Mol Neurobiol (2008) 37:164–170
DOI 10.1007/s12035-008-8030-3

Acetylcholine Receptor γ-Subunits mRNA Isoforms expressed in Denervated Rat Muscle

Amanda M. Li · Hualong Ma · Alfredo Villarroel

Received: 22 October 2007 / Accepted: 16 May 2008 / Published online: 12 June 2008
© Humana Press Inc. 2008

Abstract The fetal acetylcholine (ACh) receptor, composed of the αβγδ subunits, is expressed in fetal, neonatal, and denervated muscle. Single-channel recording has revealed three kinetically distinct classes in neonatal and denervated muscle, suggesting that at least three forms of the γ-subunit are required. To account for the kinetic classes observed, we compared the messenger ribonucleic acid (mRNA) forms expressed in neonatal and denervated muscle using reverse transcriptase polymerase chain reaction, cloning, and RNAse protection assays. We found five novel forms arising from alternative splicing, which we named γ5–γ9. The forms γ5, γ6, and γ7 lack exon 4 and 63-, 89-, and 136 bp of exon 5, respectively. A γ8 form lacks exons 3 and 4 and 19 bp of exon 5. The last, γ9, lacks exons 3, 4, and 5. Results indicate that γ4 predominates in fetal muscle and γ7 in denervated adult muscle. Some of the γ-subunit mRNAs found may generate the receptors observed in muscle.

Keywords γ-Subunits · mRNA isoform · Acetylcholine receptor · AChR · Endplate channel · Splice variant

A. M. Li · H. Ma · A. Villarroel
Department of Physiology and Biophysics,
Dalhousie University School of Medicine,
Halifax, Canada

A. M. Li
e-mail: a.li@dal.ca

A. Villarroel (✉)
Department of Physiology, Faculty of Science,
Mahidol University,
Rama VI Rd, Rajtavitee,
Bangkok 10400, Thailand
e-mail: fravl@mahidol.ac.th

Introduction

The fetal acetylcholine (ACh) receptor, composed of the $\alpha_2\beta\gamma\delta$ subunits, is expressed in skeletal muscle during embryonic stages and during the first 2 weeks after birth [1, 2]. In contrast to the adult receptor, the fetal ACh receptor produces postsynaptic currents of a slow decay time course. Such a time course guarantees the spontaneous contraction of the embryonic muscle [3]. Presumably, the slow time course of the postsynaptic current enables the depolarization to propagate along the myocyte without substantial electrotonic decrement. In addition, the γ-subunit is also expressed in adult denervated muscle where it generates muscle supersensitivity. In both neonatal and denervated muscles, the γ-subunit confers a long-lasting time course to the postsynaptic currents. Since the ACh receptor is permeable to Ca^{2+} [4, 5], a long-lasting postsynaptic current provides an opportunity for Ca^{2+} to enter into the subsynaptic space, where it has been implicated in processes of development and regeneration. Therefore, it is of interest to examine the mechanism controlling channel duration. Variability in channel kinetics has been ascribed to a splice variant of the γ-subunit with a shorter N terminus [6, 10]. Recent single-channel recording studies, however, have revealed three kinetic classes calling for at least three subtypes of the γ-subunit [7].

The γ-subunit gene contains 12 exons, six of which encode the N terminus of the subunit [8]. Exons 7–9 and exon 12 encode the four transmembrane segments in a modular fashion. Large intronic sequences between exons 4–5 and 5–6 suggest the presence of modulatory elements [9]. The examination of the messenger ribonucleic acid (mRNA) expressed in neonatal muscle suggests that more than one γ-subunit form may be expressed in rat muscle. A mRNA form encoding for a γ-subunit that lacks 43 amino

acids from the N terminus has been described [10]. The presence of an mRNA does not necessarily means that the protein will be translated. However, single-channel recording of endplate channels indicated that at least three kinetic classes exist in muscle: a neonatal one of 10 ms mean duration; a second of 7 ms duration, characteristic of denervated muscle; and a 27-ms class found in female neonatal muscle [7]. Therefore, at least three variants of the γ-subunits are predicted based on these kinetic classes. Similar to the γ2-subunit previously found, it is expected that the new forms will differ in the N terminus. In the α-subunit, the N terminus is responsible for the kinetic properties. It is possible that the N termini of other subunits also participate in gating. Different molecular forms with different N termini and a similar M2-conducting segment may provide molecular candidates for the kinetic classes observed in muscle. In this study, we tested this hypothesis using reverse transcriptase (RT) polymerase chain reaction (PCR) reaction and RNAse protection assays. The results demonstrate that there are nine mRNA forms present in the muscle during early development.

