Comparison of the Subunit Structure of Acetylcholine Receptors from Muscle and Electric Organ of *Electrophorus electricus*†

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ABSTRACT: The acetylcholine receptors of the electric organ and muscle tissues of *Electrophorus electricus* are composed of α, β, γ, and δ subunits. Receptor subunits from the two tissues were compared by peptide mapping with monoclonal antibodies, an affinity-labeling reagent, and a lectin to characterize particular peptide fragments. These experiments indicate that the corresponding receptor subunits from the two tissues are extensively homologous or identical throughout their amino acid sequences. Small differences in the electrophoresis of peptide fragments of α subunits between the two tissues occurred on fragments which bound labeled lectin. These results suggest that the acetylcholine receptors in electric organ and muscle tissues of *Electrophorus* differ in structure only by minor posttranslational modifications perhaps involving carbohydrate.

Multiple pharmacologically distinguishable receptors for a single ligand are more nearly the rule than the exception [see, for example, Goodman-Gilman et al. (1980)]. In the case of acetylcholine receptors, muscarinic receptors [reviewed in Nathason (1982)] are clearly distinguished from nicotinic receptors [reviewed in Karlin (1980) and Anholt et al. (1983)] not only by pharmacology but also by structure and function. Among nicotinic receptors in muscle there are extrajunctional and junctional forms at different developmental stages in the same cells which are distinguishable by antigenicity, function, and turnover without large apparent differences in subunit structure (Weinberg & Hall, 1979; Nathanson & Hall, 1979; Saitoh & Changeux, 1981; Sumikawa et al., 1982a,b). Such studies are hampered by the small amount of receptor in mammalian muscle and the difficulty of recovering it without substantial proteolytic degradation. Fish electric organ tissues evolved from muscle tissue (Bennett, 1970; Mellinger et al., 1978). The availability of relatively large amounts of purified acetylcholine receptor from both electric organ and muscle tissue of *Electrophorus electricus* (Lindstrom et al., 1983) permits comparison of homologous receptors which might be expected to differ at least as much as junctional and extrajunctional forms. In this paper we study the structure of acetylcholine receptors from these related tissues to determine whether they differ in structure and, if so, how they differ.

In the preceding paper (Lindstrom et al., 1983) it was demonstrated that substantial amounts of receptor could be purified from the muscles of the electric eel *Electrophorus electricus* and that it contained α, β, γ, and δ subunits corresponding immunochemically to those of receptors from the electric organs of *Electrophorus* and *Torpedo*. Elsewhere Contri-Tronconi et al. (1982; B. M. Conti-Tronconi et al., unpublished results) use this purified muscle receptor to show that the N-terminal 30 amino acids of each subunit are identical in receptor from *Electrophorus* muscle and electric organ. This strongly suggests that they are similar or identical gene products, but this analysis applies to less than 7% of their sequence. We noted some small differences in the apparent molecular weights of receptor subunits from muscle and electric organ and wanted to investigate the whole subunits of each receptor.

Previously, we showed that peptide mapping is very sensitive to even relatively small changes in amino acid sequence and that it could readily distinguish the homologous subunits of receptors from the electric organs of *Torpedo* and *Electrophorus* and bovine muscle (Gullick & Lindstrom, 1982a). Here we applied these same techniques of peptide mapping and immunochemical assay to ascertaining the extent of similarity between the subunits of receptors from *Electrophorus* electric organ and muscle. Our results suggest that the receptors from these two tissues are identical gene products differing only in small posttranslational modifications.

Materials and Methods

Receptor Purification. The purification of the receptors from the electric organs of *Torpedo californica* and *Electrophorus electricus* (Lindstrom et al., 1980a,b) have been described previously, and the purification of receptor from *Electrophorus* muscle is reported in the preceding paper (Lindstrom et al., 1983).

Electrophoretic Transfer of Proteins to Diazophenyl Thioether Paper. Receptors were denatured in sodium dodecyl sulfate (Bio-Rad) and separated on 10% (intact subunits) or 15% (proteolytic fragments) polyacrylamide gels by using the buffer system of Laemmli (1970). Electrophoretic transfer to DPT[1] paper was performed as previously described (Gullick & Lindstrom, 1982a). The paper was inactivated and then incubated in 10 mL of 50 mM Tris-HCl buffer, pH 7.4, containing 150 mM NaCl, 5 mM EDTA, 0.25% (w/v) gelatin, and 0.05% Triton X-100 (buffer A) containing either 10 μL of antiserum to a *Torpedo* receptor subunit (titer approximately 4×10^{-6} M; Lindstrom et al., 1979b), a mixture of all four anti-subunit sera, or individual mAbs (Tzartos & Lindstrom, 1980; Tzartos et al., 1981; S. J. Tzartos, L. Langeberg, S. Hochschwender, and J. Lindstrom, unpublished results).

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[1] Abbreviations: mAbs, monoclonal antibodies; [^{3}H]MBTA, [4-(*N*-maleimido)benzyl]tri[^{3}H]methylammonium iodide; PAS, periodic acid–Schiff reagent; DPT, diazophenyl thioether; Tris, tris(hydroxymethyl)-aminomethane; EDTA, ethylenediaminetetraacetic acid; $NaDodSO_4$, sodium dodecyl sulfate.

