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MONOCLONAL ANTIBODIES DETECTING REGULATORY POLYPEPTIDES OF THE INSECT NEURONAL ACETYLCHOLINE RECEPTOR

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Summary

1. Monoclonal antibodies affecting the ligand binding of the neuronal acetylcholine receptor were prepared.

2. A clone was detected which produced antibodies that increased [^{125}I]-α-bungarotoxin binding, but decreased [^{3}H]acetylcholine binding.

3. Western blot analysis established that these antibodies did not recognize the receptor protein, but labelled a 20×10^{3} M_r polypeptide.

4. This putative regulatory polypeptide was purified and was found to inhibit [^{125}I]-α-bungarotoxin binding to pretreated neuronal membranes.

Introduction

The nicotinic type of receptor for acetylcholine (ACh) mediates synaptic signal transmission at muscle and nerve cells. Acetylcholine (ACh) released from the presynaptic nerve terminal of cholinergic synapses binds to its recognition site at the acetylcholine receptor (AChR) and induces conformational changes of the receptor protein which result in opening of an integral ion channel and, consequently, in a depolarization of the postsynaptic membrane (Popot and Changeux, 1984; Hucho, 1986; Maelicke, 1984). Because ACh receptors play a central role in transduction of neuronal signals, it has been proposed that they provide a molecular basis for plasticity of neuronal function (Changeux, 1981). A model of short-term modulation of synaptic efficacy has been proposed, assuming slow allosteric transitions of the postsynaptic receptor between an activatable and an inactivatable state, which might be controlled by binding of regulatory elements to allosteric sites (Changeux and Revah, 1987). Unfortunately, the neuronal nicotinic ACh receptors from vertebrate brain have not been studied in the same

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detail as those from the peripheral nervous system, mainly because of their low concentrations and the fact that α-bungarotoxin, a valuable high-affinity ligand, does not block AChR function in most neuronal preparations (Morley and Kemp, 1981). More recent studies have established that ACh and α-bungarotoxin do not have affinity for the same proteins in vertebrate brain (Schneider *et al.* 1985; Sugiyama and Yamashita, 1986; Whiting and Lindstrom, 1986, 1987; Benke and Breer, 1989).

In contrast to the vertebrate brain, the nervous system of insects contains high concentrations of nicotinic ACh receptors which bind α-bungarotoxin (BGTX) as well as cholinergic agonists (Breer and Sattelle, 1987; Benke and Breer, 1989). The receptor proteins have been purified (Breer *et al.* 1984, 1985) and reconstituted into a planar lipid bilayer, where they form ACh-activated cation channels (Hanke and Breer, 1986, 1987). Thus, preparations from the nervous tissue of insects may provide an appropriate system for studying regulatory influences on nicotinic receptors of nerve cells, as monitored by their effects on BGTX and ACh binding. In the present report attempts have been made to obtain monoclonal antibodies for antigenic epitopes which affect ligand binding to the AChR, and thus may be involved in regulating receptor function.

Materials and methods

Isolation of neuronal membrane fractions

Crude synaptic membranes were prepared from the nervous tissue of locusts (*Locusta migratoria migratorioides*) as described by Breer *et al.* (1985). Head and thoracic ganglia were dissected and collected at 4°C. The nervous tissue of about 40 locusts was homogenized in 10 vols of ice-cold buffer [50 mmol l^{-1} Hepes/Tris, pH 6.8, 150 mmol l^{-1} NaCl, 5 mmol l^{-1} EDTA, 3 mmol l^{-1} EGTA, 0.1 mmol l^{-1} phenylmethylsulphonyl fluoride (PMSF), 0.02 % NaN_3] and centrifuged at 1000 *g* for 10 min. The supernatant (S_1) was centrifuged at 27 000 *g* for an additional 10 min at 4°C. For the binding assay the resulting pellet (P_2) was resuspended in incubation buffer (50 mmol l^{-1} Hepes/Tris, pH 6.8, 150 mmol l^{-1} NaCl, 5 mmol l^{-1} KCl, 3 mmol l^{-1} $CaCl_2$, 1 mmol l^{-1} $MgCl_2$) to give a protein concentration of 1 mg ml^{-1}.

