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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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tor subunits from TE671 cells, which show a complete homology to nucleotide sequences described for human skeletal muscle type acetylcholine receptor subunits (9).

In the present study we demonstrated a cross-activity of myasthenia gravis patient sera with acetylcholine receptor preparations from human muscle and from TE671 cells.

Furthermore, TE671 and the neuroblastoma line IMR32 (10), both of which express α-bungarotoxin binding sites, were tested to determine whether these α-bungarotoxin binding proteins represent functional acetylcholine receptors. This was done by measuring intracellular signal transduction, i.e. the increase in cAMP and the increase in intracellular Ca^{2+} concentration after stimulating cells with the acetylcholine receptor-specific agonist carbamylcholine. Finally, both cell lines were used to test whether autoantibodies from myasthenia gravis patient sera (n = 11) modulate acetylcholine receptor-induced signal transduction.

Materials and Methods

Cell culture

IMR32 (CCL 127) and TE671 (HTB 139) cells were obtained from the American Tissue Culture Cooperation (ATCC). Both lines were grown in *Dulbecco*'s modified *Eagle*'s medium using large cell culture flasks (Nunc; 850 ml). IMR32 medium was supplemented with 0.1 volumes of fetal calf serum and 3.5 g/l glucose. TE671 cells were grown in 0.05 volumes of fetal calf serum supplemented medium in the presence or absence of 2.5 µmol/l dexamethasone (Sigma). Both lines were grown in confluent monolayers and removed mechanically before use.

Gel filtration of α-bungarotoxin binding proteins

IMR32 and TE671 cells (5×10^8) were solubilized with 10 g/l Triton X100 (Sigma) in 10 mmol/l sodium phosphate buffer (pH 7.2) for 1 h, by gently shaking, and ultracentrifuged at 4 °C and 100 000 *g* in a Kontron® ultacentrifuge (Kontron TGA-65; rotortype: 50 Ti) for 40 min. The supernatant was filtered through glass wool and the resulting solubilized cell line extracts were incubated with an excess (0.185 MBq) of [^{125}I]α-bungarotoxin (Amersham & Buchler; IM-109) for approximately 16 h at 4 °C. Unbound ligand was removed by column gel filtration, using a 30 × 1.8 cm Sephadex G50 filled column (Pharmacia). Radioactivity of various fractions was measured using a gamma counter (Minaxi-gamma; Packard). Molecular masses of [^{125}I]α-bungarotoxin binding proteins in the cell lysates were determined by gel filtration, using defined standard proteins (Sigma).

Affinity chromatography of α-bungarotoxin binding proteins

Lysates of IMR32 and TE671 cells (5×10^8) were prepared as described above and incubated overnight with α-bungarotoxin-coupled (2 l Gel) Sepharose 4B (Pharmacia). Coupling was achieved as described by the manufacturer. Thereafter the gel was washed intensively with 1 mol/l sodium chloride in 10 mmol/l sodium phosphate containing 10 g/l Triton X100. The α-bungarotoxin binding proteins were then eluted with 20 g/l sodium dodecylsulphate (SDS), concentrated, and analysed under non-reducing conditions by SDS-polyacrylamide gel electrophoresis (SDS-PAGE).

SDS-PAGE of α-bungarotoxin binding proteins

Affinity purified α-bungarotoxin binding proteins were reduced at 37 °C with 30 ml/l mercaptoethanol, then applied to a 10% or 12.5–7.5% SDS-PAGE according to *Laemmli* (11). The proteins were separated at a constant current of 50 mA for 2.5 h in a vertical gel electrophoresis system (VA 150; Desaga). For the determination of protein size, high molecular mass standard proteins (Gibco-BRL; Amersham & Buchler) were run in parallel. Proteins were stained with 2 g/l Coomassie Blue (Merck).

Cross-reaction of α-bungarotoxin binding proteins with myasthenia gravis patient sera

Labelled α-bungarotoxin binding components were obtained from crude extracts of TE671 and IMR32 cells by gel filtration as described above. Pooled peak fractions were used in an immunoprecipitation assay with human myasthenia gravis sera.

[^{125}I]α-Bungarotoxin-labelled complexes from TE671 or IMR32 cells were incubated for 6 h at 4 °C with 5 µl of myasthenia gravis patient sera. Immunoprecipitation was done with an equivalent volume of goat anti-human IgG's (Lawrence Laboratories, Australia). The precipitates were pelleted by centrifugation at 11 000 *g* in an Eppendorf centrifuge, then washed twice with 10 g/l Trition X-100 containing 10 mmol/l sodium phosphate buffer. The radioactivity was measured in a gamma counter (Minaxi-gamma; Packard).