Materials and Methods

Adult Wistar rats were anesthetized by an intraperitoneal injection of 30–60 mg/kg sodium pentobarbital. Deep anesthesia was assessed by the absence of papillary reflexes. Soleus denervation was performed by sectioning the right sciatic nerve and ligating the proximal segment to prevent reinnervation. Sternomastoid muscle denervation was performed by sectioning the accessory nerve 2 mm from the muscle surface and ligating the proximal segment to prevent reinnervation. Two weeks after denervation, the animals were killed with an overdose of sodium pentobarbital, and the muscles were dissected and frozen in liquid nitrogen. All surgical procedures were performed according to the guidelines of the Dalhousie University Committee on Laboratory Animals, protocol number 99-030.

RT-PCR from muscle Frozen muscles were grounded under liquid nitrogen with mortar and pestle. Total RNA was extracted using the RNeasy kit (Quiagen), as specified by the supplier. RT reaction was performed using 5–10 μg of total RNA, denatured 7 min at 94°C, and incubated 90 min at 42°C with Moloney murine leukemia virus RT using either random hexameres or poly-dT. Then, 1 μl of RT reaction was amplified by PCR using 5′-AGCAGGTA GAACACCACCTTCT-3′ and 5′-AGCTCCTCTTGCTGCT GCTGGCTA-3′ as forward and reverse primers, respectively. The PCR program consisted of an initial denaturation (2 min 94°C) and 35 cycles (94°C, 30 s; 68°C, 30 s; 72°C, 30 s), followed by an extension period of 7 min at 72°C.

Nested PCR, performed using *Taq* deoxyribonucleic acid (DNA) polymerase (Gibco-BRL), produced further amplification. The forward and reverse primers were 5′-TCAG ATGTGGTTAATGTCAG-3′ and 5′-GAGGAGGCCCTCA CGACTAATG-3′, respectively. The temperature protocol was similar to that of the PCR reaction, except that the annealing temperature was 60°C instead of 68°C, required by the lower melting temperature of the primers. *Taq* polymerase has the advantage of providing an extra adenine in the 3′ end, which is required for the TA cloning method. PCR products were visualized in a 1.8% agarose gel stained with ethidium bromide. Bands containing the PCR fragments were excised from the gel and DNA was extracted using GeneClean (Bio101). Individual bands were cloned into the p1.2 vector (Invitrogen). To determine the proportion of the different subunit forms, 1 μl of the PCR reaction was directly ligated into the TA cloning vector.

RNAse Protection Assay

Ribonuclease protection assay was performed according to Melton et al. [12]. The antisense γ2-specific probe was synthesized from a clone in the 3′–5′ orientation [10] using the T7 promoter and purified in a polyacrylamide gel. Linearization of the vector with *Hin*dIII resulted in a complementary RNA probe of 440 bp. Similarly, sense single-strand mRNAs were synthesized for the γ1-, γ2-, γ3-, γ4-, γ5-, γ8-, and γ9-cloned γ-subunit forms and were used as positive controls for the protected fragment.

Ribonuclease protection assay was done using the RPA III™ kit from Ambion (Austin, TX, USA) following the instructions provided. Briefly, 5–20 μg of total RNA and the probe were coprecipitated overnight in 500 mM ammonium acetate and 70% ethanol at −20°C. Pelleted RNA was washed and resuspended in 10 μl of hybridization buffer provided by the kit or in 200 mM Pipes, 2 M NaCl, and 5 mM ethylenediamine tetraacetic acid, pH 7.4, mixed with formamide in 1:5 proportion. RNA was denatured at 95°C for 4 min and then incubated overnight at 42°C to hybridize the probe to its complement in the sample RNA. RNAse digestion was initiated by adding A/T1 RNAse diluted 1:100 to 150 μl of RNAse digestion buffer. After incubation at 37°C for 30 min, the RNAse was inactivated by adding 225 μl of RNase inactivation/precipitation solution to each tube. The RNA was precipitated overnight at −20°C.