Generation of hybridoma cell lines

Balb/c mice were immunized by intraperitoneal injection of a purified AChR preparation emulsified in Freund's complete adjuvant. Booster immunizations were given at 4-week intervals for 2–3 months. 3 weeks after the last injection, serum was immunoassayed. The highest-titre mice were hyperimmunized by injections of purified AChR in phosphate-buffered saline (PBS) for 3 consecutive days, rested for 1–2 days, and then killed and splenectomized. Splenocytes were fused with P3-X63 myelomas in a ratio of 1:5 splenocytes to myeloma cells using PEG 1500, according to the method of Galfre and Milstein (1981). Hybridoma cell lines were grown in modified Dulbecco's medium (Gibco), according to the

method of Littlefield (1984), plus 20% foetal bovine serum, 0.1 mmol l^{-1} hypoxanthine, 0.4 μmol l^{-1} aminopterin, 20 μmol l^{-1} thymidine, 50 μmol l^{-1} β-mercaptoethanol, 100 μg ml^{-1} penicillin and 100 μg ml^{-1} streptomycin (HAT medium). After 14 days of maintenance in HAT medium, hybridomas were switched to HT medium (HAT medium without aminopterin) and then screened for antibody secretion. For first selection, hybridomas were screened by enzyme-linked immunoassay (ELISA) using neuronal membrane preparations as antigen. Finally, positive hybridomas were tested by [^{125}I]-α-bungarotoxin binding for specific effects. Positive wells were expanded and then cloned by serial dilution.

Screening methods

ELISA

Positive hybridomas were identified with enzyme-linked immunosorbant assay, using neuronal membrane preparations (100 ng protein cup^{-1}) as antigen. For detection of antibody binding, peroxidase-labelled anti-mouse IgG was used, and the reaction was carried out using 100 μl of a reaction medium (containing 2 ml of 1 mol l^{-1} sodium acetate, pH 6.8, 10 μl of 3% H_2O_2 and 125 μl of tetramethylbenzidine made up to a volume of 20 ml with distilled water). After a certain incubation time (20–40 min) the reaction was stopped by adding 100 μl of 1 mol l^{-1} H_2SO_4 per cup. The extent of the reaction was determined by absorbance measurement at 450 nm in a Dynatech MR 600 microplate reader.

Gel electrophoresis and Western blot analysis

Electrophoresis was performed using a microelectrophoresis apparatus (Biometra). SDS–polyacrylamide gels were prepared according to the method of Laemmli (1970). Samples to be analysed were treated with an equal volume of sample buffer (150 mol l^{-1} Tris/HCl, pH 6.8, 20% glycerol, 0.002% Bromophenol Blue, 10% β-mercaptoethanol, 2% SDS). Subsequently, the mixtures were heated in a boiling water bath for 3 min and loaded onto the gel. Polypeptides were visualized by silver staining.

Following electrophoresis, the transfer of neuronal membrane polypeptides from polyacrylamide gels onto nitrocellulose sheets (Schleicher & Schüll, BA 85) was accomplished in a cooled electroblot apparatus for 6 h according to the method of Towbin *et al.* (1979). The immunoreactive polypeptides were visualized using the immunoscreening system Protoblot (Promega). After blocking the remaining sites, the blot was reacted with cell culture supernatant of the hybridoma clone. Following the antigen–antibody reaction, the blot was washed and treated with alkaline phosphatase-conjugated rabbit anti-mouse IgG. The immunoreactive polypeptides were visualized by enzyme reaction. The reaction was carried out using BCIP (5-brom-4-chlor-3-indolylphosphate; 6.6 μg ml^{-1}) as substrate and NIB (Nitrotetrazolium Blue; 3.3 μg ml^{-1}) in alkaline buffer (100 mmol l^{-1} Tris/HCl, pH 9.5; 100 mmol l^{-1} NaCl, 5 mmol l^{-1} $MgCl_2$) as coupling reagent.

Immunohistochemistry

The mesothoracic ganglia of locusts were excised and fixed for 3 h at 4 °C in 5 % paraformaldehyde, 5 % sucrose. Following extensive washes in phosphate-buffered saline containing 5 % sucrose, the tissue was frozen in a drop of Tissue Tec (Miles) and sectioned in a cryostat (20 μm). The dried sections were incubated with culture supernatant of the hybridoma clone VIII-D2. Following this incubation the slides were rinsed with phosphate-buffered saline containing 1 % normal goat serum and then incubated with goat anti-mouse IgG. Subsequently, the samples were rinsed and further incubated with a solution containing a soluble complex of peroxidase–antiperoxidase (Sternberger). After a final rinse in buffer the bound peroxidase was reacted with hydrogen peroxide and 3,3′-diaminobenzidine.