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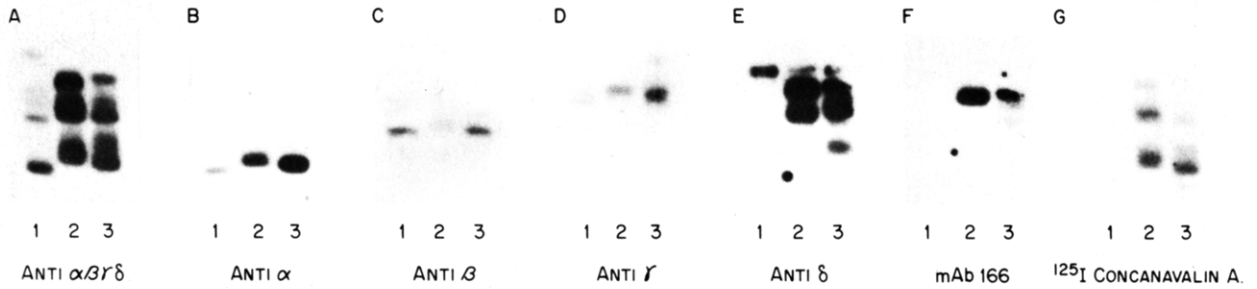

FIGURE 1: Intact receptors from *Torpedo* electric organ, *Electrophorus* electric organ, and *Electrophorus* muscle separated on a 10% polyacrylamide gel in sodium dodecyl sulfate and then transferred to DPT paper. Lane 1: *Torpedo* receptor (25 ng). Lane 2: *Electrophorus* organ receptor (200 ng). Lane 3: *Electrophorus* muscle receptor (200 ng) probed with (A) a mixture of equal amounts of antisera to *Torpedo* α, β, γ, and δ subunits, (B) anti-α serum, (C) anti-β serum, (D) anti-γ serum, (E) anti-δ serum, (F) the anti-δ mAb 166, and (G) ^{125}I-labeled concanavalin A.

The paper was then washed, incubated with ^{125}I-labeled goat anti-rat IgG, washed again, and then autoradiographed as previously described (Gullick & Lindstrom, 1982a). The paper was erased and reequilibrated into buffer A prior to subsequent probings.

Incubation with ^{125}I-labeled concanavalin A was as follows: The paper was equilibrated into 50 mM sodium phosphate buffer, pH 7.4, containing 1 mM $CaCl_2$ and 1 mM $MgCl_2$ (buffer B) and then incubated with 10 mL of buffer B containing 1 μCi of ^{125}I-labeled concanavalin A (New England Nuclear, approximately 6×10^{18} cpm/mol) for 2 h at 23 °C. The paper was then rinsed briefly with buffer B and autoradiographed for 18–36 h by using Kodak X-OMAT AR film preflashed to an optical density of 0.125 unit (Laskey & Mills, 1975).

Digestion of Electrophorus Organ and Muscle Receptors with V8 Protease. Five micrograms of each receptor in 10 μL of 10 mM sodium phosphate buffer, pH 7.4, was made 1% (w/v) in sodium dodecyl sulfate, and 1.5 μL of 1 mg/mL V8 protease (Miles, Elkhart, IN) was added. The samples were incubated at 23 °C for 1 h, and then aliquots containing 500 ng of receptor polypeptides were electrophoresed and transferred to DPT paper as described above.

Tryptic Peptide Mapping of Individual ^{125}I-Labeled Subunits from Electrophorus Organ or Electrophorus Muscle Receptors. Electrophorus receptors were iodinated by the lactoperoxidase–glucose oxidase method (Bio-Rad, Richmond, CA). The labeled subunits were isolated by preparative gel electrophoresis, and their purity was checked by analytical gel electrophoresis (Figure 4). Individual subunits were digested with trypsin (Worthington, Freehold, NJ) and the resulting fragments separated on cellulose thin-layer plates (E. Merk, Darmstadt, West Germany) according to the method of Gibson (1974). Exposure to X-ray film was for approximately 72 h.

Affinity Labeling with MBTA. Native receptors were affinity labeled with [4-(*N*-maleimido)benzyl]tri[^{3}H]methylammonium iodide ([^{3}H]MBTA; 40 Ci/mmol from New England Nuclear) (Karlin & Cowburn, 1973). Souble receptor was labeled as described by Karlin et al. (1976), resulting in labeling of half of the toxin binding sites. Peptide mapping was as previously described (Gullick et al., 1981).