Purification of acetylcholine receptor from TE671 cells

For affinity chromatography of acetylcholine receptor from TE671 and human muscle cells, α-najatoxin was prepared from crude snake toxin as described by *Karlson & Widlund* (12). Acetylcholine receptor were purified according to the method described by *Kalies & Kalden* (13). Briefly, the α-najatoxin was coupled to cyanogen bromide-activated sepharose (2 g/l gel; Pharmacia). Thereafter, cell extracts from TE671 cells or human muscle tissue were loaded on the column. After removing unspecifically bound proteins with 1 mol/l sodium chloride, acetylcholine receptors were eluted with 1 mol/l carbamylcholine. The purified acetylcholine receptors were labelled with [^{125}I]α-bungarotoxin in excess and used as antigens in a radioimmunoassay (RIA) as previously described (13, 14).

Concentrations of anti-acetylcholine receptor autoantibodies given in nmol/l were determined from the pelleted radioactivity and are equally expressed as the number fo α-bungarotoxin binding sites precipitated per litre of serum. For the calibration of the RIA a control standard serum was used as previously described (15).

Determination of cAMP

IMR32 or TE671 cells (5×10^4) were incubated for 30 min in *Dulbecco*'s modified *Eagle*'s medium, supplemented with 1 mmol/l 3-isobutyl-1-methylxanthine (Sigma) to inhibit phosphodiesterases (16). Forskolin (Sigma) was used in a concentration of 100 µmol/l for preincubation of cells for 1 h at 37 °C. Immediately before stimulation, forskolin was removed by washing cells twice with phosphate-buffered saline.

For inhibition experiments, 5×10^4 cells of IMR32 or TE671 cells were preincubated with the indicated concentrations of α-bungarotoxin (Serva). Acetylcholine receptors were stimulated with 10 mmol/l of the agonist carbamylcholine (Sigma) in phosphate-buffered saline. After 30 min, cells were pelleted and washed twice with ice-cold phosphate-buffered saline. The reaction was stopped by adding 0.1 ml of 1.5 mol/l perchloric acid (16). After neutralisation the probes were centrifuged at 5000 *g*. The supernatants were collected, stored up to 4 weeks at −70 °C or immediately tested for cAMP. cAMP was measured in duplicates (10 µl) according the method of *Gilman* (17) using a commercial test kit (Amersham & Buchler).

Determination of Ca^{2+}-mobilization after cell stimulation

Cells (0.1×10^{12}/l) were loaded with indo-1-ester (acetoxymethyl ester of 1-[2-amino-5-(6-carboxyindol-2-yl)-phenoxy]-2-(2'-amino-5'-methylphenoxy)-ethane-N,N,N',N'-tetraacetic acid, 50 µmol/l; Calbiochem) and incubated for 20 min at room temperature. Then the cell suspension was diluted 10-fold and incubated for another 60 min. Thereafter cells were pelleted, washed twice and resuspended in phosphate-buffered saline (cell count 10^{11}/l) as described by *Tsien & Rink* (18). Fluorescence before and during carbamylcholine stimulation was measured in a spectrofluorometer (Perkin Elmer) with an excitation and emission wavelength of 336 and 404 nm, respectively. As positive control 10 − 100 nmol/l A23187 (Sigma) or 0.25 − 10 µmol/l ionomycin (Calbiochem) were added to the cell suspension. $[Ca^{2+}]_i$ was calculated as described previously (19, 20). Inhibition of $[Ca^{2+}]_i$ was tested by preincubating cells with 1 − 10 µmol/l α-bungarotoxin for 30 min or for 1 h with a 1 : 100 or 1 : 500 dilution of myasthenia gravis patient sera (n = 11) or a serum obtained from a patient with *Lambert-Eaton* syndrome. Sera from healthy donors (n = 5) were used in parallel as controls.

Results

Gel filtration and affinity chromatography of α-bungarotoxin binding proteins expressed by TE671 or IMR32 cells

By incubating various fractions of Triton-X100-solubilized membranes of IMR32 or TE671 cells with [^{125}I]α-bungarotoxin, followed by removal of unbound α-bungarotoxin by Sephadex G50 gel filtration (fig. 1), it was found that IMR32 cells express about 4 times more α-bungarotoxin-binding proteins than TE671 cells (data not shown).

