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Biochemical Properties of Acetylcholine Receptor Subunits from *Torpedo californica*†

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ABSTRACT: Four polypeptide chains composing acetylcholine receptors from the electric organ of *Torpedo californica* were purified by preparative electrophoresis in sodium dodecyl sulfate. Their apparent mole ratio $\alpha/\beta/\gamma/\delta$ is 2:1:1:1. These chains are not readily distinguished by amino acid or carbohydrate composition but are distinguished by apparent molecular weight and polypeptide maps. By peptide maps, no extensive homology is evident between these chains or between any of these chains and higher molecular weight chains found in receptor-enriched membrane fragments.

Acetylcholine receptor (AcChR)[1] solubilized from the electric organ of *Torpedo californica* by Triton X-100 and purified by affinity chromatography is composed of four polypeptide chains termed α, β, γ, and δ. The apparent molecular weights of these chains depend on the electrophoresis system and standards employed but approximate 38 000, 50 000, 57 000, and 64 000 for α, β, γ, and δ, respectively (Weill et al., 1974; Raftery et al., 1975; Hucho et al., 1978; Chang & Bock, 1977; Lindstrom et al., 1978; Froehner & Rafto, 1979). α chains compose at least part of the acetylcholine binding site (Weill et al., 1974; Damle et al., 1978). δ chains are located near the acetylcholine binding site (Hamilton et al., 1978; Witzemann & Raftery, 1978). *Torpedo* AcChR's are dimerized through a disulfide bond between δ chains of adjacent monomers (Chang & Bock, 1977; Suarez-Isla & Hucho, 1977; Hamilton et al., 1977). However, the function of the β, γ, or δ chains is unknown. If the ion conductance channel regulated by acetylcholine binding is an integral component of the AcChR macromolecule, then some or all of these chains may be components of this channel.

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[1] Abbreviations used: AcChR, acetylcholine receptor; [^{125}I]-α-BGT, ^{125}I-labeled α-bungarotoxin; $NaDodSO_4$, sodium dodecyl sulfate.

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From the apparent molecular weights of these chains and AcChR monomers and the observation of two ligand binding sites per monomer, it has been proposed that the subunit composition of the monomer is $\alpha_2\beta\gamma\delta$ (Reynolds & Karlin, 1978; Damle & Karlin, 1978). Limited proteolytic digestion of α, β, γ, and δ, followed by electrophoresis on acrylamide gels, gave different patterns of peptide fragments for each polypeptide chain, suggesting that they are not derivatives of each other (Froehner & Rafto, 1979; Nathanson & Hall, 1979).

In this paper we provide direct evidence for the subunit composition $\alpha_2\beta\gamma\delta$. We show that although α, β, γ, and δ are not greatly distinguished one from another by amino acid or carbohydrate composition, two-dimensional peptide maps show that they have little if any homology with one another or higher molecular weight components from electric organ membranes. This biochemical evidence for the uniqueness of each chain is consistent with immunochemical evidence presented in the following paper (Lindstrom et al., 1979).

The purpose of this paper is to identify and partially biochemically characterize the polypeptide chains composing *Torpedo* AcChR which we use as immunogens and antigens in the following paper (Lindstrom et al., 1979). In that paper we show that polypeptide chains are found in AcChR from other species which specifically cross-react with these chains. This provides strong evidence that these chains form subunits of AcChR. Muscle AcChR is much less amenable to biochemical study than is *Torpedo* AcChR, because it is obtainable in much lower amounts. If the structure of muscle AcChR subunits is to be compared with that of *Torpedo* AcChR subunits by means of antibody templates, then biochemical characterization of the *Torpedo* subunits is doubly important.

Materials and Methods

T. californica (Pacific Biomarine) AcChR was solubilized in Triton X-100 (Sigma Chemicals) or sodium cholate (Interchim) and purified by affinity chromatography on toxin–agarose as previously described (Lindstrom et al., 1978).

