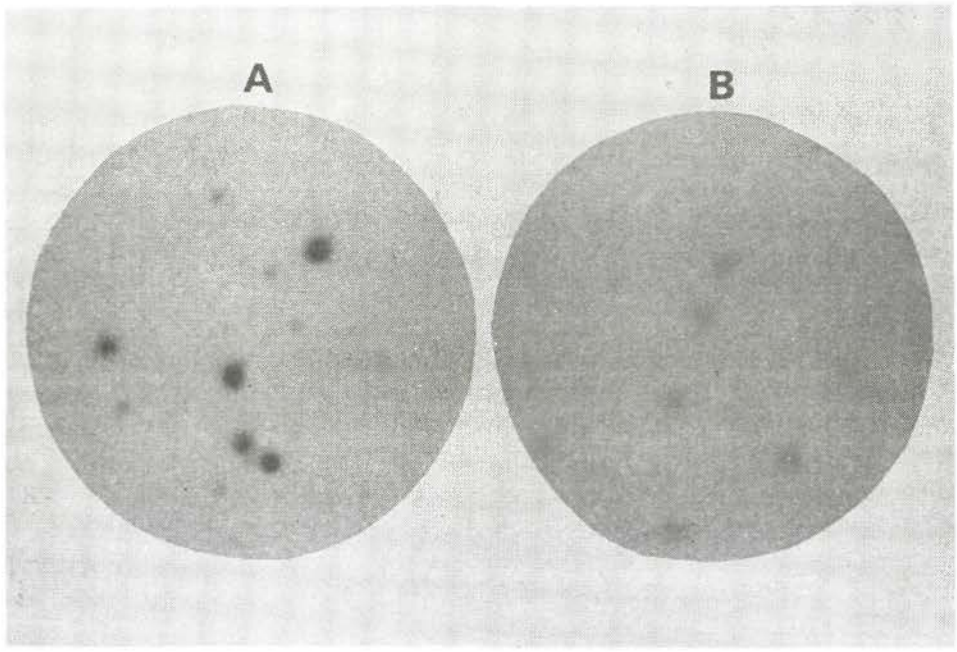

Fig. 1. Binding of (^{125}I) α-bungarotoxin (*A*) and polyclonal antibodies (*B*) to the colony blots. With the polyclonal antibodies ^{125}I protein A was used as a marker.

α-toxin and whether it binds to the nonglycosylated protein fraction in recombinant *Escherichia coli* cells.

Total cell lysates from positive clones were electrophoresed in 10% sodium-dodecylsulphate-polyacrylamide gels, stained or blotted onto nitrocellulose membranes (Hybond C, Amersham) and overlayed with ligands. Figure 3 illustrates binding of (^{125}I) α-bungarotoxin (lane A), polyclonal antibody with ^{125}I protein A (lane B) and myasthenic IgG (lane C). Both the toxin and the polyclonal antibody obviously bind to the same protein with Mr around 45,000 — 49,000. This value is identical with Mr of the protein which binds α-bungarotoxin as reported by Gershoni (1987). The discrepancy between the binding of polyclonal antibodies, α-toxin and myasthenic IgG was probably caused by different expression of cloned α-subunit proteins. In the clone analyzed in lanes A and B a protein with a larger Mr value was expressed as in the clone in lane C (Fig. 3). The presence of specific binding sites for α-toxin by both clones was tested by Scatchard analysis after chromatography on α-cobratoxin Sepharose. The value obtained by both clones – $K_D = 0.1\ \text{nmol} \cdot \text{l}^{-1}$;