Fractions 17 − 22 of TE671 cells and fractions 19 − 24 of IMR32 cells contained the initial peak of radioactivity representing α-bungarotoxin binding proteins. The increase in radioactivity after fraction 25 for each cell line represents unbound ligands. From the position of marker proteins the approximate molecular mass for α-bungarotoxin binding proteins was M_r 160 000 for IMR32 and M_r 240 000 for TE671 extracts. This value for TE671 corresponds to the known molecular mass of the acetylcholine receptor from human muscle (21).

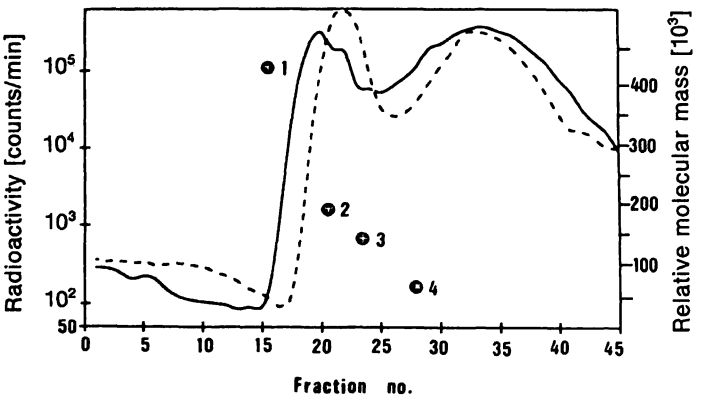

Fig. 1. α-Bungarotoxin binding proteins of TE671, but not IMR32 cells, show the molecular mass of human acetylcholine receptor in gel filtration experiments:
Triton X-100-solubilized extracts from IMR32 cells (2.5×10^8; dashed line) or TE671 cells (5×10^8; solid line) were incubated with [^{125}I]α-bungarotoxin and submitted to gel filtration on a Sephadex G50 column (flow rate: 20 ml/h). Radioactivity (counts/min) in 1 ml fractions was measured by γ-counting.
Solid dots represent the position of marker proteins (1 = apoferritin; 2 = β-amylase; 3 = alcohol dehydrogenase and 4 = albumin)

When α-bungarotoxin binding proteins from TE671 and IMR32 cells were affinity purified using α-bungarotoxin Sepharose 4B, then analysed with SDS-PAGE (fig. 2a and 2b), it was shown that the α-bungarotoxin binding protein of TE671 cells consisted of subunits of M_r 44 000, 59 000, 54 000 and 68 000, which corresponds with the known characteristics of the α-, β-, γ- and δ-subunits of the human muscle nicotinic acetylcholine receptor (22). In contrast, SDS-PAGE of the α-bungarotoxin binding protein of IMR32 cells showed one strong subunit band at M_r 50 000 and some weaker bands with higher and lower molecular masses. At present it is not clear whether these bands represent various subunits or degradation products.

Reactivity of α-bungarotoxin binding proteins with myasthenia gravis patient serum antibodies

Constant amounts of radiolabelled α-bungarotoxin binding proteins from IMR32 and from TE671 (20 000 counts per minute) were incubated with undiluted serum from myasthenia gravis patients. It was demonstrated that almost all sera from patients with myasthenia gravis precipitated high amounts of radioactively labelled α-bungarotoxin binding proteins from TE671, but not from IMR32 (tab. 1). This indicates that α-bungarotoxin binding proteins from TE671 cells immunologically represent acetylcholine receptor. This is not the case, however, for α-bungarotoxin binding proteins expressed on IMR32 cells.

