Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 7805–7809, October 1988
Neurobiology

Allelic variants of acetylcholinesterase: Genetic evidence that all acetylcholinesterase forms in avian nerves and muscles are encoded by a single gene

(membrane protein/rapidly transported protein/synaptic components)

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Communicated by George B. Koelle, June 22, 1988 (received for review March 18, 1988)

ABSTRACT **Two acetylcholinesterase (AcChoEase) polypeptide chains, α and β, are expressed in avian nerves and muscles with apparent molecular masses of 110 and 100 kDa, respectively. We now show that individual quails express α, β, or both AcChoEase polypeptide chains. By mating studies we show that the two AcChoEase polypeptides are autosomal and segregate as codominant alleles in classical Mendelian fashion. Biochemical studies of the two allelic AcChoEase polypeptides indicate that they have the same turnover number, have the same K_m for acetylcholine, are immunoprecipitated to the same extent with a monoclonal anti-AcChoEase antibody, and can assemble with equal efficiency into multimeric forms. Thus there are no obvious functional differences between the two alleles. In heterozygotes, the rates of synthesis of the two polypeptides are identical, suggesting that there are no differences in expression of these two genes. Within an individual, nerves and muscles always express the same AcChoEase alleles. Analysis of the allelic composition of the multiple AcChoEase forms isolated from muscle indicates that all AcChoEase forms are comprised of the same allelic polypeptide chains. In contrast to the nicotinic acetylcholine receptors that appear to be encoded by complex multigene families, our studies on AcChoEase show that all forms of this important synaptic component in electrically excitable cells are encoded by a single gene. Thus differences in assembly and localization of the multiple synaptic forms of AcChoEase must arise through posttranscriptional events, posttranslational modifications of a similar AcChoEase polypeptide chain or both.**

In all cholinergically innervated cells signal transduction is initiated by acetylcholine binding to one of several classes of acetylcholine receptors and subsequently terminated, at least in part, by hydrolysis of the neurotransmitter catalyzed by one of several molecular forms of acetylcholinesterase (AcChoEase; EC 3.1.1.7). In nerves and muscles, this enzyme exists as a family of forms differing in oligomeric composition, physical properties, and subcellular localization (1–4). Electrically excitable cells express four oligomeric forms of AcChoEase consisting of globular monomers (G1), dimers (G2), tetramers (G4), and a structurally asymmetric form consisting of three tetramers covalently attached to a three-stranded collagen-like tail (A12). In addition, several other structural modifications can distinguish the various molecular forms. Hydrophobic membrane-bound dimeric and tetrameric AcChoEase forms are found in the central and peripheral nervous systems of all vertebrate species examined to date (1–4). These molecules may be anchored to the plasma membrane through a specific class of covalently bound glycolipids (5) or associated with another hydrophobic polypeptide chain (6). In mammalian erythrocytes (7, 8) and nerve endings in the *Torpedo* electric organ (9), only hydrophobic dimers with glycolipid linkages are found.

The functional significance and subcellular localization of the multiple AcChoEase forms have been difficult questions to address directly. Only in simple tissue culture systems has it been possible to unequivocally determine the subcellular location and destination of the various molecular forms of AcChoEase (for review, see refs. 1–4), due in large part to the availability of specific membrane permeable and impermeable enzyme inhibitors (ref. 10; for review, see ref. 11). Tissue-cultured muscle cells express the dimeric, tetrameric, and asymmetric AcChoEase forms on their cell surfaces (12, 13), as do muscle cells *in vivo* (14). A subset of these surface molecules are highly concentrated at the synaptic basal lamina in muscle (15–17). Here, the asymmetric collagen-tailed form of the enzyme appears to predominate (18, 19), although it is also found in extrajunctional regions of muscle. On tissue-cultured nerves (20) and neuronal cell lines (21), the cell-surface AcChoEase form consists entirely of hydrophobic tetrameric AcChoEase molecules.