Binding assays

α-Bungarotoxin binding

Samples of the resuspended membranes (20–50 μg protein) were incubated in the presence of 1–3 nmol l^{-1} [^{125}I]-α-BGTX for 30–60 min at room temperature. Subsequently, the reaction was terminated by vacuum filtration through glass fibre filters (Whatman GF/C), presoaked in 1 % bovine serum albumin (BSA). The filters were washed twice with 3 ml of buffer (10 mmol l^{-1} Hepes/Tris, pH 6.8, 150 mmol l^{-1} NaCl) and the radioactivity was determined in a gamma counter. Specific binding was defined as total binding minus binding in the presence of 10^{-4} mol l^{-1} nicotine or 10^{-6} mol l^{-1} unlabelled BGTX.

Acetylcholine binding

For ACh binding, the final membrane pellet (P_2) was washed twice in buffer by centrifugation. Samples of the washed membranes (50–100 μg protein) were preincubated with 2 μmol l^{-1} atropine (to block muscarinic cholinergic receptors) and 1 μmol l^{-1} neostigmine (to prevent ACh hydrolysis) for 30 min on ice. Then 20 nmol l^{-1} [^{3}H]ACh (80 Ci mmol^{-1}) was added and the incubation was continued for an additional 45 min. Subsequently, the reaction was stopped by vacuum filtration. The filters were prewetted in 0.05 % polyethylenimine to reduce nonspecific binding. The radioactivity of the filters was determined by scintillation counting. Nonspecific ACh binding was measured in the presence of 0.1 mmol l^{-1} unlabelled ACh or nicotine.

Purification of VIII-D2 antigen

Membranes were extracted with 100 vols 1 % saponin (in 5 mmol l^{-1} Hepes/Tris, pH 6.8, 5 mmol l^{-1} EDTA) for 30 min on ice and centrifuged for 15 min at 27 000 *g* The supernatant was concentrated and applied to a Sephadex G-75 gel filtration column (2.5 cm × 60 cm) equilibrated in 20 mmol l^{-1} Hepes/Tris, pH 6.8, 100 mmol l^{-1} NaCl. Fractions (9 ml) were collected at a flow rate of 27 ml h^{-1} a[illegible] 0.1 ml of each fraction was assayed for immunoreactivity. The active fractio[illegible]

were dialysed, concentrated and submitted to ion-exchange chromatography. The column (TSK DEAE 3SW, LKB; equilibrated in 20 mmol l^{-1} Tris/Hepes, pH 7.8, 50 mmol l^{-1} sodium acetate) was run with a 0.05–1 mol l^{-1} linear sodium acetate gradient at a flow rate of 0.75 ml min^{-1}. Fractions containing the immunoreactivity were combined and dialysed overnight. The concentrated sample was finally subjected to gel filtration (TSK G 3000SW, LKB); material was eluted with 20 mmol l^{-1} Tris/Hepes, pH 7.5, 150 mmol l^{-1} sodium acetate at a flow rate of 0.5 ml min^{-1}. Fractions (1 ml) were collected and analysed for immunoreactivity.

Purification of acetylcholine receptors

AChR were affinity purified from a deoxycholate extract of neuronal membranes as previously described (Breer *et al.* 1985). For affinity chromatography, α-bungarotoxin was coupled to cyanobromide-activated Sepharose 4B (Lindstrom *et al.* 1981). The solubilized receptor protein was applied to the affinity gel and incubated overnight at 4°C. Thereafter the column was washed twice with buffer (10 mmol l^{-1} Tris/HCl, pH 7.4, 100 mmol l^{-1} NaCl, 5 mmol l^{-1} EDTA, 3 mmol l^{-1} EGTA, 0.1 mmol l^{-1} PMSF, 0.02 % sodium azide, 0.1 % sodium deoxycholate). The bound receptors were eluted from the column with buffer containing either 0.1 mmol l^{-1} *d*-tubocurarine or 1 mol l^{-1} carbamylcholine. The eluate was dialysed to remove the cholinergic ligand and was subsequently lyophilized.

Results

Effects of monoclonal antibodies on ligand binding

To obtain hybridoma clones producing antibodies affecting cholinergic ligand binding, mice were immunized with partially purified AChR preparations. After fusion of splenocytes, culture media of hybridomas which gave a positive response in ELISA tests using neuronal membranes were screened for their effects on BGTX binding. Several clones were identified having weak positive or negative effects, but one hybridoma clone (VIII-D2) was found to produce antibodies that significantly increased BGTX binding (Fig. 1). These antibodies exerted maximal effects on BGTX and ACh binding when the membranes were permeabilized with 0.05 % saponin (Fig. 2). In the presence of saponin the antibodies increased BGTX binding twofold, and significantly decreased ACh binding. The opposite nature of the effects on BGTX and ACh binding points to an indirect, allosteric effect of the antibodies. In Western blot analysis, using neuronal membrane preparations, the VIII-D2 antibodies failed to recognize the AChR polypeptides (65×10^3 M_r), but selectively labelled a 20×10^3 M_r polypeptide band. These results suggest that the antibody's effect on the AChR binding may be mediated by 20×10^3 M_r polypeptides, rather than being a direct interaction of the antibody with the AChR (Fig. 3).

