Antigenicity of the sequence 152–167 in α subunits of *Torpedo* acetylcholine receptor

Patients with myasthenia gravis (Tzartos *et al.*, 1982) and animals immunized with native acetylcholine receptors (Tzartos & Lindstrom, 1980; Tzartos *et al.*, 1981, 1983) have more than half of their antibodies to receptor directed at a small part of the extracellular surface of receptor α subunits which is termed the 'main immunogenic region.' Noda *et al.* (1982, 1983) suggested that the sequence 161–166 of α subunits might form the main immunogenic region because this was a very hydrophilic sequence on what they proposed was the extracellular surface.

However, Juillerat *et al.* (1984) and Lindstrom *et al.* (1984) observed that monoclonal antibodies to the main immunogenic region which bound to denatured α subunits did not bind to synthetic peptides containing this sequence. This indicated that this sequence was not the main immunogenic region. Further, both groups showed that this sequence was not bound by antibodies to native receptor, although it was bound by antibodies to denatured α subunits. This indicated that this sequence was not even a significantly immunogenic region in the native receptor. Still further, monoclonal antibodies to the synthetic peptide α 152–167 were made and mapped to the sequence α 152–159; these antibodies and antibodies to the main immunogenic region were shown to be able to bind to the receptor simultaneously (Lindstrom *et al.*, 1984; Criado *et al.*, 1985a). This clearly suggests that this sequence is not even close to the main immunogenic region. This was explained in part by Juillerat *et al.* (1984) who reported that antibodies to α 151–169 could not bind to soluble native receptors labelled with toxin. Our unpublished experiments agree with this. However, if these receptors are bound to plastic wells this alters their structure sufficiently so that monoclonal antibodies to α 152–159 can bind (Criado *et al.*, 1985a). These antibodies compete for binding to receptor on plastic wells (Criado *et al.*, 1985a) with monoclonal antibodies which have been demonstrated by electron microscopic techniques to bind to the cytoplasmic surface of receptors (Sargent *et al.*, 1983). This suggests that this sequence becomes exposed on the cytoplasmic surface of the receptor. This is further supported by other experiments (Criado *et al.*, 1985b). Thus, there is compelling evidence that the sequence α 151–169 is not the main immunogenic region, not a significant immunogenic region in native receptor, not on the extracellular surface of the receptor, and not clearly exposed on the surface of native receptor.

Yet, recently, McCormick *et al.* (1985a) have reported that the sequence α 159–169 contains a significant immunogenic region. They report that antisera to 'native' receptor bind to this sequence. Denaturation of some of the 'native' receptor used as immunogen could explain this, since antisera to denatured α subunits do bind to this sequence (Lindstrom *et al.*, 1984). Most investigators, including Juillerat *et al.* (1984) and ourselves, purify receptors by binding solubilized receptors to a cobra venom toxin adsorbent and eluting them by competition with an excess of another

cholinergic ligand. However, McCormick *et al.* (1985a) purified receptors by binding them to a monoclonal antibody adsorbent and eluting them under marginally denaturing conditions (0.2 M-glycine buffer, pH 10, 1% sodium cholate, 0.5 M-NaCl; Lennon *et al.*, 1980). What is 'native' is relative in any case, since although solubilizing receptors with Triton X-100 retains the ability of receptors to bind α -bungarotoxin, it alters the ligand binding affinity and inhibits the cation channel of receptors (Lindstrom *et al.*, 1980).

To answer technical questions raised in the letter from McCormick *et al.* (1985b), the synthetic peptide which we (Lindstrom *et al.*, 1984) used to screen for reaction with monoclonal antibodies was α 159–169 with an additional C-terminal tyrosine. This was separated from excess ^{125}I after iodination on a Bio-Gel P-2 column.

In summary, Juillerat *et al.* (1984) and Lindstrom *et al.* (1984) agree that α 152–169 is not a significant antigenic determinant in native acetylcholine receptors, and we suggest that antibodies to this sequence which were detected in antisera to receptors by McCormick *et al.* (1985a) were provoked by denatured receptors in their immunogen. Finally, all three groups can agree that this sequence does not contain the main immunogenic region.

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