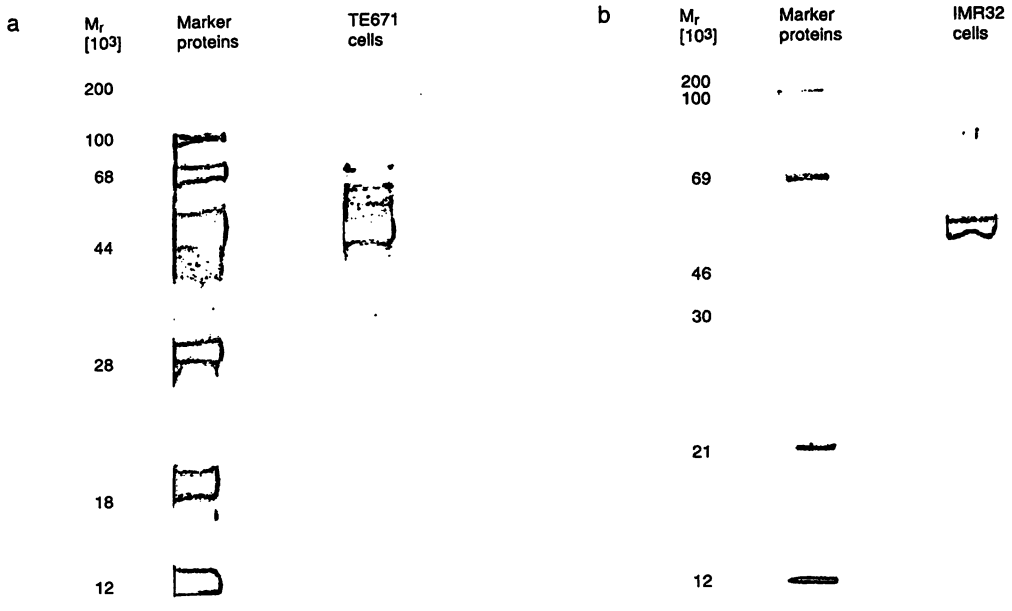

Fig. 2. Subunit composition of α-bungarotoxin binding proteins from TE671 corresponds to that of the human acetylcholine receptor in SDS-PAGE:
α-Bungarotoxin binding proteins of TE671 (2a) and IMR32 (2b) cells were separated on SDS-PAGE (10% and 12.5–7.5% gradient) under non-reducing conditions as described in Materials and Methods. Note that different sets of marker proteins were used in figures 2a and 2b. The molecular masses are given.

Tab. 1. Cross-reactivity of myasthenia gravis patient sera with α-bungarotoxin binding proteins from solubilized TE671 and IMR32 cell extracts

		Radioactivity TE671 [counts/min]	Radioactivity IMR32 [counts/min]
Myasthenia gravis patients	▲1:	861	105
	▲2:	2035	127
	▲3:	2524	97
	▲4:	1760	402
	5:	970	85
	6:	2350	81
	7:	425	73
	8:	182	201
	9:	97	94
	10:	2105	69
	11:	1430	198
Healthy controls	1:	86	121
	2:	43	83
	3:	52	nd.
	4:	75	nd.
	5:	81	98
Lambert-Eaton syndrome patient		76	109

[^{125}I]α-Bungarotoxin-labelled cellular proteins (20 000 counts/min) were incubated with constant volumes of indicated sera and thereafter precipitated with a goat anti-human IgG antibody. Radioactivity of immunoprecipitates was determined. Mean values of duplicates are shown in counts per minute. Sera which inhibit the increase in $[Ca^{2+}]_i$ are indicated by ▲; nd. = not determined.

Reactivity of myasthenia gravis patient serum autoantibodies with affinity chromatography-purified acetylcholine receptor from TE671 cells and human muscle tissue

When affinity chromatography-purified acetylcholine receptor from TE671 cells and muscle tissue were tested in a fluid phase radioimmunoassay as described (14–15), there was a very good correlation ($r = 0.96$) with the autoantibody concentrations (nmol/l) in 66 sera of patients with myasthenia gravis (fig. 3). This is further evidence for the expression of an immunologically identical acetylcholine receptor on TE671 cells, compared with that on human muscle tissue. No such correlation could be demonstrated when α-bungarotoxin binding proteins from IMR32 cells were used as substrate for this assay.

Changes in intracellular cAMP concentrations after stimulation with carbamylcholine

To test whether the α-bungarotoxin binding proteins on TE671 and IMR32 cells represent functional acetylcholine receptor, different intracellular second messenger mechanisms in carbamylcholine stimulated cells were investigated. Two different assays were used to test signal transduction in TE671 and IMR32 cells:

1) changes in intracellular cAMP concentrations, and

2) the increase in intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$).

No significant increase of cAMP in either of the tested cells were observed after stimulation with carbamylcholine. However, when TE671 cells were preincubated for 1 h with 100 µmol/l forskolin, which is known to be a potent adenylate cyclase activator, cAMP concentrations increased to approximately 130% compared with unstimulated cells. This increase was blocked by α-bungarotoxin (fig. 4a) and was enhanced when TE671 cells were grown in dexamethasone (fig. 4b). In contrast, no increase of cAMP in IMR32 cells was obtained after stimulation with carbamylcholine, no matter what culture conditions were used (with or without forskolin or dexamethasone; data not shown).