Protected fragments were separated in an 8-M urea 5% polyacrylamide gel run at 100 V for 50 min. RNA was transferred to a positively charged nylon membrane (2 h at about 200 mA in a chamber surrounded by ice) and cross-linked to the membrane in a UV Stratalinker. Detection was performed with BrightStar BioDetect kit (Ambion) following the instructions provided.

Results

RT-PCR from Denervated Muscle

First, we examined the γ-subunit forms expressed in the denervated adult soleus muscle. We used specific primers that bind to the region that encodes the N terminus of the subunit. Figure 1 shows DNA amplified using nested RT-PCR from mRNA isolated from denervated soleus muscles. Three bands can be seen: a 532-bp band, which is expected from the full-length sequences, and two extra bands of molecular size 418 and 278 bp, which correspond to the described γ2+γ3 and γ4 forms, respectively [10]. Thus, the denervated muscle, like neonatal muscle, seems to express several mRNAs corresponding to the γ-subunit.

Clone Analysis

To identify the γ-subunit forms expressed in denervated muscle, we cloned the amplified DNA into the pCR2.1 vector. Analysis with restriction enzymes and sequencing revealed the γ1–γ4 subtypes found in neonatal muscle. In addition, we found four novel forms named γ5–γ9 (Fig. 2). The form γ5 lacks exon 3, 4, and 63 bp of exon 5; γ6 lacks exon 4 and 89 bp of exon 5; γ7 lacks exon 4 and 136 bp of exon 5; γ8 lacks exon 3, 4, and 19 bp of exon 5; γ9 lacks exons 3, 4, and 5 (Fig. 3).

The γ5 and γ8 forms were almost of the same size. However, they gave a distinctive band pattern when digested with *Hae*II (not shown). A *Hae*II site at the beginning of exon 5 was present in γ8 but not in the γ5 form. Sequencing confirmed these observations.

As was the case for neonatal muscle, the different forms arise from alternative splicing, in particular in the region of exon 5. In most but not all cases, the spliced sequences correspond to an entire exon. Exonal junctions are known to contain GT in the acceptor side and an AG in the donor

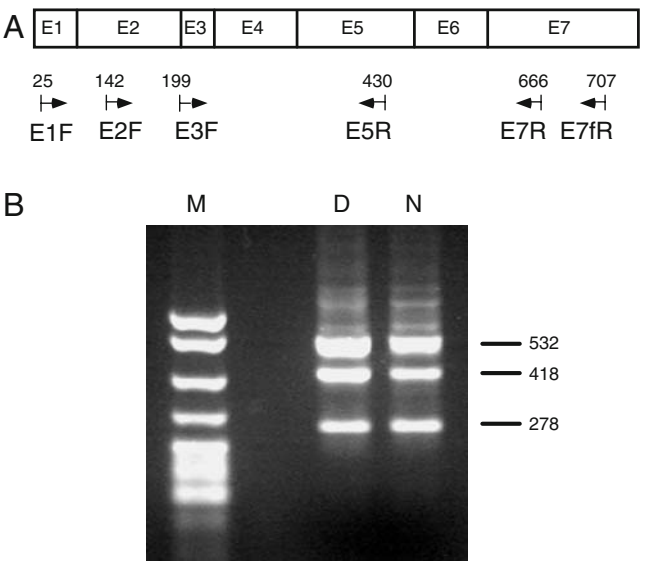

Fig. 1 PCR amplification of ACh receptor γ-subunit from denervated muscle. **a** Primers used for PCR amplification. **b** PCR products of the DNA from using nested primers E2F and E7R. *D* Denervated muscle, *N* nondenervated contralateral side. The *M lane* contains a pBR322 DNA digested with *Msp*I used as a molecular size marker

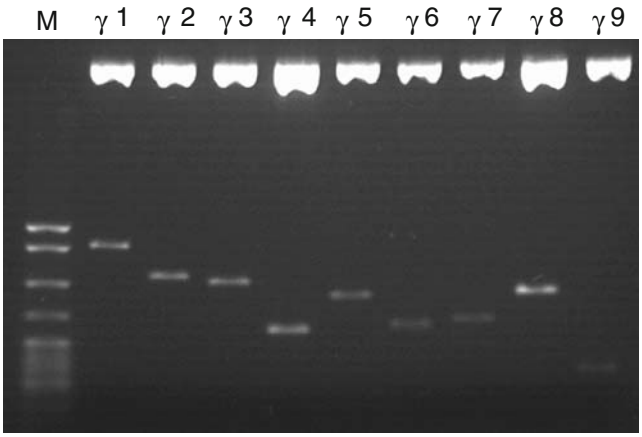

Fig. 2 Cloned γ-subunit forms. Digestion with *Eco*RI shows various fragment sizes separated in a polyacrylamide gel. The fragments shown carry 10 bp of vector sequences