To obtain more information on the localization of the antigen, subcellular fractions were analysed using the antibody as probe in dot blots. The immunoreactivity was found to be restricted to membranes and was not found in the cytoplasm.

Some of the immunoreactivity could be removed from the membranes by hypo-osmotic treatment; addition of detergent (1% saponin) extracted it more effectively, possibly indicating its hydrophobic interaction with the membrane (Table 1). Furthermore, the antigen is obviously specifically associated with

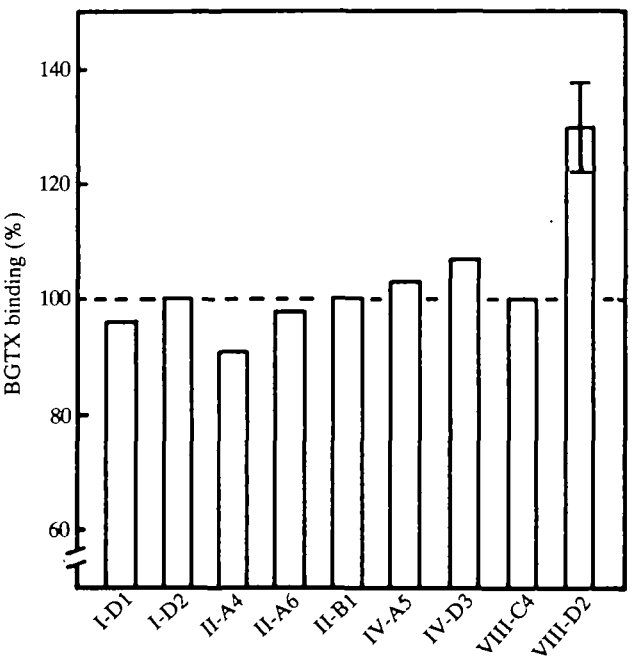

Fig. 1. Effects on BGTX binding of culture media derived from various hybridoma clones. Neuronal membranes were incubated with the indicated hybridoma supernatant in the presence of 1 nmol l^{-1} [^{125}I]-α-bungarotoxin for 60 min at room temperature and subsequently the bound toxin was determined by vacuum filtration. Nonspecific binding was determined in the presence of 1 μmol l^{-1} unlabelled BGTX. The data for the hybridoma clone VIII-D2 are the mean±S.D. of three determinations.

Table 1. *Subcellular localization of the VIII-D2 antigen*

Preparation	Immunoreactivity
P_2 membranes	+
S_2 supernatant	−
P_2 lysate*	+
P_2 extract†	+

*Neuronal membranes (P_2) were extracted with 5 mmol l^{-1} Hepes/Tris, pH 6.8, 5 mmol l^{-1} EDTA. The membranes were pelleted by centrifugation (27 000 *g*, 15 min) and the supernatant was tested for immunoreactivity in the dot blot assay.

†Neuronal membranes (P_2) were extracted with 5 mmol l^{-1} Hepes/Tris, pH 6.8, 5 mmol l^{-1} EDTA, 0.5% saponin and the supernatant was tested for immunoreactivity in the dot blot assay.