Glycosidase Treatment of Native Receptors. Electrophorus organ or *Electrophorus* muscle receptors (20 μL; 0.5 mg/mL) were treated with either 2 μL of a mixture of glycosidic enzymes (Bose et al., 1976; kindly provided by Dr. J. Kyte, University of California, San Diego) in 100 mM phosphate–citrate buffer, pH 6.0, or 10 milliunits of neuraminidase (Calbiochem, San Diego, CA) in 50 mM sodium acetate buffer, pH 5.5, containing 150 mM NaCl and 10 mM $CaCl_2$, or 10 milliunits of endoglycosidase H (Miles, Elkhart, IN) in

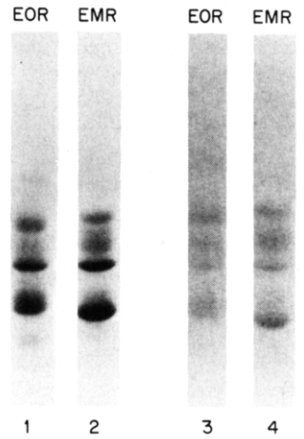

FIGURE 2: Intact receptors from *Electrophorus* organ or *Electrophorus* muscle separated on a 10% polyacrylamide gel in sodium dodecyl sulfate stained for protein or for carbohydrate. Lanes 1 and 3: *Electrophorus* organ receptor (10 μg) stained with Coomassie blue for protein or with periodate–Schiff reagent for carbohydrate. Lanes 2 and 4: *Electrophorus* muscle receptor (10 μg) stained for protein or carbohydrate.

100 mM sodium citrate buffer, pH 5.0, for 18 h at 37 °C. Control samples were incubated without enzyme. The products were analyzed on 10% polyacrylamide gels containing sodium dodecyl sulfate.

Results

Figure 1 shows samples of *Torpedo california* receptor, *Electrophorus* organ receptor, and *Electrophorus* muscle receptor separated by electrophoresis on a 10% polyacrylamide gel containing sodium dodecyl sulfate, transferred to DPT paper, and then probed with a variety of antisera, a mAb, and ^{125}I-labeled concanavalin A. We confirm here using anti-subunit sera the subunit identifications determined in Lindstrom et al. (1983) using mAbs (Figure 1A–F). Incubation of the same transfer with ^{125}I-labeled concanavalin A (Figure 1G) labels each *Electrophorus* receptor subunit, indicating that they are all glycoproteins containing mannose residues. The α subunits in each case are the most strongly labeled. *Torpedo* receptor subunits are also known to contain mannose (Lindstrom et al., 1979a), and the native molecule binds well to immobilized concanavalin A (Lindstrom et al., 1980), but no bound concanavalin A could be detected here, presumably due to a lower amount of receptor material or a lower affinity for the lectin. Samples of both types of *Electrophorus* receptor were run on a 10% polyacrylamide gel and stained either for protein (Figure 2, lanes 1 and 2) or with periodic acid–Schiff reagent (PAS) for carbohydrate (Gullick et al., 1981). The PAS stain is rather insensitive but does visualize each *Electrophorus* receptor subunit, confirming the presence of car-