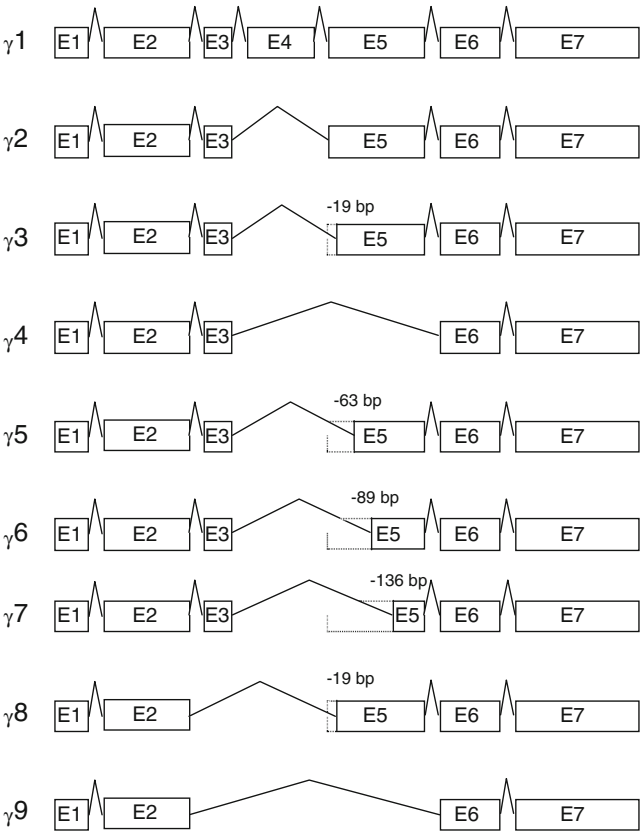

Fig. 3 Exonic composition of the γ-subunit variants. Each *box* represents the sequences of an exon. Notice that in the case of γ3, γ5, γ6, γ7, and γ8 only part of exon 5 is retained

side [13]. We examined the γ-subunit genomic sequence [8] to splice junction regions. Except for γ5 and γ6, all splice junctions have a GT in the acceptor side and an AG in the donor side. Examination of the next 12 nucleotides in the splice junction revealed four matches for the γ1 subunit and two to five matches for the other forms (Fig. 4). These observations indicate that the junctions are bona fide splice sites [11, 13].

Comparative Abundance

To compare the relative abundance of each clone, we excised the portion of a gel corresponding to molecular sizes shorter than 527 bp to exclude γ1. Then, we extracted the amplified DNA to clone it into the pCR2.1 vector for analysis. The result of examining 95 clones from denervated muscle is summarized in Table 1. The percentages reported correspond to the relative abundance of the γ2–γ9 subtypes. In the neonatal muscle, it was estimated that ~45% of the total forms corresponds to subtypes other than γ1 [10]. Table 1 shows that besides γ1, γ2 is the most abundant subtype in the denervated muscle, which corresponds to one third of the subtypes. The next most abundant form was γ7. In addition to the expected increase in γ-subunit mRNA, we also found a moderate increase in expression in the nondennervated muscle of the contralateral side of the body. Such an increase in expression has been described in other systems [14]. The most remarkable feature of the expression in the contralateral side is the large relative abundance of the γ7 form. Therefore, the γ7 form seems to be characteristic of the denervated muscle.