The component polypeptide chains were purified by preparative electrophoresis in $NaDodSO_4$ using a slight modification of the previously described method (Lindstrom et al., 1978). Larger slab gels were used (40 × 30 × 0.5 cm), and electrophoresis was performed for 17–20 h at 120 mA in a 4 °C room. Both staining with 0.25% Coomassie brilliant blue and destaining were done in 50% methanol–0.5% acetic acid over a period of less than 15 min to minimize fixation of protein. Stained bands sliced from the gel were each homogenized in a Waring blender for 60 s in 300 mL of water. Then 1.5 mL of 10% $NaDodSO_4$ was added. Homogenates were agitated overnight at 4 °C and then for 2 h at room temperature before gel fragments were removed by vacuum filtration. The extracts were then lyophilized, transferred to smaller tubes in 10 mL of water, and relyophilized. Dye was removed by three extractions with 5 mL of methanol at −20 °C. Then 2 mL of water was added, followed by overnight dialysis against 0.05% $NaDodSO_4$, 10 mM sodium phosphate buffer, pH 7.5, and 10 mM sodium azide at room temperature. Any insoluble material was removed by 2-min centrifugation in a microfuge. The yield of purified chains varied from 30 to 90% of the protein applied to the gel.

Electrophoresis on slab gels (19 × 18 × 0.1 cm) using a 4.75% acrylamide stacking gel, a 10% acrylamide running gel, and the discontinuous buffer system with 0.1% $NaDodSO_4$ described by Laemmli (1970) was conducted at 30 mA for 3–5 h.

Amino acid composition of AcChR chains was determined on a Beckman Model 121 analyzer after hydrolysis in 5.7 N HCl for 24 h at 110 °C. Cysteine was not determined. Tryptophan was determined spectrophotometrically (Edelhoch, 1967).

Carbohydrate composition of purified AcChR chains was determined by gas–liquid chromatography after methanolysis and preparation of trimethylsilyl ether derivatives of *O*-methyl glycosides (Clamp et al., 1971; Etchison & Holland, 1975). The protein amount was determined by the Lowry (1951) method, using bovine serum albumin as the standard. After dialysis against water, samples were lyophilized. Aliquots of 0.25–0.5 mg of lyophilized chains along with 25 nmol of mannitol as the internal standard were subjected to anhydrous methanolysis for 18 h (Clamp et al., 1971). Samples were neutralized and N-acetylated according to the procedure of Etchison & Holland (1975) for better recovery of amino sugars. The dry, vacuum-desiccated *O*-methyl glycosides were trimethylsilylated and analyzed by using a Hewlett-Packard 7610A gas chromatograph equipped with a glass column (6 ft × 1/4 in. in o.d.) packed with 3% SE-30 on 80/100 Supelsport support. The instrument was programmed to run isothermally at 130 °C for 10 min, followed by a 1 °C/min increase to 200 °C final temperature. Quantitation of individual monosaccharides was performed by comparing the peak area (calculated by Spectro-Physics System I electronic integrator) of the internal standard to that of various sugars multiplied by a detector response factor.

Analysis of lipid content associated with detergent-solubilized macromolecular AcChR was also determined by gas chromatography. First the AcChR was methanolyzed as described above, and then the sample was extracted 3 times with 1 mL of hexane. The fatty acid content of the hexane extract was analyzed by using heptadecanoic acid in methyl ester as the internal standard. A 6-ft glass column containing 10% DEGS on 80/100 chromasorb-W was used at 160 °C under isothermal conditions (Yogeeswaran et al., 1972).