Neurons synthesize and rapidly transport at least three classes of AcChoEase forms including dimers, tetramers, and asymmetric molecules (22, 23). A subset of these molecules may be targeted to the neuromuscular junction and other synapses. By using electron microscopic histochemical methods and biochemical techniques in conjunction with denervation studies, it has been possible to correlate the location of several AcChoEase molecular forms with specific regions of the neurons (24, 25). These studies suggest that the monomeric AcChoEase molecules are localized within the neuronal perikarya whereas the multimeric forms are transported away from the perikarya and localized along the axonal plasma membrane and at sites of nerve contact on both the pre- and postsynaptic membranes. However, it has not yet been possible to determine precisely which versions of the enzyme are the physiologically relevant ones at identified synapses. Only in the *Torpedo* electric organ has it been possible to show that both asymmetric collagen-tailed AcChoEase, associated with the basal lamina (26), and hydrophobic AcChoEase dimers, associated with the presynaptic plasma membrane, coexists at the synapse (9).

It is now clear that different cell types express different AcChoEase forms on their cell surfaces and that they localize them by different mechanisms (1–4). To understand the molecular basis for this diversity of enzyme forms and their mechanisms of localization we have studied the expression of the AcChoEase polypeptides in avian nerves and muscle. We now show that two allelic forms of the AcChoEase polypep-

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Abbreviations: AcChoEase, acetylcholinesterase; BSA, bovine serum albumin.

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tide are expressed in avian nerves and muscle and that all forms of AcChoEase arise from a single gene. Thus the differences in AcChoEase polypeptides specifying assembly or subcellular localization must arise either through alternative splicing events and/or different posttranslational modifications of the AcChoEase polypeptide chains. A preliminary report of this work has been published in abstract form.†

MATERIALS AND METHODS

Cell Culture and Metabolic Labeling. Primary muscle cultures were prepared from 10-day-old quail embryos (Truslow Farms, Chestertown, MD), essentially as described by Konigsberg (27), and grown in Eagle's minimal essential medium supplemented with 10% (vol/vol) horse serum and 2% (vol/vol) chicken embryo extract (EMEM-210). Neuronal cultures were prepared from whole embryonic brains of the same age by triturating the tissue in complete EMEM-210 medium. Cell suspensions were passed through a 10-μm nylon mesh, diluted to 8 ml with EMEM-210 containing 1 μM cytosine arabinoside, and plated on poly(L-lysine)/collagen-coated culture dishes. Cells were metabolically labeled in methionine-free EMEM with 10% (vol/vol) horse serum (3 ml per 100-mm dish) supplemented with [^{35}S]methionine at 50–100 μCi/ml (New England Nuclear; specific activity >1300 Ci/mmol; 1 Ci = 37 GBq) for 3–7 hr, depending upon the experiment.

Immunoprecipitation of AcChoEase. Labeled cultures were extracted in borate extraction buffer (EB) consisting of 20 mM sodium borate (pH 9.0), 1 M NaCl, 5 mM EDTA, 0.5% Triton X-100, 0.5% bovine serum albumin (BSA), and protease inhibitors at 4 ml per culture dish as described (13, 28). The extracts were homogenized and centrifuged for 20 min at 27,000 × g, and the supernatants were incubated for 2 hr with 20 μg of anti-AcChoEase monoclonal antibody 1A2 (28). The immune complexes were precipitated with 20 μl of a 1:1 (vol/vol) suspension of protein A-Sepharose CL-4B beads (Pharmacia), washed, resuspended, and heated in 50 μl of SDS sample buffer containing 100 mM dithiothreitol, as described (28). Aliquots of the samples were analyzed by electrophoresis on 10% polyacrylamide slab gels (29) and fluorographed by using Fluorenhance (RPI). Alternatively, samples were analyzed by two-dimensional gel electrophoresis under nonreducing conditions in the first dimension followed by reduction of the samples in the tube gel by heating in sample buffer containing 100 mM dithiothreitol prior to the second dimension.

Immunotitration of AcChoEase. Goat anti-mouse IgG [Cooper Biomedical, Malvern, PA; 25 μg/ml in isotonic phosphate-buffered saline (pH 7.2; PBS), 100 μl per well] was added to each well of a Falcon MicroTest III flexible assay plate and incubated 6 hr at room temperature. The wells were washed three times with 200 μl of PBS containing BSA at 2 mg/ml (PBS/BSA) and incubated overnight with 200 μl of the same solution to block residual binding sites. To each well was added 100 μl of PBS/BSA containing the monoclonal anti-AcChoEase 1A2 serially diluted 1:2 and thus containing from 0.1 ng to 50 ng, incubated overnight at 4°C, and washed four times with 200 μl of PBS/BSA. To each well was added 50 μl of quail brain AcChoEase supernatant obtained from quail homozygous for either α or β or from heterozygous quail and matched for total AcChoEase enzyme activity and incubated for 2 hr at 4°C. After washing wells four times with 200 μl of PBS/BSA, bound AcChoEase activity was assayed by adding 50 μl of buffer/substrate solution containing 600 μM [^{14}C]acetylcholine to each well, incubating 15 min at room temperature, and measuring radioactivity in 40-μl aliquots by the two-phase scintillation counting system developed by Johnson and Russell (30). This assay system is linear with respect to time and enzyme concentration and gives a relative and proportional measure of the number of AcChoEase enzyme molecules bound per well.