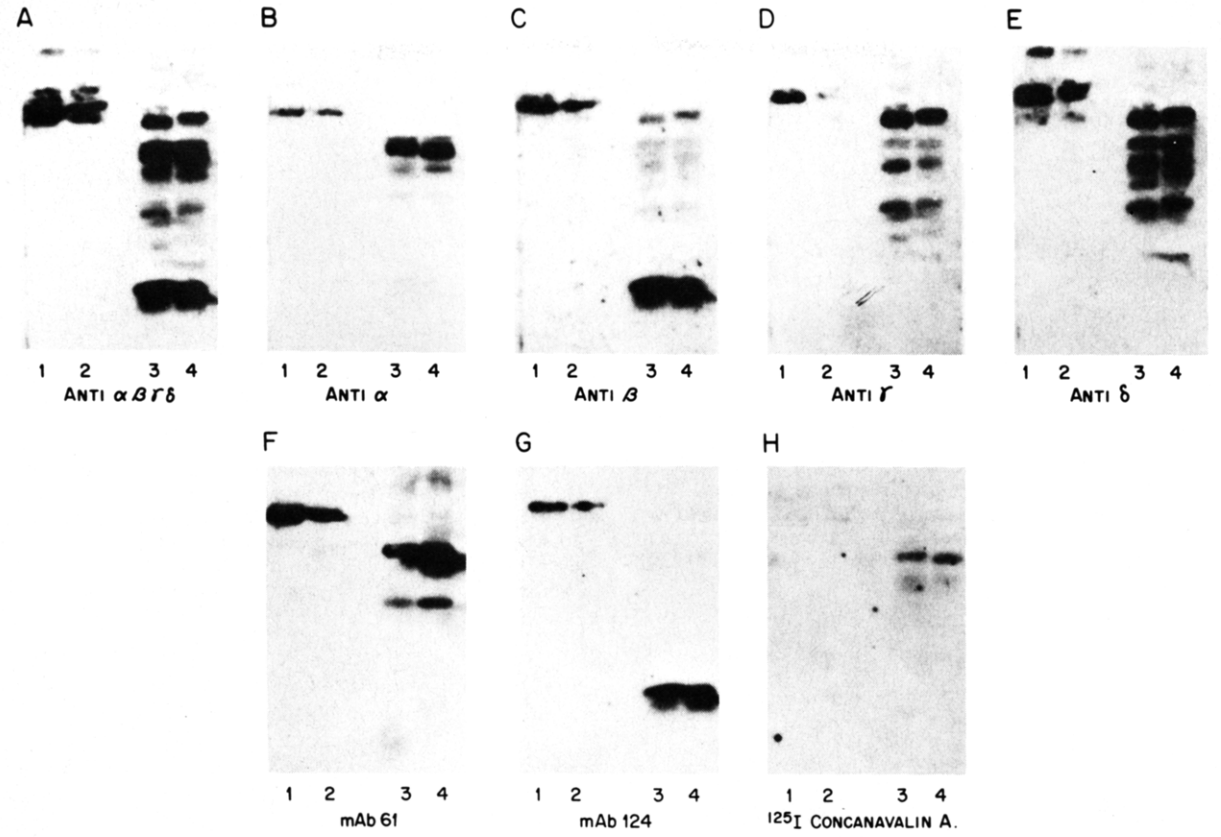

FIGURE 3: Receptors from *Electrophorus* organ or *Electrophorus* muscle separated on a 15% polyacrylamide gel in sodium dodecyl sulfate and then transferred electrophoretically to DPT paper. Lane 1: *Electrophorus* organ receptor (200 ng). Lane 2: *Electrophorus* muscle receptor (200 ng). Lane 3: *Electrophorus* organ receptor (500 ng), digested with V8 protease (1:10 w/w). Lane 4: *Electrophorus* muscle receptor (500 ng) digested with V8 protease (1:10 w/w) and probed with (A) a mixture of equal amounts of antisera to *Torpedo* α, β, γ, and δ subunits, (B) anti-α serum, (C) anti-β serum, (D) anti-γ serum, (E) anti-δ serum, (F) the anti-α mAb 61, (G) the anti-β mAb 124 and (H) ^{125}I-labeled concanavalin A.

bohydrate indicated by binding of ^{125}I-labeled concanavalin A.

Comparison of the mobilities of equivalent pairs of *Electrophorus* receptor subunits reveals that there are some small differences in apparent size. For example, the *Electrophorus* muscle receptor α ran slightly faster than the α from *Electrophorus* organ receptor (Figure 1). For greater sensitivity, their primary structures were compared by peptide mapping with V8 protease and electrophoresis of the fragments on acrylamide gels (Cleveland et al., 1977; Gullick et al., 1981; Gullick & Lindstrom, 1982a). For precise reproducibility the peptide fragments from acrylamide gels in sodium dodecyl sulfate were transferred to DPT paper in order to allow subunit fragments from a single peptide map to be identified by exposures of the map to antisera or mAbs to subunits of receptor from *Torpedo*, eluting these antibodies and reexposing the same map to other antibodies. The specificity of these reagents was determined both by the experiments presented in Figure 1 and also by including unproteolyzed subunit patterns as internal controls (Figure 3, lanes 1 and 2). Even rather limited alterations in the primary sequence of equivalent subunits would substantially change the peptide maps obtained as shown by similar studies of the homologous subunits in acetylcholilne receptors from *Torpedo*, *Electrophorus*, and cattle (Gullick & Lindstrom, 1982a). Selective visualization using subunit-specific antisera and mAbs revealed very similar peptide fragment patterns from receptors purified from both electric organ and muscle (Figure 3A–G, lanes 3 and 4). These patterns contained a majority of various equally sized pairs of fragments together with one pair in each visualization whose mobilities differed slightly (Figure 3B–E, lanes 3 and 4). mAb 124, specific for β subunits under these conditions (S. J. Tzartos et al., unpublished results; Gullick & Lindstrom, 1982a) (Figure 3G, lanes 1 and 2) bound to a fragment of the same size from both receptors (Figure 3G, lanes 3 and 4). The two highest molecular weight fragments in Figure 3C (lanes 3 and 4), which differ in size, are visualized rather weakly and may be derived from either the γ and δ subunits, since there is some cross-reaction of the anti-β serum with γ in this case (Figure 3C, lanes 1 and 2), and these fragments are much more strongly visualized with either anti-γ or anti-δ serum (Figure 3D,E, lanes 3 and 4). Thus the β subunits and their fragments do not appear to differ in mobility. Anderson & Blobel (1981) have observed that glycosylated or nonglycosylated β subunits from *Torpedo* comigrate on NaDodSO$_4$ gels, whereas glycosylated or nonglycosylated α, γ, or δ subunits differ in relative mobilities. The patterns revealed by anti-γ sera (Figure 3D) or anti-δ sera (Figure 3E) are more difficult to interpret due to cross-reaction as already shown (Figure 1E) (Figure 3D,E, lanes 1 and 2). The overall picture is the same, however, with most of the fragments being of the same size from both receptors, but with at least one fragment from each subunit differing slightly in electrophoretic migration between the two subunits. The same paper was incubated with ^{125}I-labeled concanavalin A which in this instance reacted rather weakly (Figure 3H, lanes 1 and 2) but did visualize predominantly a pair of fragments (Figure 3H, lanes 3 and 4) which are derived from the α subunit (cf. Figure 3B,F, lanes 3 and 4). Note that these fragments are the only α subunit fragments that differ in size. This suggests that differences in the carbohydrate on the α subunits of receptors from electric organ and muscle might account for the small