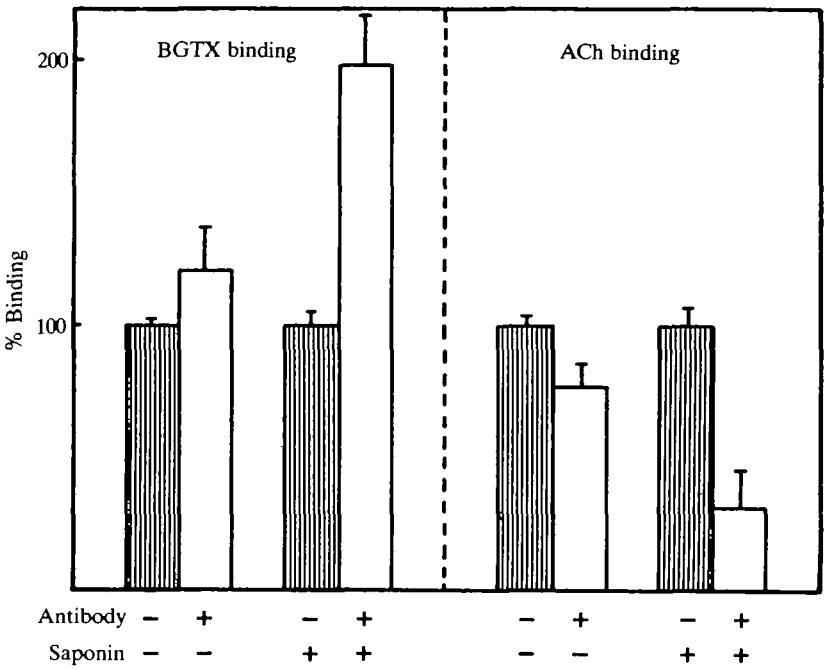

Fig. 2. Effect of VIII-D2 hybridoma clone supernatant on [^{3}H]acetylcholine and [^{125}I]BGTX binding. Neuronal membranes were preincubated with VIII-D2 hybridoma clone supernatant in the presence or absence of 0.01 % saponin for 15 min at room temperature and then [^{3}H]acetylcholine and [^{125}I]-α-BGTX binding were determined. Control experiments were performed by substitution of the hybridoma supernatant with culture medium. Data are the means of three determinations+S.D.

Table 2. *Tissue distribution of VIII-D2 immunoreactivity*

Tissue*	Immune reaction
Cerebral ganglia	+
Thoracic ganglia	+
Abdominal ganglia	+
Thoracic muscle	–
Leg muscle	–
Malpighian tubules	–
Labial gland	–
Fat body	–
Gut	–

*The indicated tissue was homogenized in buffer, centrifuged at 1000 *g* for 10 min and the supernatant was tested for immunoreactivity in dot blot assays.

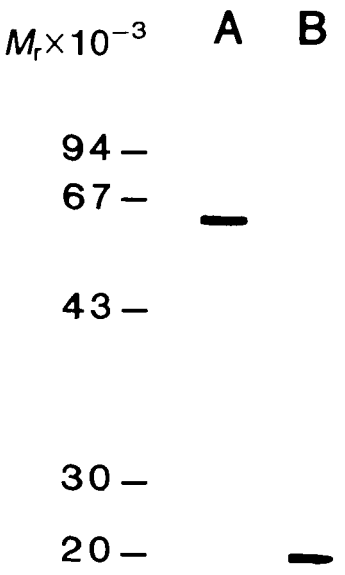

Fig. 3. Immunoblot staining of acetylcholine receptor polypeptides and VIII-D2 antigen using a monospecific antiserum directed against affinity-purified ACh receptor protein (A) or VIII-D2 supernatant (B). Neuronal membranes were solubilized in sample buffer for electrophoresis and run on 12.5% SDS–polyacrylamide slab gels; immunoreactive polypeptides were identified using the Western blot analysis.

neuronal membranes, since several non-neuronal tissues showed no significant immunoreactivity (Table 2).

Immunohistochemical investigation showed that the VIII-D2 antigen was localized in the insect ganglia. In tissue sections from locust thoracic ganglia intensive labelling was found in the central neuropile, known to be very rich in synaptic contacts (Fig. 4). Furthermore, immunoreactivity was detected in some neuronal fibres.

Purification of the antigen

Several chromatographic steps were employed to purify the VIII-D2 antigen. First, a detergent extract from neuronal membranes was applied to a Sephadex G-75 gel filtration column and the eluted material in each fraction was tested for immunoreactivity. Immunoreactivity was exclusively found in the void volume, indicating a relative molecular mass (M_r) greater than 70×10^3 (Fig. 5). The positive fractions were pooled and subsequently subjected to HPLC anion-exchange chromatography. Immunoreactive material adsorbed quantitatively to the resin and was eluted with a linear salt gradient (Fig. 6). Finally, the positive fractions were desalted and subjected to HPLC gel filtration. The immunoreactivity eluted as a single peak with an apparent M_r of about 80×10^3 (Fig. 7). The