Analysis of AcChoEase Oligomeric Forms by Velocity Sedimentation. Individual muscle cultures (100-mm dishes) were metabolically labeled and extracted in 500 μl of 2× EB as described above. The supernatants were analyzed by velocity sedimentation on 5–20% (wt/vol) sucrose gradients in an SW 41 rotor at 40,000 RPM for 16 hr at 4°C. Aliquots of each fraction were assayed for AcChoEase activity by a modification of the radiometric method of Johnson and Russell (30) as described (31) with [^{3}H]acetylcholine (New England Nuclear; specific activity, 3.5 mCi/mmol) as substrate.

Mating Studies. This study was done with the participation of our quail supplier (Truslow Farms), whose assistance is gratefully acknowledged. Juvenile quail were isolated prior to sexual maturity and subsequently paired and mated in separate pens. Fertilized eggs were collected from each mated pair over a period of 10 days, coded, and shipped together with the marked parents. To determine the genotypes, adult quails were killed by decapitation and the brains were removed and homogenized in 10 vol of EB. After centrifugation, the AcChoEase polypeptide composition was determined by immunoprecipitation and analysis by SDS/gel electrophoresis as described above. The fertilized eggs were incubated for 16 days and the allelic forms of AcChoEase expressed were determined as described for the adults.

RESULTS AND DISCUSSION

Expression of the α and β AcChoEase Alleles in Embryonic Quail Muscle. AcChoEase consists of two types of polypeptide chains, α and β, with apparent molecular masses of 110 and 100 kDa, respectively (28, 32). Randall *et al.* (33) reported that the two AcChoEase polypeptide chains expressed in chicken brain were in fact allelic variants of the same locus. To determine whether the two AcChoEase polypeptides found in quail muscle cultures were allelic variants, primary cultures made from single embryos were labeled with [^{35}S]-methionine and the newly synthesized AcChoEase molecules were immunoprecipitated and analyzed by SDS/gel electrophoresis and fluorography. The results (Fig. 1) show that some embryos express only the α AcChoEase polypeptide, some only β, and some both, suggesting that these are allelic variants of the same genetic locus. It should be noted that in the heterozygotes both alleles are always expressed to the same extent, suggesting that there are no quantitative differences in the regulation of these two alleles.

The AcChoEase Polypeptides Segregate as Codominant Autosomal Alleles. To formally test the hypothesis that the α and β AcChoEase polypeptides were allelic variants of the same locus quails were mated and the genotypes of both parents and progeny were determined. Although AcChoEase constitutes only 0.01% of the total protein in the avian brain (28), sufficient AcChoEase protein can be immunoprecipitated from a single adult brain to be readily visualized on Coomassie blue-stained gels. The results of this experiment (Table 1) indicate that the two AcChoEase polypeptides are codominant autosomal alleles and segregate in classical Mendelian fashion. Segregation of the progeny from the α/α × α/β matings, although not 0.5 as would be predicted, does not show a statistically significant deviation from this value given the small size of our experimental population sample. From these studies, we conclude that a single gene encoding the AcChoEase polypeptide exists in quails.

The α and β AcChoEase Alleles Are Functionally Equivalent. The existence of multiple alleles at a given locus could reflect the need for functional variability of that gene product arising as an adaptation to a broad range of environmental

†Rotundo, R. L., Gomez, A. M., Fernandez-Valle, C. & Randall, W. R., 17th Meeting of Society for Neuroscience, Nov. 16–21, 1987, New Orleans (abstr.).