These observations indicate that carbamylcholine treatment stimulated an increase of cAMP in TE671 cells under certain conditions. It is likely that this effect is mediated through a human acetylcholine receptor expressed on TE671 cells. In contrast, no such effect was observed with IMR32 cells. The increasing effect of dexamethasone treatment in TE671 cells is consistent with experiments by *Luther & Lindstrom* who described an increase of acetylcholine receptor

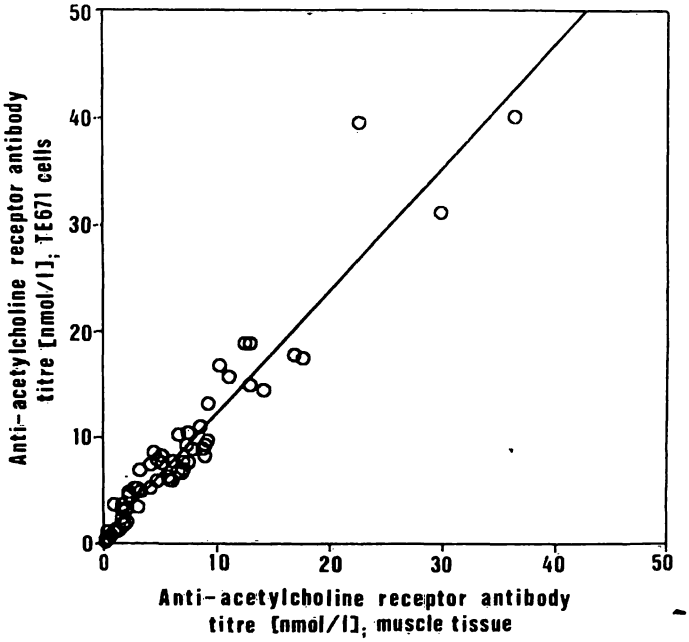

Fig. 3. Correlation of reactivity of sera from patients with myasthenia gravis towards affinity acetylcholine receptor from human muscle or TE671 cells:
Sera from 66 patients with myasthenia gravis were tested for their autoantibody concentration against acetylcholine receptor from TE671 cells (y-axis) and muscle tissue (x-axis) in a RIA. The presented data represent the mean value of duplicates in the RIA. The correlation coefficient was r = 0.96.

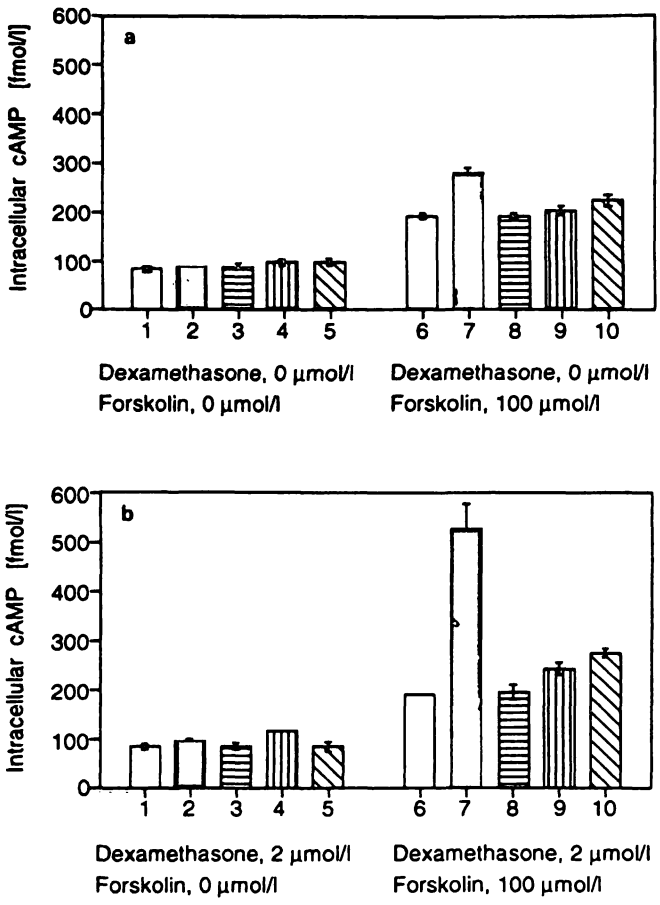

Fig. 4. Stimulation with carbamylcholine increases cAMP concentrations in TE671 cells:
Figure 4a gives data obtained without dexamethasone, figure 4b with the addition of 2 µmol/l dexamethasone to the medium. Columns 1 to 5 represent results with forskolin, columns 6 to 10 after preincubation of TE671 cells with 100 µmol/l forskolin.
☐ unstimulated cells; ☐ cells stimulated with 10 mmol/l carbamylcholine; ▤ carbamylcholine-stimulated cells preincubated with 100 µmol/l α-bungarotoxin; ▥ carbamylcholine-stimulated cells preincubated with 10 µmol/l α-bungarotoxin; ▨ carbamylcholine stimulated cells preincubated with 1 µmol/l α-bungarotoxin.
In all experiments TE671 cells were grown in RPMI medium supplemented with 1 mmol/l isobutylmethylxanthine to inhibit phosphodiesterases.

surface expression in TE671 cells after dexamethasone exposure (22). Similar results were reported by *Kaplan & Blau* on cultured human muscle cells (23).