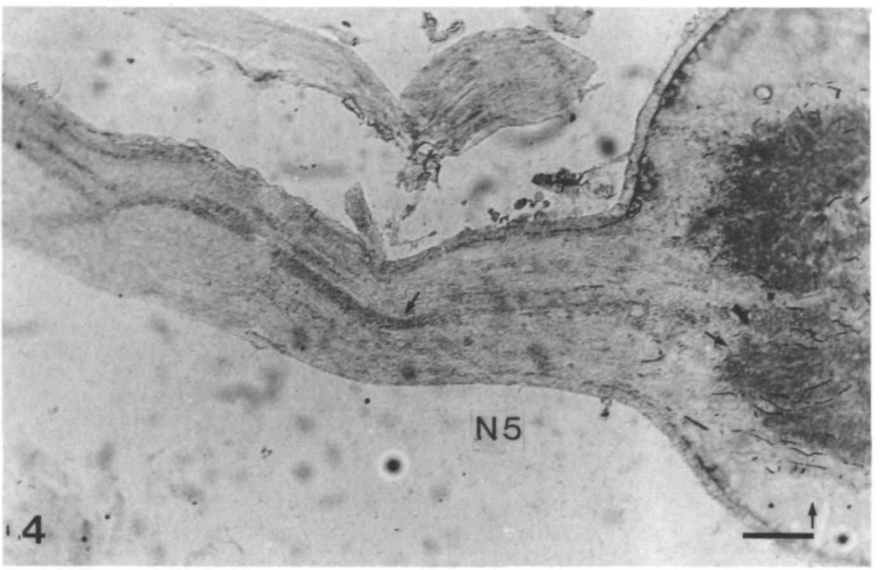

Fig. 4. Immunocytochemical localization of VIII-D2 antigen in the mesothoracic ganglia of locust. Cross-sections were treated with hybridoma supernatant and antigenic sites were visualized by the peroxidase–antiperoxidase reaction. Horizontal section (20 μm) with peripheral nerve (N5). Note the heavy labelling of the neuropile; some nerve fibres are also labelled. Arrow points towards anterior. Scale bar, 100 μm.

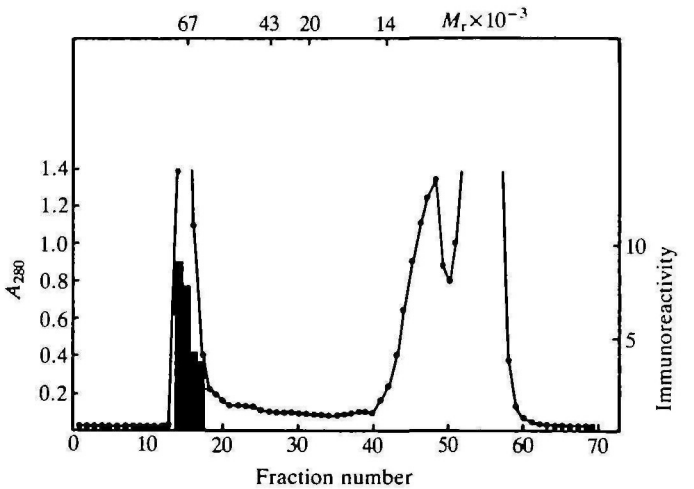

Fig. 5. Gel filtration of a crude detergent extract of neuronal membranes. The extract was concentrated and applied to a Sephadex G-75 gel filtration column (2.5 cm×60 cm, equilibrated in 20 mmol l^{-1} Hepes/Tris, pH 6.8, 100 mmol l^{-1} NaCl). 9 ml fractions were collected at a flow rate of 27 ml h^{-1} and 0.1 ml of each fraction was assayed for immunoreactivity. The column was calibrated using the following marker proteins: BSA (67×10^3 M_r), ovalbumin (43×10^3 M_r), trypsin inhibitor (20×10^3 M_r) and lactalbumin (14×10^3 M_r). (●) absorbance at 280 nm; filled columns indicate immunoreactivity (in relative units) of the fractions.