Analysis of tryptic peptides from purified AcChR chains basically followed the method of Gibson (1974). Solutions containing 1 mg of the chain (and sometimes tracer quantities of the ^{125}I chains labeled with ^{125}I as described in the following paper) were treated with 20% trichloroacetic acid at 4 °C, and the precipitated protein was collected by centrifugation at 20000*g* for 0.5 h. Protein pellets were washed twice with 95% ethanol at −20 °C and finally with absolute acetone at −20 °C. After drying, the protein pellets were performic acid treated and trypsin digested at room temperature for 18 h by two additions of 10 μg of L-1-tosylamido-2-phenylethyl chloromethyl ketone (TPCK) treated trypsin (Worthington). Tryptic digests were lyophilized several times and finally dissolved in electrophoresis solvent, spotted on microcrystalline cellulose thin-layer plates (EM Laboratories), and electrophoresed in one dimension and chromatographed in the other as described by Gibson (1974). ^{125}I-Labeled peptides were visualized by autoradiography (Gibson, 1974). Unlabeled peptides were visualized by spraying the plates with fluorescamine (Hoffmann-La Roche Inc.) as described by Felix & Jimenez (1974). Fluorescamine-labeled peptides were photographed under ultraviolet illumination as described by Stephens (1978).

Results

The apparent molecular weights reported for α, β, γ, and δ have depended on the electrophoresis system used and the values assigned to standard proteins [e.g., Weill et al. (1974) and Froehner & Rafto (1979)]. The electrophoretic migration

Table I: Mole Ratio of Purified α, β, γ, and δ Chains Recovered from Preparative Acrylamide Gel Electrophoresis of Purified *Torpedo* AcChR

no. of prepn	% yield[a]	mole ratio of $\alpha/\beta/\gamma/\delta$ (av ± SD)
7	30–40	4.4 ± 1.5:2.0 ± 0.40:1:1.3 ± 0.40
2	50–65	2.9 ± 0.10:1.3 ± 0.13:1:1.1 ± 0.16
6	73–100	2.1 ± 0.27:1.1 ± 0.24:1:1.1 ± 0.09

[a] Percent yield = [(protein recovered from the gel)/(protein applied to the gel)]100.

of these chains with respect to bovine serum albumin, human IgG heavy chain, ovalbumin, and horseradish peroxidase in the gel system used in these papers indicates apparent molecular weights for α, β, γ, and δ of 38 000, 50 000, 57 000, and 64 000, respectively. Immunochemical methods described in the following paper provide a way to clearly, qualitatively distinguish these *Torpedo* AcChR chains and their analogues in AcChR from other species.

The apparent mole ratio of $\alpha/\beta/\gamma/\delta$ purified by preparative electrophoresis depended on the total yield of protein recovered in all chains after electrophoresis (Table I). The apparent mole ratio obtained when recovery of protein from the preparative acrylamide gel was only 30–40% approximates the 4:2:1:1 ratio reported by Raftery et al. (1975) on the basis of scans of stained gels. However, in six preparations of chains in which the yield averaged 85%, the mole ratio very closely approximated the $\alpha_2\beta\gamma\delta$ composition proposed by Reynolds & Karlin (1978). Evidently, loss of protein in the acrylamide gel by fixation during staining and destaining is selective, and α is more readily eluted than the higher molecular weight chains.