Increase of $[Ca^{2+}]_i$ in TE671 cells after stimulation with carbamylcholine

A significant increase in $[Ca^{2+}]_i$ was observed in TE671 cells after stimulation with various concentrations of carbamylcholine. This agonist-mediated increase of $[Ca^{2+}]_i$ was completely blocked by the preincubation of TE671 cells with α-bungarotoxin (tab. 2). No $[Ca^{2+}]_i$ increase was observed in IMR32 cells (data not shown).

Autoantibodies in some sera of myasthenia gravis patients inhibit the carbamylcholine-induced increase of $[Ca^{2+}]_i$ in TE671 cells

Sera of 11 patients with myasthenia gravis were tested for their ability to inhibit carbamylcholine-induced increases in $[Ca^{2+}]_i$ in TE671 cells. In 4 of these patient sera we found a dose-dependent inhibition of maximal $[Ca^{2+}]_i$ to about 50% (fig. 5a–d). Interestingly, the serum of a patient with *Lambert-Eaton* syndrome was also able to suppress the increase in $[Ca^{2+}]_i$ (fig. 5e). The fact that this patient's serum was negative in conventional radioimmunoassay for the presence for anti-acetylcholine receptor antibodies is consistent with reports by *Sher & Clementi* (24) suggesting that *Lambert-Eaton* syndrome patient sera contain autoantibodies reacting with calcium channels. No inhibition of the carbamylcholine-induced increase in $[Ca^{2+}]_i$ in TE671 cells was found when sera from healthy donors (n = 5) were used (fig. 5f). None of the other tested sera from myasthenia gravis patients (n = 7) showed any inhibition of the calcium response. There was no correlation between acetylcholine receptor antibody titres in myasthenia gravis patient sera and their correlation to inhibit the calcium response. This might be due to the fact that autoantibodies against the acetylcholine receptor are directed against different epitopes of the receptor complex (25, 26) and might therefore differ in their functional capabilities.

Tab. 2. Carbamylcholine-induced acetylcholine receptor activation in TE671 cells measured by Ca^{2+}-mobilization

		Concentration of $[Ca^{2+}]_i$
Unstimulated cells		128[a]
Ionomycin	(0.25 µmol/l)	172
	(1.00 µmol/l)	351
	(10.0 µmol/l)	10758
Carbamylcholine	(1 mmol/l)	207
	(10 mmol/l)	409
	(25 mmol/l)	467
α-Bungarotoxin + Carbamylcholine	(10 µmol/l) (25 mmol/l)	137
α-Bungarotoxin + Carbamylcholine	(1 µmol/l) (25 mmol/l)	140

INDO-1 loaded TE671 cells were stimulated with ionomycin and carbamylcholine in indicated concentrations. $[Ca^{2+}]_i$ was determined as described in Material and Methods using a spectrofluorometer. Maximal values for $[Ca^{2+}]_i$ after stimulation with the indicated stimuli are given. For inhibition experiments cells were preincubated with α-bungarotoxin for 30 min at 37 °C in indicated concentrations.

[a] Figures are given in nmol/l. Results of one out of four independent experiments are shown.

Discussion

The binding of the snake venom α-bungarotoxin is a characteristic property of the human acetylcholine receptor. In this paper we describe two human tumour cell lines that were previously demonstrated to bind