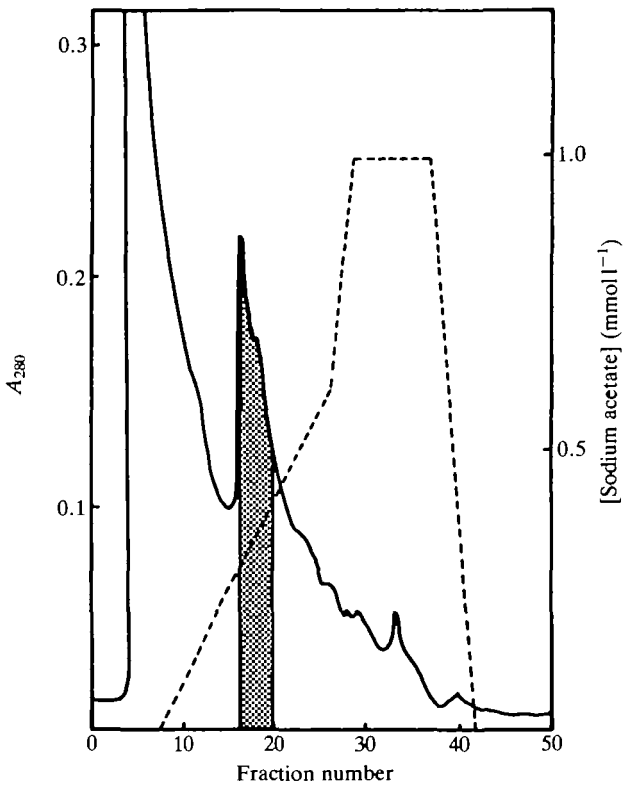

Fig. 6. HPLC anion-exchange chromatography of immunoreactive fractions from Sephadex G-75 gel filtration. The active fractions of the Sephadex G-75 chromatography were dialysed, concentrated and submitted to a DEAE ion-exchange column. The column (UltroPac TSK DEAE 3SW, LKB; equilibrated in 20 mmol l^{-1} Tris/Hepes, pH 7.8, 50 mmol l^{-1} sodium acetate) was run with a 0.05–1 mol l^{-1} linear sodium acetate gradient at a flow rate of 0.75 ml min^{-1}. Left ordinate indicates the absorbance at 280 nm; dashed lines and right ordinate indicate the sodium acetate gradient; immunoreactive fractions are shaded.

purified material was subsequently analysed by gel electrophoresis under denaturating conditions, followed by silver staining. A single polypeptide band (20×10^3 M_r) was found (Fig. 8). These results suggest that either the polypeptides aggregate during the isolation procedure or the native protein may represent an oligomeric complex of 20×10^3 M_r subunits.

To verify the modulatory effect of the purified antigen on the AChR, BGTX binding to pretreated neuronal membranes was determined in the presence or absence of the purified polypeptides. In the presence of purified antigen the BGTX binding was significantly reduced (Fig. 9).

Discussion

Using hybridoma techniques to obtain monoclonal antibodies affecting ligand

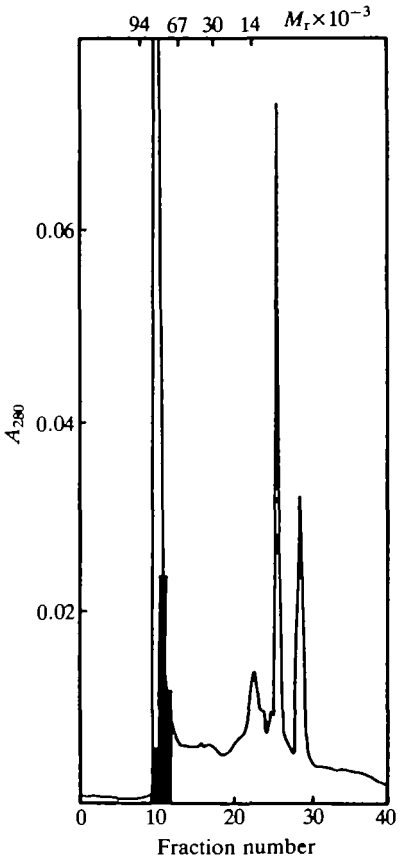

Fig. 7. HPLC gel filtration of immunoreactive fractions of the DEAE ion-exchange chromatography. Positive fractions eluted from the DEAE column were combined, dialysed overnight and the concentrated sample was finally injected onto a gel filtration HPLC column (TSK G 3000SW, LKB). Material was eluted with 20 mmol l^{-1} Tris/Hepes, pH 7.5, 150 mmol l^{-1} sodium acetate at a flow rate of 0.5 ml min^{-1}. Fractions (1 ml) were collected and analysed for immunoreactivity. Left ordinate indicates the absorbance at 280 nm; filled columns indicate the immunoreactivity of the fractions; marker proteins were BSA (64×10^3 M_r), ovalbumin (43×10^3 M_r), carbonic anhydrase (30×10^3 M_r), trypsin inhibitor (20×10^3 M_r) and lactalbumin (14×10^3 M_r).

binding of the neuronal AChR, one clone (VIII-D2) was isolated producing antibodies that increase BGTX binding, but decrease ACh binding. Since ACh and BGTX occupy distinct, only partially overlapping, binding sites on the insect AChR (Benke and Breer, 1989) this bimodal effect on ligand binding suggests an indirect action of the antibody, possibly by altering the conformational state of the AChR protein. In this regard, binding of the antibody to its antigenic sites appears to induce conformational transitions of the receptor complex, which result in a masking of the ACh recognition sites and exposure of additional BGTX binding sites. Although the VIII-D2 clone was obtained by immunizing mice with partially