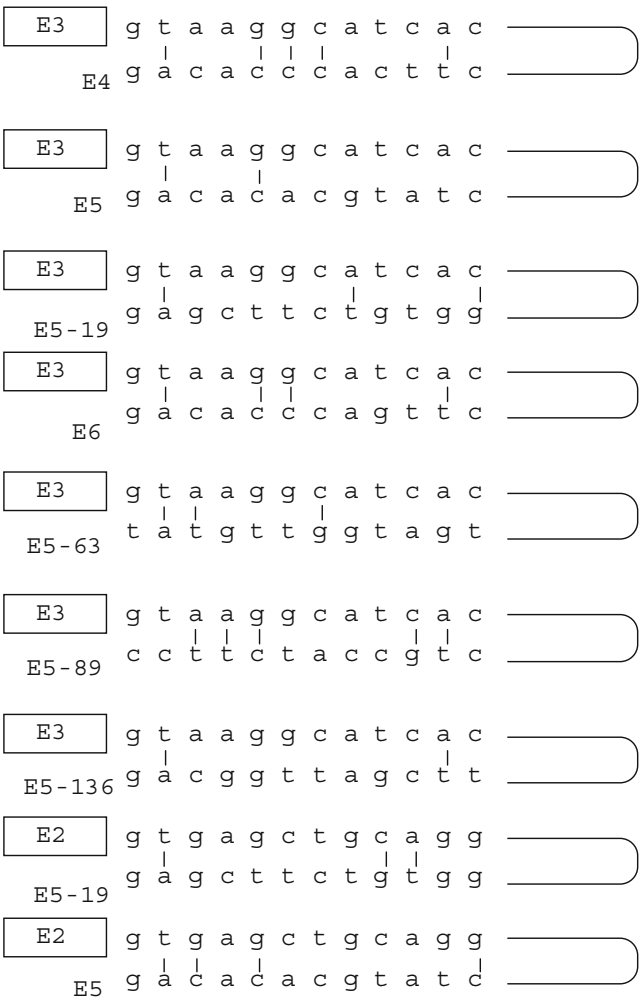

Fig. 4 Splice sites according to the γ-subunit genomic sequence [8]. The figure shows the putative splice junction containing the conserved GT…AG sequences. Except for γ8 and γ9, splicing occurs at the end of exon 3

Table 1 Repetoire of γ-subunit forms in devervated soleus muscle

	Ipsilateral (denervated)	Contralateral
γ2	32 (65%)	23 (50%)
γ3	3 (6%)	5 (11%)
γ4	2 (4%)	
γ5		2 (4%)
γ6	1 (2%)	
γ7	9 (18%)	15 (33%)
γ8	1 (2%)	
γ9	1 (2%)	1 (2%)

Comparison to Neonatal Muscle

Since we found new forms in adult dennervated muscle, we re-examined the repertoire on the neonatal muscle to see if the new forms were also present there. Table 2 shows the repertoire of subtypes γ2–γ9 determined using the same procedure as for the denervated muscle. All the other forms, except for γ5 and γ6, were present. In neonatal muscle, the most abundant form was γ2, confirming previous observations. The second most abundant was γ4 in the case of the female muscle and γ3 in the case of the male muscle.

Table 2 Repetoire of γ-subunit forms in neonatal muscle

	Female	Male
γ2	48 (69%)	27 (44%)
γ3	5 (7%)	15 (24%)
γ4	7 (10%)	9 (15%)
γ5		
γ6		
γ7	4 (6%)	9 (15%)
γ8	1 (1%)	
γ9	4 (6%)	1 (1%)

Comparison among Different Muscles

In addition to the soleus, we examined the expression of subtypes in the sternomastoid muscle. Neonatal sternomastoid muscle showed a low abundance of subtypes. Out of 121 clones, 113 belonged to the γ1 form (93%). In denervated sternomastoid, out of 80 clones, 73 belonged to the γ1 type (91%). In both cases, the sternomastoid muscle showed low abundance of γ-subunit subtypes in comparison to other muscles.

RNAse Protection Assay

Our identification of subunit subtypes and relative abundance relies on PCR analysis. An alternative method by the RNA protection assay was used to confirm the PCR results. As a probe, we used a 440 nucleotides antisense RNA corresponding to the γ2 form. This probe protects a 372-nucleotide fragment in the γ2 form. Assuming that the RNAse will digest the regions that do not match, it is expected that the probe will protect two fragments around the mismatch region. The sizes of the expected fragments are: 75 and 316, 297, 161, 253, 227, or 180 bp for the γ1, γ3, γ4, γ5, γ6, and γ7 forms, respectively. For the γ8 and γ9 forms, fragments of 30 and 297 or 161 bp, respectively, are expected. Using the cloned forms, we constructed sense RNA standards for the most abundant forms to verify the presence of expected fragments and to use as a reference for the analysis of mRNA from the muscle. Figure 5a shows the protected fragments for six of the standards.