We have solubilized and purified AcChR in cholate because (1) Triton X-100 is known to inhibit AcChR function (Fischbach & Lass, 1978), (2) we had thought cholate-solubilized AcChR might be amenable to reconstitution into membranes after removal of the cholate by dialysis (Epstein & Racker, 1978), and (3) pure cholate, unlike Triton X-100, has very little A_{280}, thereby readily allowing measurement of protein by A_{280}. When AcChR is solubilized in 1% sodium cholate and purified in 0.2% sodium cholate rather than in Triton X-100, variable amounts of two closely spaced bands of higher molecular weight are observed in addition to α, β, γ, and δ. The critical micelle concentration of cholate is 0.6% (Helenius & Simon, 1975); thus, 0.2% cholate would not be expected to be as effective as concentrations in excess of 0.6%. The two bands ϵ and ζ can be removed almost completely by washing the affinity column after AcChR is bound with 1.5 volumes of buffers containing either 2% cholate or 0.5% Triton X-100. ϵ and ζ are prominent components of preparations containing AcChR-rich membranes (Figure 1). ζ comigrates on these gels with the high molecular weight chain of *Electrophorus electricus* Na^+/K^+-dependent ATPase (Lindstrom et al., 1979), suggesting that it may be the enzymatically active subunit of the *Torpedo* sodium pump. Hucho et al. (1978) report that the ϵ and ζ bands are most prominent in membrane fractions least enriched in α, β, γ, and δ, suggesting that AcChR and the sodium pump may at least in part be in different membrane fragments, and Hamilton et al. (1979) report AcChR-rich membrane fractions free of ϵ and ζ. We did not find evidence of lipid in AcChR purified in Triton X-100. However, in four preparations of *Torpedo* AcChR solubilized in 1% cholate and purified in 0.2% cholate which we hydrolyzed and assayed for fatty acids, we found variable ratios of 14–24 carbon saturated and unsaturated fatty acids in concentrations from 7 to 75 total nmol of fatty acids per mg of protein. Thus, although we know that in 0.2% cholate *Torpedo* AcChR is solubilized and dissociated into the same monomer–dimer pattern [e.g., Chang & Bock (1977)] after sucrose gradient centrifugation that is observed with AcChR in Triton X-100 (data not shown), in 0.2% cholate, both contaminating lipid and protein components of the membrane are more difficult to completely remove than when 2% cholate or Triton X-100 is used.

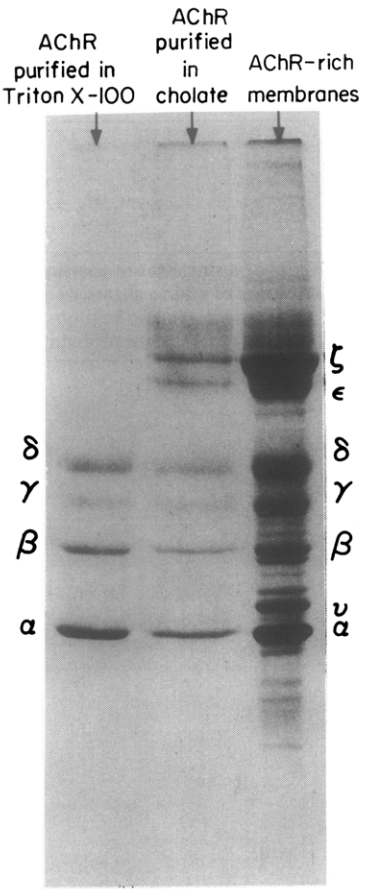

FIGURE 1: Electrophoresis of AcChR purified in Triton X-100, AcChR purified in 0.2% sodium cholate, and membrane fractions enriched in AcChR.

Table II: Amino Acid Composition[a] of *T. californica* AcChR Subunits

amino acid	mole percent			
	α	β	γ	δ
aspartic acid	11.5	12.6	12.5	13.6
glutamic acid	9.3	10.3	11.4	9.8
histidine	2.8	1.9	1.8	2.5
lysine	6.2	5.4	6.5	5.3
arginine	4.1	4.7	4.7	5.0
tyrosine	4.1	3.9	3.8	4.1
phenylalanine	4.8	4.7	4.7	5.1
tryptophan	1.4	1.9	1.3	1.4
serine	6.0	5.7	5.3	5.6
threonine	6.9	6.6	6.0	5.4
glycine	4.9	3.7	5.3	5.2
alanine	4.0	5.5	5.1	5.1
valine	7.9	7.6	6.0	6.6
leucine	8.7	10.0	10.6	10.0
isoleucine	8.4	6.8	7.0	7.3
proline	5.6	6.9	6.9	6.5
cysteine				
methionine	3.0	1.7	1.6	2.0

[a] The amino acid composition shown is the average of at least two determinations on independent preparations of subunits. The average deviation between duplicates was 7%.

Table III: Carbohydrate Composition[a] of *Torpedo* AcChR

sugar	nmol/mg of protein			
	α	β	γ	δ
mannose	90	120	140	110
galactose	18	35	80	79
glucose	20	23	23	21
glucosamine	57	62	74	25
sialic acid	⩽2	⩽6	9.6	10

[a] These are the results of a single set of gas–liquid chromatography analyses. Amounts of amino sugars were particularly variable between runs. The traces of sialic acid observed in α and β are ⩽0.3 mol/mol of chain and thus are probably insignificant.