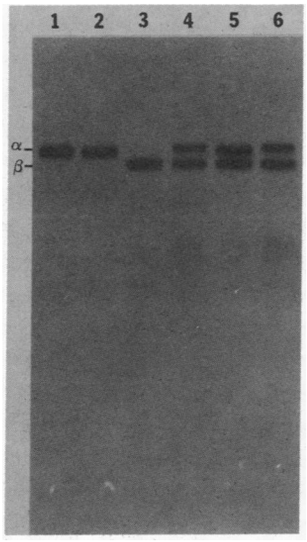

FIG. 1. Expression of the α and β AcChoEase alleles in quail embryo muscle cultures. Myoblasts were obtained from individual 11-day-old quail embryos and cultured in 100-mm collagen-coated culture dishes. After 1 week in culture, the cells were labeled for 4 hr with [^{35}S]methionine at 50 μCi/ml. The cultures were then extracted, the AcChoEase was immunoprecipitated, and the polypeptides were analyzed by SDS/gel electrophoresis and fluorography. AcChoEase polypeptides expressed by six individual embryo muscle cultures showing the characteristic pattern in individuals homozygous for α (lanes 1 and 2) or for β (lane 3), and heterozygous for both (lanes 4–6).

requirements. Alternatively, multiple alleles could arise as a consequence of "neutral" mutations and be maintained in populations through their linkage to other selectable loci. To examine this question we compared the catalytic properties of the two allelic AcChoEase forms. We already know that the α and β AcChoEase alleles exhibit identical epitopes determined by immunoblotting with monoclonal antibodies (28, 33). Embryonic quail brains were homogenized in EB and the genotypes were determined by immunoprecipitation and SDS/gel electrophoresis. Aliquots of the supernatants obtained from homozygous α, homozygous β, or heterozygous individuals were diluted 1:30 to 1:50 in a solution of 50 mM Tris·HCl (pH 7.0), 150 mM NaCl, 1 mM EDTA, and 0.5% Triton X-100 to give equivalent amounts of catalytic activity per unit volume. These stock solutions were then used for all subsequent studies of AcChoEase activity.

The K_m for acetylcholine was determined for each allelic form by using [^{14}C]acetylcholine at substrate concentrations from 25 μM to 600 μM, as described (31). Both allelic forms of AcChoEase have identical affinities for their substrate (K_m = 235 μM; data not shown). Immunotitration of the two AcChoEase polypeptides with our monoclonal anti-AcChoEase antibody 1A2 indicates that the same number of enzyme molecules produce equivalent enzyme activities (Fig. 2). Thus the turnover numbers of the two allelic AcChoEase forms are identical. Finally, to determine whether differences in the assembly of these molecules existed, quail muscle cultures made from pooled embryonic myoblasts were labeled with [^{35}S]methionine, the AcChoEase was immunoprecipitated, and the extent of disulfide bond formation between the two types of polypeptide chains was determined by two-dimensional gel electrophoresis under nonreducing conditions in the first dimension and reducing conditions in the second. If the two types of AcChoEase polypeptide chains can assemble with equal probability, then the frequency of appearance of α/α, α/β, and β/β dimers should reflect their relative abundance in the population. The results of this experiment indicate that the assembly of α and β AcChoEase polypeptide chains is random (Fig. 3). We have also analyzed the composition of disulfide-linked dimers in cultures made from heterozygous individuals. These studies show that assembly of the two allelic subunits is completely random (R.L.R., unpublished results). Because of the prevalence of monomeric and dimeric AcChoEase forms in these muscle cells and the large size of the tetrameric AcChoEase form, we limited our comparison to the assembly of AcChoEase polypeptides into dimeric molecules.

Table 1. Segregation of AcChoEase alleles

Parents		Progeny, no.			
Male	Female	α/α	α/β	β/β	Total
α/β	α/α	3	6	0	9
α/β	α/α	1	4	0	5
β/β	α/α	0	7	0	7
α/α	β/β	0	4	0	4
α/α	α/β	2	3	0	5

Allelic forms of AcChoEase expressed in five mated pairs of quails and their progeny were determined by immunoprecipitating the enzyme protein from brain tissue and analysis by SDS/gel electrophoresis (for an example, see Fig. 1). In all five cases the AcChoEase polypeptides segregated as codominant alleles in classical Mendelian fashion. The frequencies of the α and β AcChoEase alleles, based upon the analysis of >100 individuals during the course of our experiments, is 0.7 and 0.3, respectively.

All Oligomeric Forms of Muscle AcChoEase Are Encoded by the Same Gene. Muscle cells synthesize and assemble four oligomeric forms of AcChoEase. If the AcChoEase catalytic subunit is encoded by a single gene then all forms of the enzyme should be assembled from the same allelic polypeptide chain. Conversely, if more than one gene encodes the various AcChoEase forms, then individual forms may express different allelic compositions as a result of recombination events.