The analysis of mRNA from denervated muscle is shown in Fig. 5b. Lanes R1–R3 contain RNA from the soleus muscle extracted at 7, 12, and 16 days after denervation, respectively. The lanes R1–R3 show a band of 345 bp consistent with the γ1- or γ2-protected fragment. In addition, there is a band of 160 bp consistent with the γ4- or γ9-protected fragment. Based on the clone abundance, this band is likely to be predominantly γ4.

Conclusions

We conclude that the denervated and the neonatal muscles express several mRNA forms encoding for the γ-subunit. The characteristic feature of the denervated muscle was the γ7 subunit.

Discussion

The results demonstrate that the denervated skeletal muscle contains nine mRNA forms encoding for the γ-subunit. Seven of these forms are also present in the neonatal muscle. Alternative splicing is the main mechanism

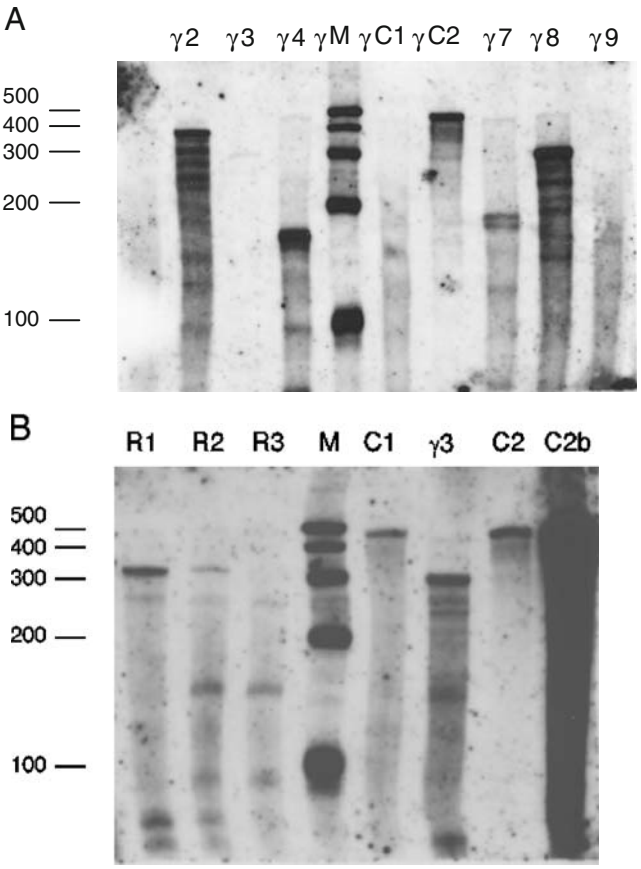

Fig. 5 RNA protection assay. **a** Standards showing expected protected fragment sizes. Control reactions with digested (*C1* 100% loaded) and undigested (*C2* 25% loaded) probes are also indicated. **b** The first three lanes containing bands of 345, 159, 84, and 64 bp are RNAs from the denervated muscle (*R1* and *R2* female, *R3* male) of 7, 12, and 16 days of denervation, respectively. Control reactions with digested (*C1* 100% loaded) and undigested (*C2*, 0.125%; and *C2b* 25% loaded) probe are also indicated. *C2* shows the position of the undigested probe

generating such diversity. From the forms described, four (γ1, γ3, γ7, and γ8) predict a complete subunit peptide lacking part of the N terminus. Five forms, if translated, will generate truncated peptides resulting from a stop codon.

The form γ3 lacks 43 amino acids from the N terminus: Q59–E101 (numbers refer to the mature peptide). The form γ7 lacks 82 amino acids: Q59–Q140. The form γ8 lacks 58 amino acids: N34–E101. Thus, among the three residues K34, S111, and F172, located in the γ–α interface, which are important for agonist binding [15], the residue S111 is missing in γ7. This residue belongs to region II, which is not conserved among different species, suggesting that it affects ligand selectivity rather than expression efficiency [15]. It is interesting to note that the three forms, γ3, γ7, and γ8, carry the N141 residue, which once glycosylated guarantee the proper insertion into the membrane [16]. Thus, all three forms are likely to be translated into protein

where they may form channels presumably with altered agonist affinity.