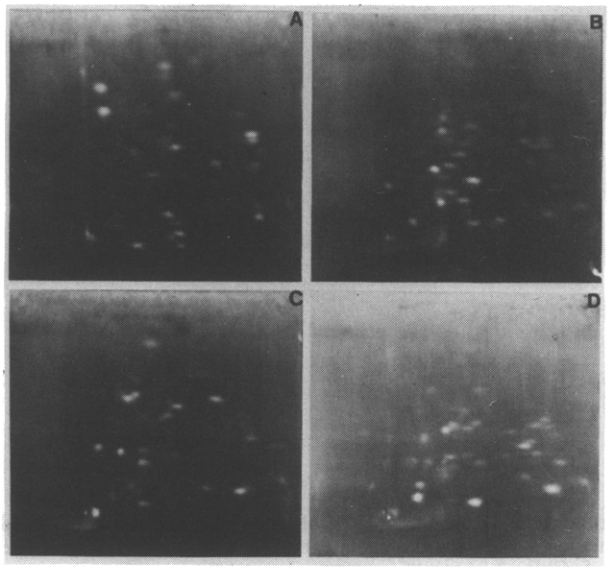

FIGURE 2: Tryptic maps of α, β, γ, and δ chains of *T. californica*. The origin is in the lower left. Peptides are visualized by fluorescamine staining. (A) α; (B) β; (C) γ; (D) δ.

Table II shows the amino acid composition of α, β, γ, and δ. None is distinguished remarkably from the other by amino acid composition. All closely resemble the amino acid compositions previously reported for intact *Torpedo* AcChR (Michaelson et al., 1974; Eldefrawi et al., 1975; Karlin et al., 1975) and for *Torpedo* subunits (Vandlen et al., 1979). The large amounts of glutamic and aspartic acids and small amounts of histidine, lysine, and arginine observed are consistent with the low (4.5–4.8) isoelectric point reported for native AcChR (Eldefrawi & Eldefrawi, 1973; Biesecker, 1973; Brockes & Hall, 1975). Cysteine was not determined. We know from the work of Karlin and collaborators that at least α, β, and δ contain structurally and/or functionally important disulfide bonds and that at least β, γ, and δ contain free sulfhydryl groups as well (Hamilton et al., 1979).

Table III shows the carbohydrate composition of α, β, γ, and δ. As expected, because each band on acrylamide gels stains for carbohydrate with the Schiff reagent (data not shown), each subunit contains carbohydrate. The amount of carbohydrate totals 4–7% by weight and represents 7–20 residues per chain. Although there appear to be some differences in carbohydrate composition between chains, these data do not represent an exhaustive study and may not in detail accurately reflect the exact carbohydrate composition of each subunit.

Tryptic maps of the chains and their ^{125}I-labeled derivatives revealed distinct patterns for each chain and did not reveal extensive homologies among them (Figures 2–4). This suggests that α, β, γ, and δ are not overlapping proteolytic