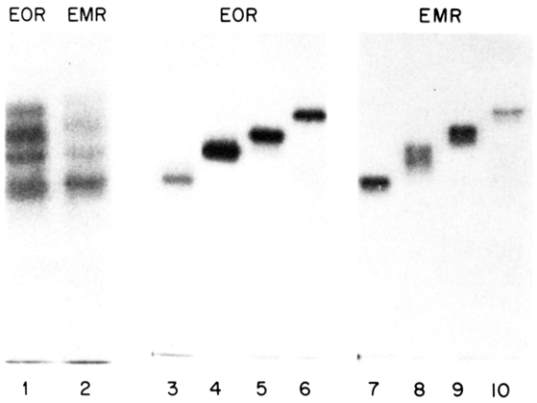

FIGURE 4: Autoradiograph of *Electrophorus* organ and *Electrophorus* muscle receptors labeled with ^{125}I and isolated individual subunits separated on a 10% polyacrylamide gel in sodium dodecyl sulfate. Lane 1: ^{125}I-Labeled *Electrophorus* organ receptor. Lane 2: ^{125}I-Labeled *Electrophorus* muscle receptor. Lanes 3–6: *Electrophorus* organ α, β, γ, and δ subunits. Lanes 7–10: *Electrophorus* muscle α, β, γ, and δ subunits.

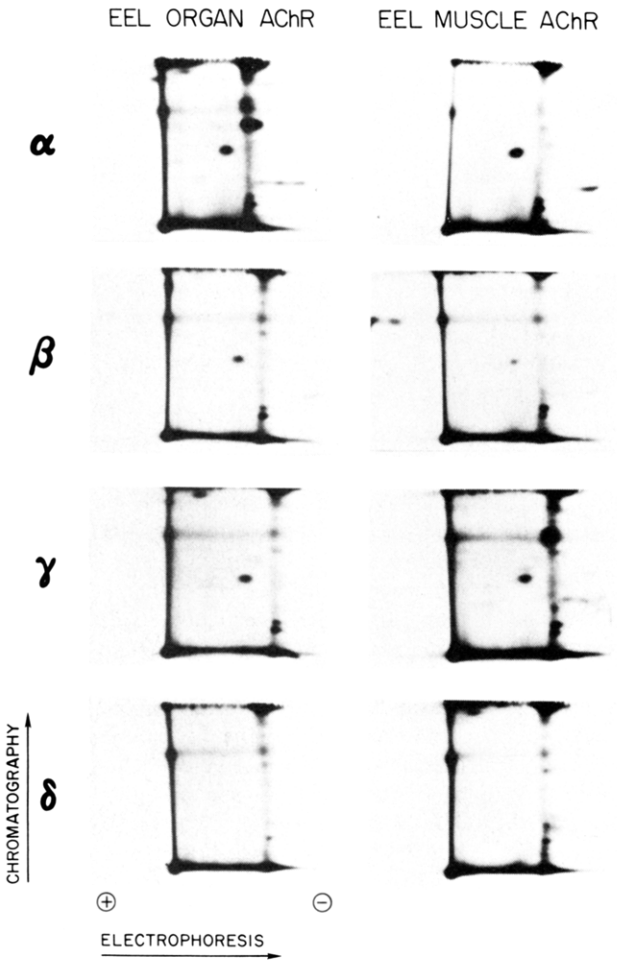

FIGURE 5: Two-dimensional tryptic peptide maps of ^{125}I-labeled individual subunits from *Electrophorus* organ or *Electrophorus* muscle receptor. Plates were autoradiographed for 72 h.

difference in their electrophoretic mobility.

The primary structure of the two types of *Electrophorus* receptor subunits were also compared by two-dimensional peptide mapping of tryptic peptides of iodinated material. Iodination of the *Electrophorus* receptors did not cause significant breakdown (Figure 4, lanes 1 and 2). Individual subunits were prepared by gel electrophoresis of total ^{125}I-labeled receptors. The wet gels were autoradiographed, and the bands were cut out, passively eluted, and concentrated.

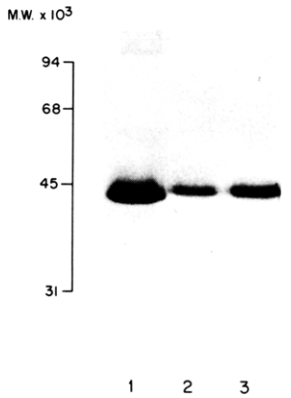

FIGURE 6: Native receptors from *Torpedo* electric organ, *Electrophorus* organ, and *Electrophorus* muscle labeled with [^{3}H]MBTA resolved into subunits on a 10% polyacrylamide gel containing sodium dodecyl sulfate and visualized by fluorography. Lane 1: *Torpedo* electric organ receptor (10 μg). Lane 2: *Electrophorus* electric organ receptor (5 μg). Lane 3: *Electrophorus* muscle receptor (5 μg).