Based on the cloning data, the most abundant form, after the complete form, is the putative soluble form, γ2, which accounted for 33% of all mRNA encoding for the γ-subunit. The finding is unlikely a result of a cloning artifact since two forms differing in only 19 bp generate proportions of 33% and 6%, respectively. The possibility that large sequences are easily incorporated into the pCR®2.1 vector is unlikely. In addition, we have used specific primers to amplify the corresponding N-terminal region of the ε-subunit. We found two forms, the complete form and the splice variant lacking exon 5, which has been described in mice [15]. In the rat, the absence of exon 5 would preserve the reading frame. It is interesting to note that a γ-subunit lacking exon 5 has been found in the C2C12 cell line and in mouse muscle [6]. The γ-subunit splice variant lacking exon 5 seems to be exclusively expressed in mice, as was absent from neonatal as well as denervated rat muscle.

Splice variants have been described for other subunits of the ACh receptor. The ε-subunit of mice has a short form lacking exon 5 [17]. The α-subunit of human but not that of dog or monkey shows an interesting splice variant in which a sequence is inserted between exons 3 and 4 [18]. Such a long form of the α-subunit has been implicated in the regulation of the ACh receptor expression [19]. Two forms of the β-subunits have been identified by the RNA protection assay; they differ in 25 nucleotides [20]. The exonic composition is unknown.

The rich repertoire of splice variants found here provides molecular candidates for the fetal type channels found in neonatal and denervated muscle. Single-channel recording of neonatal endplates showed two kinetic classes of 10 and 27 ms, respectively [7]. The slow one was found only in the female muscle. The splice variants of the γ-subunit reported here might generate these kinetic classes. The γ-subunit repertoire of the neonatal muscle was found to be different from that of the denervated muscle where the variant γ7 was particularly abundant. Accordingly, neonatal channels had a somewhat faster kinetic with a mean burst duration of 7.4 ms.

The different kinetic classes are probably due to the different forms of the ACh receptor γ-subunit described here. In mice, a γ-subunit lacking the region encoded by exon 5 generates channels with long open time when expressed in *Xenopus* oocytes [6], indicating that truncated subunits are capable of generating functional receptors.

The forms γ2, γ4, γ5, γ6, and γ9 predict mature truncated peptides of 75, 79, 110, 93, and 64 amino acids, respectively. It is possible that these peptides are processed and secreted. Secreted forms of membrane receptors arising from alternative splicing are known. Examples include laminin [21] and acetylcholinesterse [22]. In the case of dystrophin, mutations in this gene result in mRNA with early stop signals. The consequence of this defect is a truncated dystrophin molecule, which can be detected by immunoblotting [23]. These examples indicate that the muscle might translate the truncated peptides of the γ-subunit.

Truncated subunits may also play a role in synaptic transmission. A peptide that resembles the N terminus of the α-subunit has been reported to be released from glial cells [24, 25]. This peptide contains the ACh-binding site and may bind ACh. It is plausible that some of the γ-subunit truncated forms, if secreted, generate a complete binding site.

If this is the case, one can speculate that this soluble peptide may act as a retrograde signal indicating that the γ-subunit is being transcribed. This signal could attract nerve endings eager to make synaptic contact with the muscle. Once the contact becomes established and the ε-subunit is induced, no other nerve ending will be attracted. This interpretation can explain reinnervation without involving a multiplicity of signaling pathways and molecules.

Future studies using the expression system would be needed to clarify whether some or all of the splice variants described here translate into functional proteins and form a functional ACh receptor pentamer. The expression of these forms in oocytes would clarify their functional properties.

Acknowledgments This work was partially supported by NSERC Canada (Grant 227298-02 to AV). We are grateful to Dr. P. Murphy for discussions and critical reading of the manuscript and to Ms J. Froom for expert technical assistance.

References

1. Mishina M, Takai T, Imoto K, Noda M, Takahashi T, Numa S, Methfessel C, Sakmann B (1986) Molecular distinction between fetal and adult forms of muscle acetylcholine receptor. Nature 321:406–411
2. Kues WA, Sakmann B, Witzemann V (1995) Differential expression patterns of five acetylcholine receptor subunit genes in rat muscle during development. Eur J Neurosci 7:1376–1385
3. Jaramillo F, Vicini S, Schuetze SM (1988) Embryonic acetylcholine receptors guarantee spontaneous contractions in rat developing muscle. Nature 335:66–68
4. Vernino S, Amador M, Luetje CW, Patrick J, Dani J (1992) Calcium modulation and high calcium permeability of neuronal nicotinic acetylcholine receptors. Neuron 8:127–134
5. Villarroel A, Sakmann B (1996) Calcium permeability increase of endplate channels in rat muscle during postnatal development. J Physiol (Lond) 496(2):331–338
6. Mileo AM, Monaco L, Palma E, Grassi F, Miledi R, Eusebi F (1995) Two forms of acetylcholine receptor g subunit in mouse muscle. Proc Natl Acad Sci USA 92:2686–2690
7. Villarroel A (2001) Sex differences in the acetylcholine receptor kinetics of postnatal and denervated rat muscle. J Physiol (Lond) 531:175–180