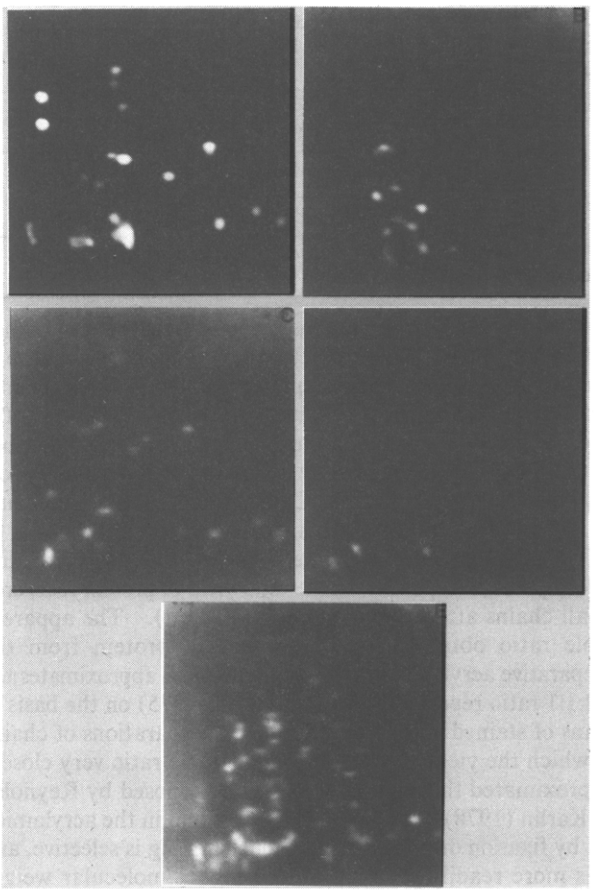

FIGURE 3: Tryptic maps of α, β, γ, δ, ε, and ζ chains from a second preparation of subunits. The peptides were stained with fluorescamine as described in Figure 2. (A) α; (B) β; (C) γ; (D) δ; (E) ε and ζ chains found in trace amounts when AcChR is purified in cholate rather than in Triton X-100.

derivatives of one another (Figures 2–4). It further suggests that α, β, γ, and δ are not derived by proteolysis of the ε and ζ chains observed in preparations of AcChR purified in cholate (Figures 3 and 4). Peptides which seem to be present in more than one chain (e.g., a series of three in the center-right portion of the maps of β, γ, and δ in Figure 2) are present in very different relative amounts, suggesting that their presence is due to cross contamination. An important caveat in interpreting the peptide maps is that as much as 50% of the trypsin digest remained insoluble in electrophoresis buffer. If the insoluble material contained a unique population of peptides, we could not assess the possible homology or lack of it contained in those species.

Discussion

We observe four polypeptide chains (α, β, γ, and δ) in *T. californica* AcChR solubilized and purified in Triton X-100. These correspond in approximate apparent molecular weight, [^{3}H]MBTA labeling of the α chain (following paper), and disulfide cross-linking of the δ chain (data not shown) to the chains in *T. californica* reported by others (Weill et al., 1974; Raftery et al., 1975; Hucho et al., 1978; Chang & Bock, 1977; Froehner & Rafto, 1979; Vandlen et al., 1979).

T. californica AcChR appears to have the subunit composition $\alpha_2\beta\gamma\delta$. Our direct measurements of the relative amounts of each chain are in agreement with the mole ratio predicted on the basis of chain and monomer molecular weight and ligand binding stoichiometry (Reynolds & Karlin, 1978) and by kinetics of reaction with oxidizing agents (Hamilton et al., 1979).