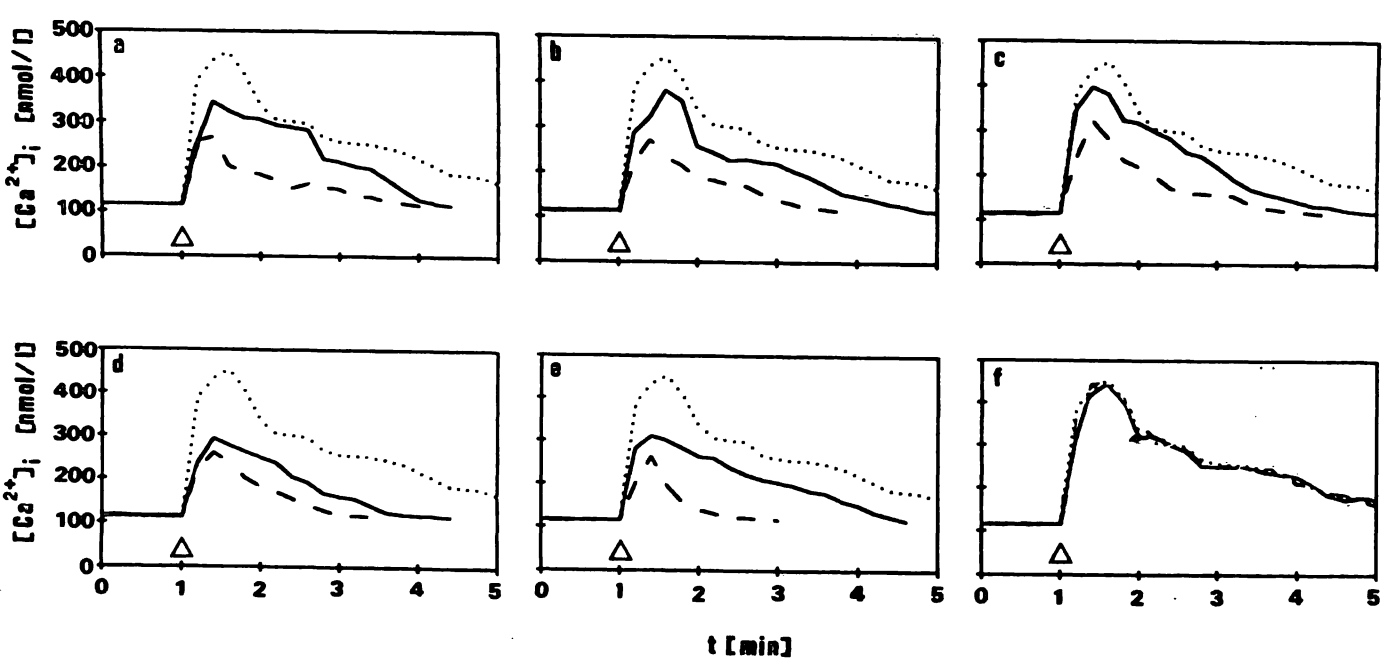

Fig. 5. Myasthenia gravis and *Lambert-Eaton* syndrome patient sera inhibit Ca^{2+}-mobilization in TE671 cells after stimulation with carbamylcholine:
Serum dilutions of 1 : 100 (dashed line) and 1 : 500 (solid line) from patients with myasthenia gravis (a–d) and one patient with a *Lambert-Eaton* syndrome (e) and serum of a healthy donor (f) were preincubated with TE671 cells. Untreated TE671 cells were used as controls (dotted line). $[Ca^{2+}]_i$ was measured after stimulation with 25 mmol/l carbamylcholine as described. Carbamylcholine was added at 1 min and is indicated (△).

α-bungarotoxin in vitro. One of these cell lines, TE671, which was originally described as a medulloblastoma cell line (7), is derived from a rhabdomyosarcoma (8). The second cell line investigated, IMR32, originates from a human neuroblastoma (10).

Applying gel filtration techniques, the α-bungarotoxin binding protein from TE671 cells exhibited a considerably higher molecular mass than the α-bungarotoxin protein isolated from IMR32 cells. When α-bungarotoxin binding proteins from the two cell lines were analysed by SDS-PAGE, the molecular mass and subunit composition of the α-bungarotoxin binding structure from TE671 cells showed a similar pattern to that known for the nicotinic acetylcholine receptor isolated from human muscle (21). The acetylcholine receptor purified from TE671 consists of subunits of M_r 44 000, 50 000, 54 000 and 68 000, which is consistent with the known characteristics of the α-, β-, γ- and δ-subunits of the human muscle nicotinic acetylcholine receptor. In contrast, the α-bungarotoxin binding protein from IMR32 cells showed a distinct pattern of one strong band at M_r 50 000 accompanied by weaker bands with higher and lower molecular masses. At present it is not clear if these differences in the protein pattern are due to the presence of different receptor subunits or degradation products.

When the immunological cross-reactivity was tested using sera from myasthenia gravis patients, a high cross-reactivity was found between α-bungarotoxin binding proteins from TE671 cells and human muscle acetylcholine receptor, whereas no cross-reactivity was found when α-bungarotoxin binding proteins from IMR32 cells were used as antigen. These data suggest that the α-bungarotoxin binding protein expressed on TE671 cells represents biochemically and immunologically intact human nicotinic acetylcholine receptors. This is apparently not true for the α-bungarotoxin binding protein from IMR32 cells.