8. Herlitze S, Villarroel A, Witzemann V, Koenen M, Sakmann B (1996) Structural determinants of channel conductance in fetal and adult rat muscle acetylcholine receptors. J Physiol 492:775–787
9. Lopez J (1998) Alternative splicing of pre-mRNA: developmental consequences and Mechanisms of regulation. Annu Rev Genet 32:279–305
10. Villarroel A (1999) Alternative splicing generates multiple mRNA forms of the acetylcholine receptor γ-subunit in rat muscle. FEBS Lett 443:381–384
11. Wu SP, Romfo CM, Nilsen TW, Green MR (1999) Functional recognition of the 3′ splice site AG by the splicing factor $U2AF_{35}$. Nature 402:832–835
12. Melton DA, Krieg PA, Rebagliati MR, Maniatis T, Zinn K, Green MR (1984) Efficient in vitro synthesis of biologically active RNA and RNA hybridization probes from plasmids containing a SP6 promoter. Nucleic Acids Res 12:7035–7056
13. Moore MJ (2000) Intron recognition comes of AGe. Nat Struct Biol 7:14–16
14. Koltzenburg M, Wall PD, McMahon SB (1999) Does the right side know what the left is doing? Trends Neurosci 22(3):122–127
15. Sine SM, Kreienkamp HJ, Bren N, Maeda R, Taylor P (1995) Molecular dissection of subunit interfaces in the acetylcholine-receptor—identification of determinants of α-conotoxin m1 selectivity. Neuron 15:205–211
16. Gehle M, Sumikawa K (1991) Site-directed mutagenesis of the conserved N-glycosylation site on the nicotinic acetylcholine receptor subunits. Molec Brain Res 11:17–25
17. Koishi K, McLennan IS (1998) A murine specific splicing variant of the acetylcholine receptor epsilon-subunit gene transcript. Biochim Biophys Acta 1401(3):315–318
18. MacLennan C, Beeson D, Vincent A, Newsom-Davis J (1993) Human nicotinic acetylcholine receptor alpha-subunit isoforms: origins and expression. Nucleic Acids Res 21:5463–5467
19. Shieh BH, Ballivet M, Schmidt J (1987) Quantitation of an alpha subunit splicing intermediate: evidence for transcriptional activation in the control of acetylcholine receptor expression in denervated chick skeletal muscle. J Cell Biol 104:1337–1341
20. Goldman D, Tamai K (1989) Coordinate regulation of RNAs encoding two isoforms of the rat muscle nicotinic acetylcholine receptor beta-subunit. Nucleic Acids Res 17(8):3049–3056
21. Kamiguchi H, Hlavin ML, Yamasaki M, Lemmon V (1998) Adhesion molecules and inherited diseases of the nervous system. Annu Rev Neurosci 21:97–125
22. Aziz-Aloya B, Sternfeld M, Soreq H (1993) Promoter elements and alternative splicing in the human ACHE gene. Prog Brain Res 98:147–153
23. Engel AG, Yamamoto M, Fischbeck KH (1994) Dystrophinopathies. In: Engels AG, Franzini-Armstrong C (eds) Myology. vol. 2. McGraw-Hill, New York
24. Smit AB, Syed NI, Schaap D, van Minnen J, Klumpermank J, Kits KS, Lodder H, van der Schors RC, van Elk R, Sorgedrager B, Brejc K, Sixma TK, Geraerts WPM (2001) A glia-derived acetylcholine-binding protein that modulates synaptic transmission. Nature 411:261–268
25. Brejc KA, Dijk WJ, Klaassen RV, Schuurmans M, van der Oost J, Smit AB, Sixma TK (2001) Crystal structure of an ACh-binding protein reveals the ligand-binding domain of nicotinic receptors. Nature 411:269–276