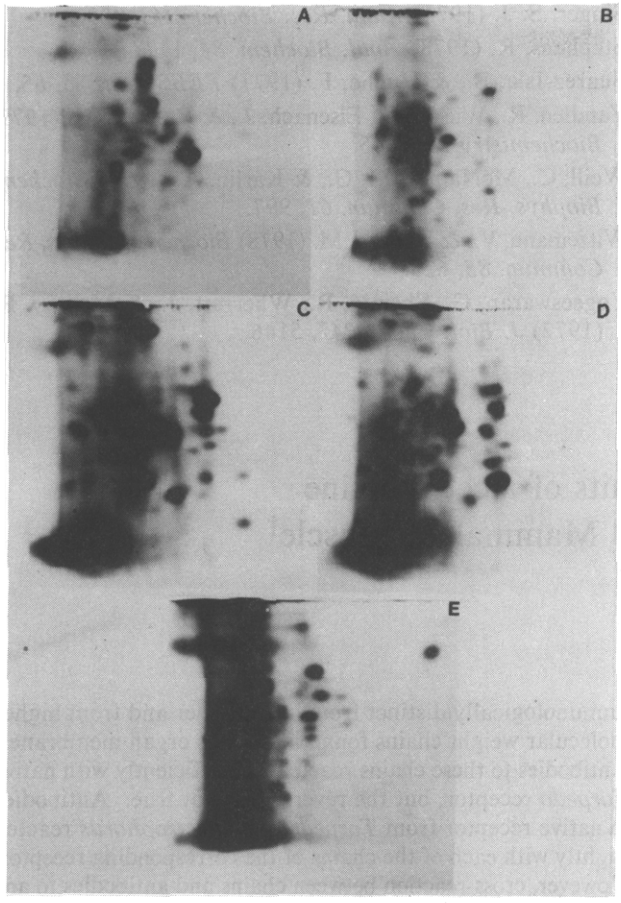

FIGURE 4: Autoradiogram of the peptide maps shown in Figure 3. Trace amounts of ^{125}I-labeled chains were included with unlabeled chains whose proteolytic fragments were visualized with fluorescamine in Figure 3. The incorporation of ^{125}I changed the electrophoretic and chromatographic properties of the peptides sufficiently that they cannot be identified with any of the noniodinated species. (A) α; (B) β; (C) γ; (D) δ; (E) ϵ and ζ.

α, β, γ, and δ are each acidic glycopeptides. Thus, unlike the Na^+/K^+-dependent ATPase, carbohydrate is not concentrated in a single subunit (Kyte, 1972). The presence of carbohydrate on each chain suggests that at least the carbohydrate-bearing part of each chain is exposed to the extracellular surface (Singer, 1974; Marchesi et al., 1976). The observation that immunization of rats with any of these chains induces an autoimmune response which cross-reacts in vivo with AcChR in rat muscle also suggests that both α, β, γ, and δ and their analogues in rat muscle AcChR are in part exposed on the extracellular surface (Lindstrom et al., 1978, 1979). None of the chains has a uniquely hydrophobic amino acid composition, suggesting that none is completely buried within the macromolecule or the membrane.

Peptide maps obtained of α, β, γ, and δ are more detailed than those reported by Froehner & Rafto (1979) or Nathanson & Hall (1979) but support the same conclusion. Extensive homology between subunits is not observed, so it is unlikely, despite their similarity in amino acid composition, that these chains are derivatives of one another. Further, they also do not appear to be proteolytic derivatives of the ϵ and ζ chains. These conclusions are supported by the immunochemical studies in the following paper (Lindstrom et al., 1979). The α, β, γ, and δ chains and their analogues in *Electrophorus electricus* AcChR are readily distinguished by antibodies to these chains.

ϵ and ζ chains are prominent components of *Torpedo* membranes, especially those poor in AcChR (Hucho et al., 1978). ζ chains may be a subunit of Na^+/K^+-dependent ATPase (following paper). Both ϵ and ζ are apparently contaminants of AcChR purified in low concentrations of cholate. When concentrations of cholate below its critical micelle concentration are used during affinity chromatography of AcChR, both ϵ and ζ and some membrane lipids are observed. Neither is observed when AcChR is purified in 0.5% Triton X-100. Eldefrawi et al. (1978) have reported that some material binding [^3H]perhydrohistrionicotoxin, which they attribute to the AcChR ion channel, is associated with AcChR purified in cholate but not in Triton X-100. This [^3H]perhydrohistrionicotoxin binding material may be a component of the lipids or ϵ and ζ chains which are found in preparations of AcChR purified in cholate. The choice of detergent for solubilization and purification is difficult, aside from technical considerations. Both Triton X-100 and cholate preserve the α-BGT binding capacity of AcChR, but Triton X-100 is known to directly inhibit AcChR ion channels (Fischbach & Lass, 1978), and addition of exogenous lipid to cholate is required to prevent denaturation of the ion channel of AcChR on solubilization (Anholt and Lindstrom, unpublished experiments).