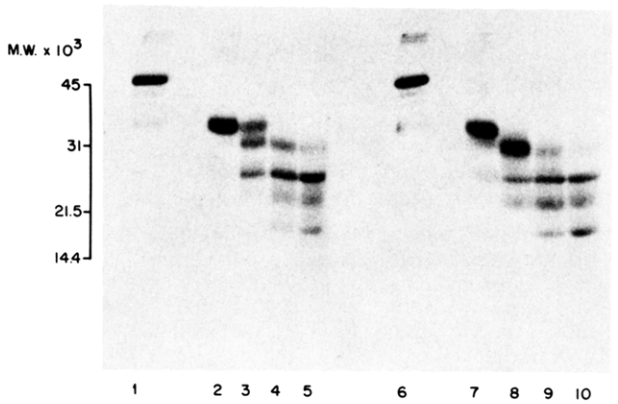

FIGURE 7: Peptide maps on a 15% polyacrylamide gel containing sodium dodecyl sulfate of [^{3}H]MBTA-labeled receptors from *Electrophorus* organ and *Electrophorus* muscle. Lane 1: *Electrophorus* organ receptor (3.25 μg). Lane 6: *Electrophorus* muscle receptor (2.6 μg). Lanes 2–5: *Electrophorus* electric organ receptor (4.55 μg) digested for 30 min at room temperature with 0.1, 1, 5, and 10 μg of V8 protease, respectively. Lanes 7–10: *Electrophorus* muscle receptor (3.25 μg) digested as for lanes 2–5. The gel was fluorographed and exposed at −70 °C for 48 h.

The purity and integrity of each subunit are shown in lanes 3–10 of Figure 4. Samples were digested with trypsin and the resultant peptides separated by electrophoresis and chromatography. As in the case of the one-dimensional peptide maps of Figure 4, the two-dimensional peptide maps of Figure 5 show that the corresponding subunits of receptors from muscle and electric organ are very similar.

We next labeled the native receptors from *Electrophorus* electric organ, *Electrophorus* muscle, and, for comparison, *Torpedo* electric organ with the acetylcholine binding site specific reagent [^{3}H]MBTA. The conditions were such that only one of the two α subunits present in *Torpedo* receptor would be labeled (Karlin et al., 1976). The whole receptors were denatured in sodium dodecyl sulfate and run on a 10% polyacrylamide gel. Each receptor was labeled only on its α subunits (Figure 6). The α subunit from *Electrophorus* muscle receptor must therefore contain a readily reducible disulfide in the proximity of a site which has a high affinity for this affinity label analogous to those on receptors from the electric organs of *Torpedo* and *Electrophorus* (Karlin et al., 1975). Since the labeling was confined to the α subunits, we denatured MBTA-labeled receptors from *Electrophorus*

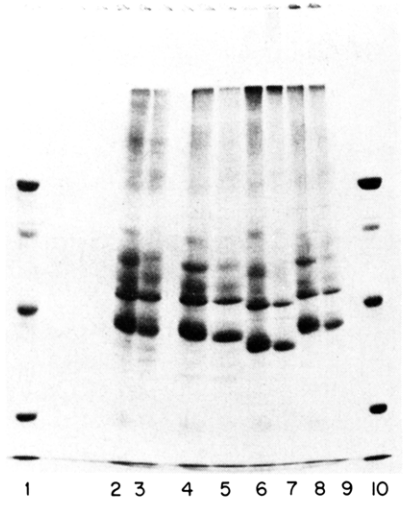

FIGURE 8: Treatment of native receptors from *Electrophorus* organ or *Electrophorus* muscle with glycosidic enzymes. Lanes 1 and 10: molecular weight standards, phosphorylase B, 94 000, bovine serum albumin, 68 000, ovalbumin, 43 500, and carbonic anhydrase, 31 000. Lanes 2, 4, 6, and 8: *Electrophorus* electric organ receptor (10 μg). Lanes 3, 5, 7, and 9: *Electrophorus* muscle receptor (10 μg) treated with (lanes 2 and 3) no treatment, (lanes 4 and 5) endoglycosidase H, (lanes 6 and 7) neuraminidase, and (lanes 8 and 9) mixture of glycosidases. The receptor subunits were separated on a 10% polyacrylamide gel containing sodium dodecyl sulfate and stained with Coomassie blue R250.

electric organ and muscle with sodium dodecyl sulfate and digested them with increasing weight ratios of V8 protease to obtain Cleveland type peptide maps (Figure 7). The patterns of labeled fragments obtained were visualized by fluorography and are clearly very similar. Thus, the sequences surrounding the active site label on the α subunits are very similar or identical.

Since all the *Electrophorus* receptor subunits are glycoproteins, we treated the native molecules with glycosidic enzymes to see if this would affect the mobility of their denatured subunits in polyacrylamide gels. Two highly purified enzymes or a mixture of glycosidases were employed. Endoglycosidase H which removes simple mannose type sugars from proteins (Tarentino et al., 1974) had no obvious effect on the subunit mobilities (Figure 8), lanes 4 and 5). This was surprising since it has been used effectively in elegant experiments on the receptor from a clonal muscle cell line (Merlie et al., 1982), and the *Electrophorus* subunits should contain mannose since they can bind concanavalin A (Figure 1G). It is possible that the native conformation of the molecules presented steric hindrance to the action of the enzyme. The mixture of glycosidases likewise had no obvious effect (Figure 8, lanes 8 and 9). Purified neuraminidase from *Vibro colerae*, however, did alter the subunit band pattern, increasing the mobility of all of *Electrophorus* receptor subunits (Figure 8, lanes 6 and 7). The receptor from *Torpedo* contains sialic acid (Lindstrom et al., 1979a), and these results suggest that the *Electrophorus* receptor subunits do also.

