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Immunological and Functional Properties of the Acetylcholine Receptor Expressed on the Human Cell Line TE671[1])

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Summary: In the first part of the present study we compared the antigenicity of affinity-purified acetylcholine receptors from the cell line TE671 and from human skeletal muscle. The reactivities of the two acetylcholine receptor preparations showed a strong correlation (r = 0.96) in a radioimmunoassay using sera from myasthenia gravis patients. In additional functional studies, carbamylcholine stimulated cAMP production in TE671 cells to 130%. This increase was even more pronounced when TE671 cells were grown in the presence of dexamethasone. α-Bungarotoxin completely blocked this carbamylcholine-induced cAMP increase. Using the Ca^{2+} indicator, indo-1, it was shown that intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$) were elevated in TE671 cells after stimulation with carbamylcholine. This effect was also completely blocked by α-bungarotoxin. To test the functional activity of autoantibodies against the acetylcholine receptor, TE671 cells were preincubated with sera from myasthenia gravis patients. In one third of sera a significant inhibition of the agonist-stimulated $[Ca^{2+}]_i$ increase was detected, possibly caused by antibodies directed to functionally important areas of the acetylcholine receptor. There was no correlation between the inhibition rate of $[Ca^{2+}]_i$ and anti-acetylcholine receptor antibody titres in these patient sera.

Introduction

Autoantibodies direct against the human acetylcholine receptor are known to play an important role in the pathogenesis of myasthenia gravis. However, isolation of the target structure, the acetylcholine receptor from human muscle tissue, is difficult and various preparations differ in their antigenicity. As an alternative we isolated the acetylcholine receptor from the human cell line TE671 and investigated its reaction with human autoantibodies immunologically and functionally.

The nicotinic acetylcholine receptor, initially purified from electric organs of certain fish species has been characterized biochemically, pharmacologically and immunologically (1, 2). For immunological studies in myasthenia gravis these receptors are less useful because of their low cross-reactivity to human acetylcholine receptor (3, 4). Therefore, human acetylcholine receptor from amputated leg muscle tissue has usually been applied as autoantigen in different assays. However, the supply of human muscle is limited and more importantly, during the preparation human muscle acetylcholine receptor is easily degraded (5, 6). The human TE671 cell line expresses significant numbers of acetylcholine receptors and is therefore a useful alternative source for acetylcholine receptor preparations. This cell line was initially described as a human medulloblastoma line by *McAllister & Gardner* (7). It has recently been reported to have muscle like features, and probably originates from a human rhabdomyosarcoma cell (8). *Luther & Lindstrom* generated cDNA probes specific for acetylcholine recep-

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