In additional studies, second messenger mechanisms in TE671 cells and IMR 32 cells were tested after the cells had been stimulated with the acetylcholine receptor agonist carbamylcholine. In TE671 cells, an increase in cAMP concentrations was induced by carbamylcholine, but only when cells were pretreated with forskolin. This effect was inhibited by the addition of various concentrations of α-bungarotoxin. In addition, there was a pronounced increase in intracellular cAMP concentrations in cells grown in media supplemented with dexamethasone, which is known to induce and increase the acetylcholine receptor expression (22, 23). No increase in cAMP was seen in identically treated IMR32 cells.

As another mechanism of intracellular signal transduction, changes in $[Ca^{2+}]_i$ were determined after stimulation of TE671 cells with carbamylcholine. The resulting increase $[Ca^{2+}]_i$ showed a maximum about one minute after stimulation with carbamylcholine, and this increase was inhibited by the addition of α-bungarotoxin. These data suggest the presence of a functional acetylcholine receptor on TE671 but not on IMR32 cell lines. *Safran & Fuchs* described phosphorylation of *Torpedo californica*-derived acetylcholine receptor by a Ca^{2+}-dependent protein kinase C (27). The stimulation of acetylcholine receptor leads to the action of protein kinase and a cAMP-dependent protein kinase, followed by a desensitization of receptors (28, 29). Therefore, the agonist-induced decrease in channel currents of muscle nicotinic acetylcholine receptor may be related to intracellular Ca^{2+} concentrations (30). All these findings support the role of Ca^{2+} and cAMP in the muscle activation process. In one part of our experiments we measured an increase in intracellular Ca^{2+} after stimulation of TE671 cells with the agonist carbamylcholine. Whether this increase in $[Ca^{2+}]_i$ is caused by external Ca^{2+} influx or by release from intracellular stores remains to be investigated.

In addition, *Bencheriff & Lukas* described the existence of muscarinic acetylcholine receptors on the TE671 cell line (31). However, it is unlikely that the $[Ca^{2+}]_i$ increase in TE671 cells is mediated through muscarinic acetylcholine receptor, because this should not be blocked by α-bungarotoxin. Many questions remain concerning signal transduction pathways in malignant cell lines. For example *Morris & Newsom-Davis* described two α-subunit isoforms of the acetylcholine receptor in TE671 cells (32). A differential expression of these isoforms therefore may lead to an altered intracellular signal transduction pathway.

To investigate the role of acetylcholine receptor-function-inhibiting autoantibodies, we preincubated TE671 cells with myasthenia gravis patient sera prior to stimulation with carbamylcholine. Four out of 11 myasthenia gravis patients sera had an inhibiting effect on the maximal amplitude and duration of $[Ca^{2+}]_i$ increase in TE671 cells. However, no correlation could be obtained with acetylcholine receptor serum antibody titres. This finding could be explained by the well documented effect that acetylcholine receptor directed antibodies in myasthenia gravis patients sera are characterized by a considerable heterogeneity in terms of their reactivity to different epitopes on various subunits of the acetylcholine receptor. This observation is also in agreement with data published by *Lang & Newsom-Davis* (33), who demonstrate that

acetylcholine receptor antibodies inhibiting Na^+ influx in stimulated TE671 cells were present in only about 30% of myasthenia gravis patients' sera (34). Interestingly, the serum from one patient with a *Lambert-Eaton* symdrome also showed an inhibitory effect on the intracellular Ca^{2+} response in carbamylcholine-stimulated TE671 cells. This patient serum did not contain any detectable anti-acetylcholine receptor antibodies, which is in concordance with a report by *Sher & Clementi* (24) who described specific antibodies against Ca^{2+}-channels in sera of *Lambert-Eaton* patients, with the functional capacity to cause myasthenia gravis-like symptoms.

In summary, our data suggest that the α-bungarotoxin-binding proteins in TE671 cells represent immunologically and functionally intact human nicotinic acetylcholine receptors. Therefore, TE671 cells are not only useful for the isolation of acetylcholine receptors, but also for functional studies on the effects of various types of anti-acetylcholine receptor antibodies in myasthenia gravis patient sera. It remains to be ascertained whether the observation that about one third of myasthenia gravis patient sera were capable of modulating second messenger pathways in the TE671 cell line is of clinical relevance.

Acknowledgement

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