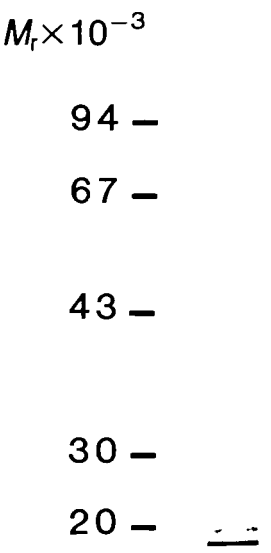

Fig. 8. Electrophoretic analysis of immunoreactive fractions eluted from the HPLC gel filtration column. Immunoreactive fractions were concentrated, treated with sample buffer for electrophoresis and applied to a 12.5 % SDS–polyacrylamide slab gel. Polypeptides were visualized by silver staining. For details see Materials and methods.

purified AChR protein, the antigenic recognition site is not a constituent of the AChR itself, but of a distinct 20×10^3 M_r polypeptide. This suggests that this polypeptide may be closely associated with the AChR and that during the purification procedure some of the 20×10^3 M_r polypeptide remains attached to the receptor complex, sufficient to induce the production of specific antibodies. This view of a close association between the AChR and the 20×10^3 M_r polypeptides was further supported by the following observations. (1) The VIII-D2 antigen is exclusively present in neuronal tissue, but not in nonneuronal preparations. (2) The VIII-D2 antibodies labelled exactly the same areas of the central neuropile which react with polyclonal antibodies raised against the purified locust ACh receptor (Breer *et al.* 1985). (3) The AChR as well as the VIII-D2 antigen are co-localized in the membrane fraction. But, in contrast to the ACh receptor, the VIII-D2 antigen seems to be a peripheral protein, as it was partially removed from the membranes by hypo-osmotic treatment in the presence of EDTA and was removed by mild detergent extraction, whereas the ACh receptor remains in the membranes under these conditions.

All these results point to the existence of membrane-associated polypeptides that might regulate ligand binding to the ACh receptor. In this system, binding of the VIII-D2 antibodies would result in an inactivation of these putative regulatory polypeptides. As a consequence, the ACh binding sites would be masked, whereas

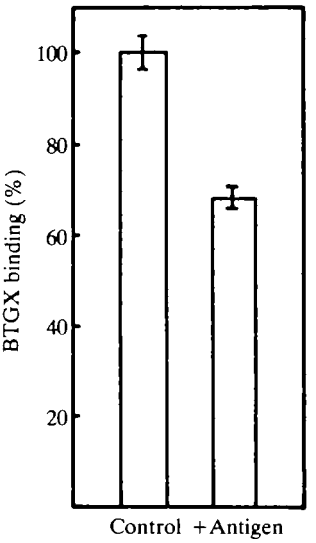

Fig. 9. Effect of purified VIII-D2 antigen on [^{125}I]-α-BGTX binding. Following pretreatment with 1% saponin, the membranes were washed three times with incubation buffer prior to the binding assays. The pretreated membranes were incubated in the presence or absence of purified antigen with 1 nmol l^{-1} [^{125}I]-α-BGTX for 60 min at room temperature and specific BGTX binding was determined by vacuum filtration followed by gamma counting. The data are the means of three determinations ±S.D.

additional BGTX binding sites would be exposed. This thesis implies that removal of the regulatory polypeptides ought to exert the same effect on ligand binding; we have recently demonstrated that removal of membrane-associated polypeptides by mild detergent treatment reversibly increases the number of BGTX binding sites and decreases the number of binding sites for ACh (D. Benke & H. Breer, unpublished results). The regulatory components were purified and independently identified as polypeptides of about 20×10^3 M_r. In addition, it was found that the VIII-D2 antibodies crossreact with these modulatory polypeptides, indicating some molecular relatedness of the VIII-D2 antigen and the regulatory polypeptides. The polypeptides detected by the VIII-D2 antibodies may be involved in regulating the number of functional and nonfunctional ACh receptor sites in nerve cells, as recently proposed by Margiotta *et al.* (1987*a*,*b*). Such a mechanism would allow precise control of cholinergic chemosensitivity of nerve cells and thus enable the postsynaptic neurone to respond rapidly to microenvironmental changes.

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