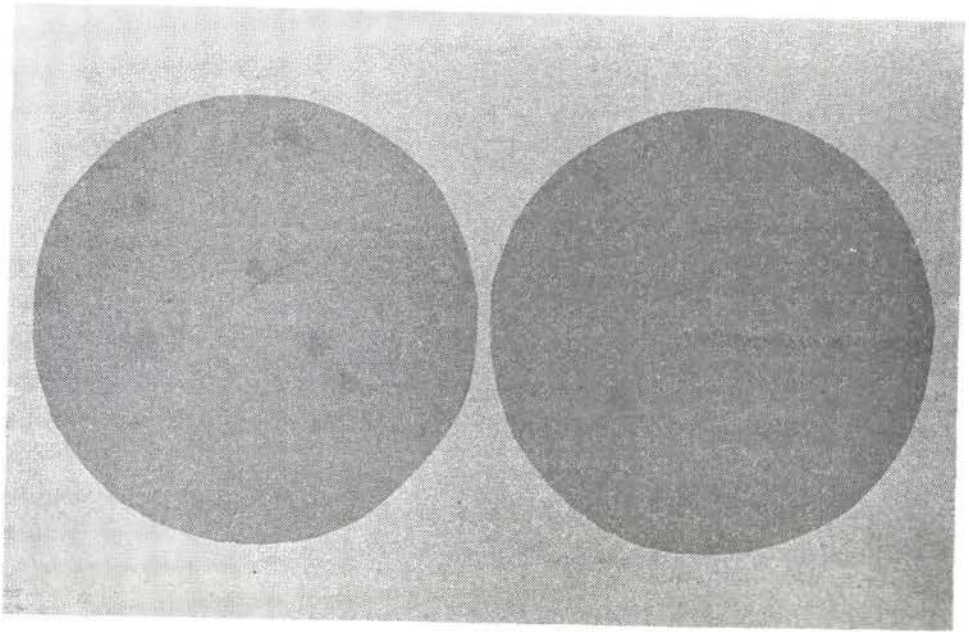

Fig. 2. Binding of myasthenic IgG to the colony blots. All procedures were done as described by Huynh et. al (1985).

$B_{max} = 1.24$ pmol . mg^{-1} protein, suggest that proteins with high affinity binding sites for α-toxins were exprimed.

The present results allow to conclude that myasthenic IgG binds to the proteins exprimed in *Escherichia coli*, independently of glycosylation sites.

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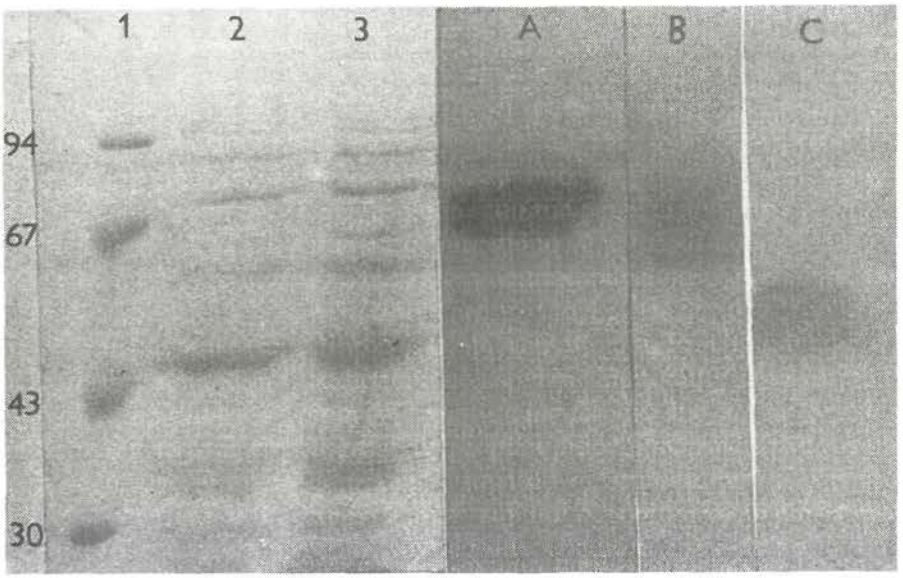

Fig. 3. Whole cell lysates from positive clones were electrophoresed in 10% polyacrylamide gels and stained with Coomassie Brilliant Blue (lanes 2 and 3) or blotted on a nitrocellulose membrane. Lane *A*: (^{125}I) α-bungarotoxin bound to blotted proteins; lane *B*: the same bound of polyclonal antibody ($^{+}$with ^{125}I protein A); lane *C*: binding of myasthenic IgG ($^{+}$with ^{125}I protein A). 94—30: low molecular weight kit (Pharmacia). Numbers at the left represent molecular weights x10^3.

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