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Immunochemical Similarities between Subunits of Acetylcholine Receptors from *Torpedo*, *Electrophorus*, and Mammalian Muscle†

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ABSTRACT: Polypeptide chains composing acetylcholine receptors from the electric organs of *Torpedo californica* and *Electrophorus electricus* were purified and labeled with ^{125}I. Immunochemical studies with these labeled chains showed that receptor from *Electrophorus* is composed of three chains corresponding to the α, β, and γ chains of receptor from *Torpedo* but lacks a chain corresponding to the δ chain of *Torpedo*. Experiments suggest that receptor from mammalian muscle contains four groups of antigenic determinants corresponding to all four of the *Torpedo* chains. Binding of ^{125}I-labeled chains was measured by quantitative immune precipitation and electrophoresis. Antisera to the following immunogens were used: denatured α, β, γ, and δ chains of *Torpedo* receptor, native receptor from *Torpedo* and *Electrophorus* electric organs and from rat and fetal calf muscle, and human muscle receptor (from autoantisera of patients with myasthenia gravis). The four chains of *Torpedo* receptor were immunologically distinct from one another and from higher molecular weight chains found in electric organ membranes. Antibodies to these chains reacted very efficiently with native *Torpedo* receptor, but the reverse was not true. Antibodies to native receptor from *Torpedo* and *Electrophorus* reacted slightly with each of the chains of the corresponding receptor. However, cross-reaction between chains and antibodies to any native receptor was most obvious with the α chain of *Torpedo* or the corresponding α' chain of *Electrophorus*. Antiserum to α chains exhibited higher titer against receptor from denervated rat muscle. Antibodies from myasthenia gravis patients did not cross-react detectably with ^{125}I-labeled chains from electric organ receptors. Most interspecies cross-reaction occurred at conformationally dependent determinants whose subunit localization could not be determined by reaction with the denatured chains.

Acetylcholine receptor (AcChR)[1] purified from the electric organ of the marine elasmobranch *Torpedo californica* is composed of four glycopeptide chains in the apparent mole ratio $\alpha_2\beta\gamma\delta$ (Lindstrom et al., 1979; Weill et al., 1974; Raftery et al., 1975; Hucho et al., 1978; Chang & Bock, 1977; Froehner & Rafto, 1979; Vandlen et al., 1979). Receptor purified from the electric organs of the fresh water teleost *Electrophorus electricus* appears on polyacrylamide gel electrophoresis in $NaDodSO_4$ to be composed of two sharp bands of apparent molecular weights approximating 41 000 and 52 000 (Lindstrom & Patrick, 1974) as well as a more diffuse band approximating 60 000 (Karlin & Cowburn, 1973). Both the α chains of *Torpedo* (38 000 apparent molecular weight) and the 41 000 apparent molecular weight chain of *Electrophorus* are specifically labeled by an affinity labeling reagent directed at the acetylcholine binding site ([^{3}H]MBTA (Karlin & Cowburn, 1973; Weill et al., 1974). The function of the other chains in either AcChR is unknown, though it is suspected that they may be components of the ion conductance channel regulated by binding of acetylcholine, because it is known that pure AcChR can be prepared under conditions in which it retains full ion conductance activity (Moore et al., 1979; Lindstrom, Einarson, Anholt, and Montal, unpublished experiments). Previously, it had not been possible to determine whether these chains in the AcChR from each species were even structurally analogous. Substantial variations in the apparent molecular weights of the chains from either species further hampered this determination. Here we report that antisera to the α, β, γ, and δ chains from *Torpedo* AcChR can be used to identify the three chains from *Electrophorus* AcChR by cross-reaction as α', β', and γ', corresponding in immunochemical structure and presumably function to the corresponding chains from *Torpedo*.

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[1] Abbreviations used: AcChR, acetylcholine receptor; [^{125}I]-α-BGT, ^{125}I-labeled α-bungarotoxin; $NaDodSO_4$, sodium dodecyl sulfate; MG, myasthenia gravis; EAMG, experimental autoimmune myasthenia gravis; [^{3}H]MBTA, [^{3}H]-4-(*N*-maleimido)-α-benzyltrimethylammonium iodide.

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