Muscle cultures made from single embryos were metabolically labeled and the four oligomeric AcChoEase forms were

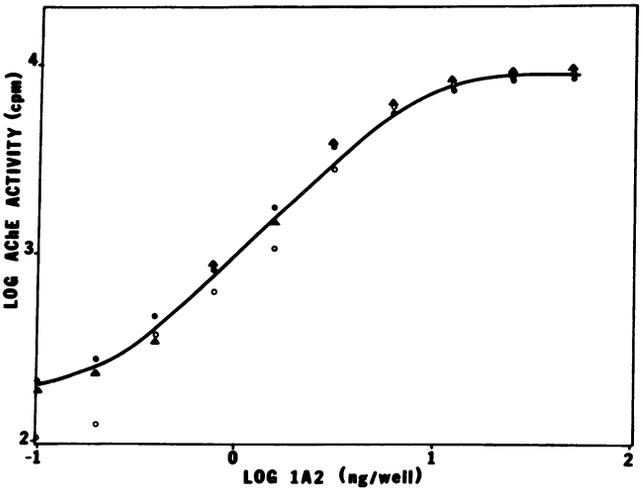

FIG. 2. α and β AcChoEase polypeptides are catalytically identical. Extracts containing the same amounts of catalytically active AcChoEase (AChE) were obtained from individual quails of the α/α (●), β/β (○), or α/β (▲) genotype and incubated with various amounts of immobilized anti-AcChoEase monoclonal antibody 1A2 in a solid-phase radioimmunoassay. Bound AcChoEase was determined by using a modification of the standard radiometric assay. Similar amounts of active AcChoEase enzyme molecules of each genotype were bound over a 500-fold dilution range of antibody, indicating that the catalytic sites of the two allelic variants have the same turnover number. Since both alleles have the same affinity for substrate (K_m = 235 μM) and catalytic efficiency, they must be functionally identical.

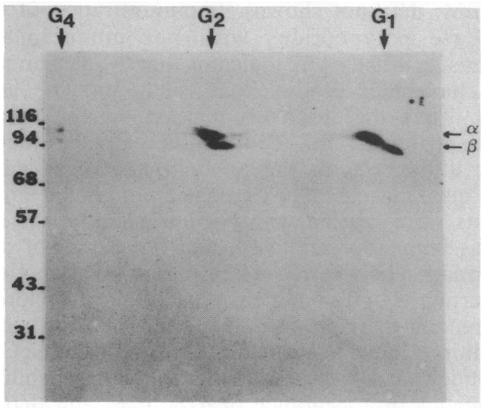

FIG. 3. AcChoEase subunits are randomly assembled into oligomeric forms. Mixed muscle cultures were prepared by pooling myoblasts from randomly selected embryos. When the myotubes were mature, the cultures were labeled with [^{35}S]methionine and the AcChoEase was immunoprecipitated. Aliquots of the SDS-treated immunoprecipitated AcChoEase were electrophoresed under nonreducing conditions in tube gels for the first dimension and then reduced and electrophoresed on a 10% polyacrylamide slab gel for the second dimension. Most of the newly synthesized AcChoEase consists of the monomeric (G1) and disulfide-bonded dimeric (G2) forms with only a small amount of disulfide-bonded tetrameric forms being apparent (G4). The G2 forms separate into α/α, α/β, and β/β forms in the first dimension, and their allelic composition is resolved in the second dimension.

isolated by velocity sedimentation on 5–20% (wt/vol) sucrose gradients. The positions of the four oligomeric AcChoEase forms were determined by assaying aliquots of each fraction for enzyme activity by using [^{3}H]acetylcholine as substrate. The fractions were then immunoprecipitated and analyzed by SDS/polyacrylamide gel electrophoresis and fluorography (Fig. 4*A*). Alternatively, peak fractions corresponding to each oligomeric form were pooled and the labeled AcChoEase was immunoprecipitated and analyzed in the same manner (Fig. 4*B*). In every case all oligomeric forms shared the same AcChoEase subunits as would be expected if all molecular forms are encoded by the same gene. In addition, we have also compared the cell-associated and secreted forms of AcChoEase synthesized by muscle cells, and they also share the same subunit composition (data not shown).