Discussion

The results described here indicate that the homologous subunits of acetylcholine receptors purified from *Electrophorus* electric organ and muscle are extremely similar. These similarities are (1) apparent subunit molecular weights, (2) reaction with subunit-specific antisera raised against subunits of receptor from *Torpedo*, (3) reaction with subunit-specific mAbs, (4) reaction of α subunits with [^{3}H]MBTA, (5) peptide maps composed of fragments which are of similar sizes and in specific cases identical by reaction with antisera, mAbs, [^{3}H]MBTA, or concanavalin A, (6) presence of sugars on all subunits, (7) binding of concanavalin A, especially to α subunits, and (8) increasing the mobility of all subunits by treatment with neuraminidase. In addition, very small differences were noted in the electrophoretic migration of intact α, γ, and δ subunits between the receptors from these two tissues and of some of the peptide fragments from these subunits.

The observed differences may result from any one or any combination of the following mechanisms: (1) the expression of different members of a gene family (Blundel & Humbel, 1980), (2) rearrangement of genes during differentiation (e.g., immunoglobulins), (3) alternative RNA processing (e.g., calcitonin; Amara et al., 1982), or (4) posttranslational modifications (Wold, 1981). The first three mechanisms require that the primary structure of the receptor polypeptides differ due to a priori differnces in the DNA sequence of the genes or subsequent rearrangements, deletions, or insertions. The last mechanism requires that the protein sequences be the same (although proteolysis may remove N- or C-terminal regions) but that they be treated differently by modifying enzymes present in the two tissues. The known posttranslational modifications include glycosylation, phosphorylation, methylation, proteolysis, addition of prosthetic groups, and many others [reviewed by Wold (1981)]. The receptor from *Torpedo* is known to be glycosylated (Lindstrom et al., 1979a; Vandlen et al., 1979), can act as a substrate for endogenous phosphorylation (Saitoh & Changeux, 1981; Davis et al., 1982) and methylation (Flynn et al., 1982), and has signal sequences removed during synthesis (Anderson & Blobel, 1981; Anderson et al., 1982; Ballivet et al., 1982; Noda et al., 1982, 1983; Sebbane et al., 1983). The significance of phosphorylation or methylation in vivo has yet to be fully established. Any one of these or any combination may be responsible for the small apparent molecular weight differences observed here.

In order to determine which of the above mechanisms have operated here, we compared the structures of the subunits of the two forms of *Electrophorus* receptor by immunological and peptide mapping techniques using unlabeled, iodinated, or affinity-labeled material. We treated each denatured *Electrophorus* receptor with V8 protease, separating the resultant fragments, and transferred them to DPT paper. The paper was then probed with antisera or mAbs to receptor subunits. Each equivalent subunit pair was fragmented to give a majority of identical-sized pieces. mAbs visualized subsets of individual subunit fragment pairs. Thus, the amino acid sequences of equivalent subunits must be very similar, if not identical, since the enzyme must both recognize and cleave the same amino acids at the same points along the sequence and produce fragments which possess the same antigenic determinants within them (Gullick & Lindstrom, 1982a). Both two-dimensional tryptic peptide maps of iodinated individual *Electrophorus* receptor subunits and Cleveland type peptide maps of MBTA-labeled α subunits confirm this by showing very similar fragment patterns.

There were, however, a pair of fragments derived from the α subunits and from either the γ or δ subunits which differed slightly in size. The differences in mobility between these fragments reflected the differences observed with the intact subunits. There were, additionally, some spots on the two-dimensional peptide maps that were not common to equivalent subunits. ^{125}I-Labeled concanavalin A visualized all of the intact *Electrophorus* receptor subunits, and they could be specifically stained for carbohydrate in gels, indicating that

they are all glycosylated. Since the receptor is an integral membrane protein that protrudes into the extracellular space, this is not surprising. Using the same approach, we incubated the fragment pattern transfer with ^{125}I-labeled concanavalin A and found that the two fragments derived from the α subunit that differ in size were specifically labeled. This result indicates that both of the fragments are glycosylated but does not indicate whether they have identical or nonidentical carbohydrate compositions.

We then treated each intact *Electrophorus* receptor with glycosidic enzymes to see if we could influence the electrophoretic mobilities of their subunits. Neither the mixture of glycosidases nor endoglycosidase H had any apparent effect. This may be because the sugars are O-linked or that the enzymes did not act efficiently on the native receptor molecules. Treatment with neuraminidase, which removes terminal sialic acid residues from glycoproteins, caused an increase in mobility of all the *Electrophorus* subunits. The γ and δ subunits of *Torpedo* receptor are known to contain sialic acid (Lindstrom et al., 1979a), and this charged sugar may have a considerable but unpredictable effect on protein apparent molecular weight (Gahmberg & Anderson, 1982). The binding of concanavalin A and the effect of neuraminidase thus indicate the presence of both mannose and sialic acid on the *Electrophorus* receptor subunits.