Neurons and Muscle Cells Express the Same AcChoEase Gene. To determine whether the same allele was being expressed in both nerves and muscle, we prepared matched neuronal and muscle cultures from individual embryos and metabolically labeled the AcChoEase polypeptides with [^{35}S]methionine. The labeled AcChoEase was immunoprecipitated and analyzed by SDS/gel electrophoresis and fluorography (Fig. 5). In every case neurons and muscle cells isolated from the same individual synthesized the same allelic AcChoEase polypeptide chains, indicating that the same gene is expressed in both tissues.

SUMMARY AND CONCLUSIONS

The molecular basis for the multiple AcChoEase forms in vertebrates could arise by several mechanisms, including multiple genes, as has been described in the nematode *Caenorhabditis elegans* (ref. 34 and references therein), or a single gene as has been found in *Drosophila* (35, 36). Cloning of cDNAs encoding the catalytic subunits of AcChoEase from *Torpedo californica* (37) and *Torpedo marmorata* (38) and analysis of genomic sequences (38, 39) indicate that AcChoEase is encoded by a single gene in these species. Sequence analysis of individual cDNA clones from these species (39, 40) and amino acid sequencing of the AcChoEase polypeptide chains (41) indicate the presence of at least two

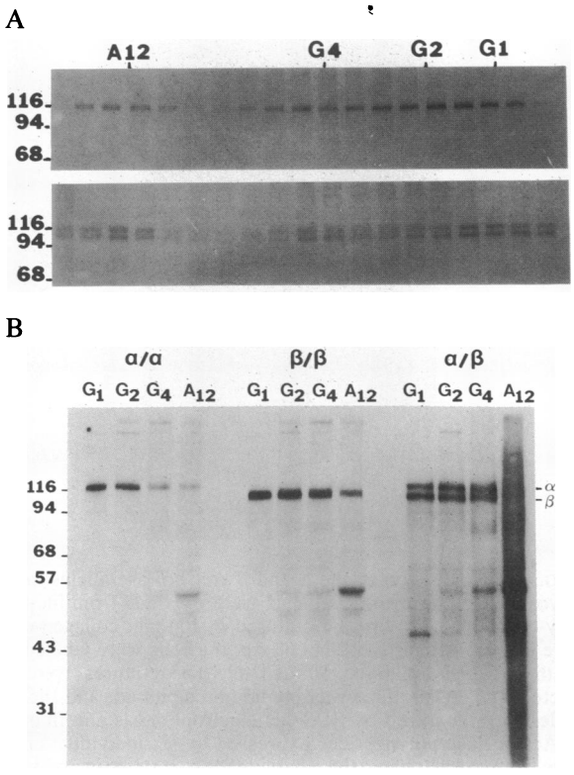

FIG. 4. Subunit composition of muscle AcChoEase oligomeric forms expressed by individual α/α, β/β, and α/β embryos. Tissue-cultured muscle cells from individual embryos were grown in 100-mm culture dishes and labeled for 7 hr with [^{35}S]methionine at 100 μCi/ml. At the time of culturing the brain from each embryo was removed and the AcChoEase was immunoprecipitated, analyzed by SDS/gel electrophoresis, and stained with Coomassie blue to determine which alleles were being expressed in each culture. The AcChoEase oligomeric forms expressed in each culture dish were isolated by velocity sedimentation, immunoprecipitation, and gel electrophoresis and visualized by fluorography. (*A*) Immunoprecipitates of all AcChoEase activity-containing fractions from sucrose gradients of either an α-expressing muscle culture (*Upper*) or heterozygous culture (*Lower*). The positions of the peak AcChoEase activity fractions corresponding to the monomeric (G1), dimeric (G2), tetrameric (G4), and asymmetric collagen-tailed (A12) forms are shown. (*B*) Immunoprecipitates of the pooled peak AcChoEase fractions from sucrose gradients of homozygous α, homozygous β, or heterozygous cultures. The bands below the α and β AcChoEase polypeptides are nonspecific bands that occasionally contaminate the immunoprecipitates.

mRNA transcripts encoding the asymmetric and hydrophobic AcChoEase subunits, respectively. Thus several mechanisms can be identified that contribute to the diversity of AcChoEase molecular forms. These now include the choice of alternative splice sites to generate different transcripts and a variety of posttranslational modifications that are most likely involved in the assembly and targeting of these enzyme molecules to specific functional domains on the neuronal and muscle cell surfaces.

We now report the existence of two allelic variants of the AcChoEase polypeptide chain in quail. Although differing in apparent molecular mass by ≈10 kDa, these allelic polypeptides appear to be functionally identical by several criteria including their affinity for acetylcholine, turnover number (Fig. 2), and their ability to assemble into the same multimeric forms (Figs. 3 and 4). Thus the allelic differences appear to be neutral ones from a functional standpoint, yet extremely useful in that they now permit the application of genetics as a means of studying the biogenesis and regulation of an identified synaptic component.

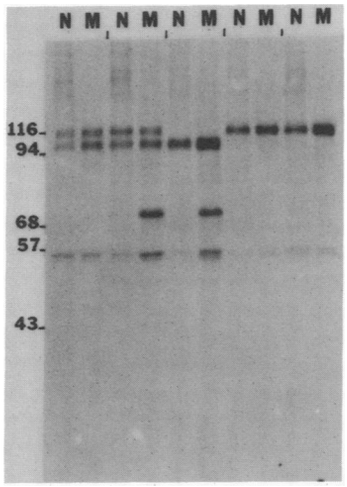

FIG. 5. Neurons and muscle cells express the same AcChoEase alleles. Neuronal and muscle cell cultures were prepared from individual embryos as described, except that neurons were grown on poly(L-lysine)/collagen-coated culture dishes in the presence of 1 μM cytosine arabinoside and labeled after 2–3 days in culture. All labeling and immunoprecipitation were as described above. Each pair of lanes shows the AcChoEase alleles immunoprecipitated from neurons (N) or muscle (M) cells obtained from the same embryo. These are five representative samples from a total of 15 embryos examined. In all cases nerves and muscle cells expressed the same AcChoEase alleles, indicating that the same gene is expressed in both tissues.

The conclusion that all forms of AcChoEase in nerves and muscle are encoded by the same gene is supported by three lines of evidence. (*i*) Our mating study shows that the two AcChoEase polypeptides are encoded by the same locus and that these alleles segregate according to classic Mendelian genetics (Table 1). (*ii*) All oligomeric forms of AcChoEase, regardless of origin, are comprised of the same allelic AcChoEase polypeptide (Fig. 4). (*iii*) Individuals always express the same allelic forms of the AcChoEase polypeptide in nerves and muscle (Fig. 5). Although the existence of two or more very closely linked structural genes encoding different classes of AcChoEase in nerves and muscle is a formal possibility, the likelihood of such genes occurring is exceedingly low.

Nerves and muscle cells assemble the AcChoEase polypeptides into similar oligomeric forms. Monomeric (G1) AcChoEase catalytic subunits are assembled into dimeric (G2) and tetrameric (G4) forms in the rough endoplasmic reticulum and subsequently transported to the Golgi apparatus where a subset of these molecules is covalently attached to a collagen-like tail to form the asymmetric (A12) AcChoEase molecule (13). However, the mechanisms employed by nerves and muscle cells to localize these AcChoEase molecules on their surfaces is considerably different. Muscle cells localize predominantly the tetrameric and asymmetric forms on their surface, the latter being associated with the extracellular matrix, whereas neurons appear to localize only the hydrophobic tetrameric forms of the enzyme on their surface (20). These hydrophobic forms can arise either through the addition of covalently linked glycolipids (5, 7, 8) or by association with a smaller hydrophobic polypeptide chain (6). Tissue-cultured neurons secrete soluble dimeric, tetrameric, and asymmetric collagen-tailed forms of the enzyme, whereas muscle cells retain the asymmetric forms on their surface, along with some tetrameric enzyme while secreting relatively large amounts of the dimeric and tetrameric forms (ref. 20 and R.L.R., unpublished results). The monomeric form appears to be entirely an intracellular precursor to the oligomeric forms. Since we now know that the entire family of AcChoEase molecular forms in birds is encoded by a single gene, the relative contributions of alternative splicing events and posttranslational modifications to this diversity of forms remain to be determined.

We thank Dr. Douglas M. Fambrough for helpful discussions. We also gratefully acknowledge the generous assistance of Ms. Margaret Brown and Mr. Rousby Quesenberry of Truslow Farms for selecting and mating the quails for us. This work was supported by grants from the National Institutes of Health (AG-05917), the Muscular Dystrophy Association, and a Sloan Foundation Fellowship in Neurosciences to R.L.R. C.F.-V. is a recipient of a Florida Pre-Doctoral Scientist Award.

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