In summary, there are few, if any, differences in the primary amino acid sequences of equivalent *Electrophorus* receptor subunits. There are considerable differences between subunits composing each individual type of receptor, since their peptide maps are dissimilar and specific visualization of each subunit with antisera or mAbs is possible. The small differences observed in apparent molecular weight between equivalent subunit pairs probably results from posttranslational modification at a limited region of the sequence. Carbohydrate may be at least in part responsible for these differences.

Our conclusion that the acetylcholine receptors expressed in two closely related tissues are distinguished only by posttranslational glycosylation is very similar to the conclusion recently drawn by Hall et al. (1983) to explain the difference between fetal and adult forms of acetylcholine receptor in muscle. The fetal and adult forms of receptor can be distinguished by a myasthenia gravis autoantibody against carbohydrate located near the acetylcholine binding site with highest affinity for curare (Hall et al., 1983). These results suggest that only a single gene codes for the receptors expressed in *Electrophorus* electric organ and muscle or in mammalian fetal and adult muscle, which is consistent with the evidence for only single genomic copies of receptor subunit genes in *Torpedo* (Davidson et al., 1983) or mammals (Merlie et al., 1983; Noda et al., 1983). A gene family involving less homologous receptor subunits may, however, account for the observation of a putative acetylcholine receptor in neurons of the lateral spiriform nucleus that binds mAbs specific for all four receptor subunits but does not bind α-bungarotoxin (Swanson et al., 1983).

Acknowledgments

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Ligand-Induced Effects at Regions of Acetylcholine Receptor Accessible to Membrane Lipids†

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ABSTRACT: The effectiveness of fluorescence quenching of pyrene-1-sulfonyl azide, a hydrophobic probe used to photolabel acetylcholine receptor (AcChR)-rich electroplax membranes [Sator, V., Gonzalez-Ros, J. M., Calvo-Fernandez, P., & Martinez-Carrion, M. (1979) *Biochemistry 18*, 1200], is used to study the accessibility of the covalently attached fluorophore to extramembranous quenchers as a function of occupancy of cholinergic receptor binding sites. In these membranes, binding of water-soluble cholinergic ligands to specific sites at the extracellular side affects the fluorophore located in a distant topographical area of the AcChR molecule. When a neurotransmitter analogue (carbamylcholine) is present, the susceptibility of the covalently attached fluorophore to quenching with externally added nitromethane decreases in comparison with that of the same membranes in the absence of carbamylcholine. This neurotransmitter agonist effect is, however, reversible as removal of carbamylcholine by dialysis restores the quenching effectiveness to that of resting nonliganded membranes. The presence of bound α-bungarotoxin produces an opposite effect to that of carbamylcholine and induces an increase in susceptibility to quenching agent. These results are interpreted in terms of long-range effects induced by occupancy of cholinergic sites which are detected by covalently bound fluorophore located at regions of the AcChR protein accessible through the lipid matrix of the *Torpedo* membrane. Such effects are presumably due to molecular rearrangements within the membrane-bound AcChR structure.

The role of the acetylcholine receptor (AcChR)[1] in promoting increased cation permeability upon agonist binding at the cholinergic binding sites in postsynaptic muscle membranes is well documented (Adams, 1981; Conti-Tronconi & Raftery, 1982; Martinez-Carrion et al., 1982). Nevertheless, little is known regarding molecular mechanisms for formation of the ion channel or induction by cholinergic agonists of reversible transitions of the receptor between "sensitized" (low-affinity) and "desensitized" (high-affinity) states. In this regard, conformational changes of the AcChR upon ligand binding were postulated early (Nachmansohn, 1955). Consistent with this hypothesis were reports of changes both in the intrinsic fluorescence of the receptor (Bonner et al., 1976; Barrantes, 1976) and in its increased labeling with a water-soluble probe, ethidium azide, in the presence of cholinergic ligands (Witzemann & Raftery, 1978). More recently, Kaneda et al. (1982) have reported ligand effects on the intrinsic fluorescence of purified, detergent-solubilized *Narke* (another electric fish) receptors which they attributed to conformational changes occurring within the AcChR protein.

We previously reported pyrene-1-sulfonyl azide (PySA) as a nonperturbing hydrophobic fluorescent probe useful in the identification of AcChR subunits accessible from the hydrophobic membrane matrix (Sator et al., 1979a). In this and in a subsequent study (Gonzalez-Ros et al., 1979b), the distribution of PySA photoproducts in labeled membranes was extensively characterized. It seems that in this compound the

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[1] Abbreviations: AcChR, acetylcholine receptor; PySA, pyrene-1-sulfonyl azide; α-Bgt, α-bungarotoxin; Tris, tris(hydroxymethyl)aminomethane.

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