Two Types of ACh Receptors Contribute to Fast Channel Gating on Mouse Skeletal Muscle

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Shepherd, Dawn and Paul Brehm. Two types of ACh receptors contribute to fast channel gating on mouse skeletal muscle. J. Neurophysiol. 78: 2966-2974, 1997. Single-channel recordings from mouse C2 myotubes indicate that maturation of skeletal muscle is accompanied by the appearance of two types of fast acetylcholine (ACh) receptor channels that are each functionally distinct from the embryonic receptor type present at early stages of differentiation. The embryonic receptor type has a low conductance (45 pS) and long channel open time, rendering slowly decaying synaptic currents. One fast channel type that appears during muscle maturation is distinguished from the embryonic receptor type on the basis of both higher conductance (65 pS) and shorter open time. However, single-channel recordings from differentiated mouse skeletal muscle cell line (C2) point to the existence of a slow receptor type, which has a conductance similar to the embryonic receptor type (45 pS), yet significantly reduced mean channel open time. Analyses of individual channel function at high ACh concentrations directly demonstrate the coexistence of two kinetically distinct types of 45 pS ACh receptors. Openings by fast type and slow embryonic type of 45 pS receptors occurred in bursts, allowing distinction on the basis of both mean open time and open probability for individual receptors. The embryonic type of 45 pS receptor has an open time approximately twofold longer than the fast-receptor counterpart. Additional differences were reflected in the open probability distributions for fast and slow 45 pS receptor types. Both types of 45 pS receptor were kinetically distinguishable from the 65 pS receptor. We found no support for the idea that the slow and fast 45 pS receptor types result from the interconversion of dual gating modes involving the same receptor protein. Our results are consistent with the idea that the acquisition of fast synaptic current decay, required at mature neuromuscular synapses, is the result of the up-regulation of two distinct fast types of nicotinic ACh receptors during skeletal muscle development.

INTRODUCTION

Much of the functional heterogeneity observed for nicotinic acetylcholine (ACh) receptors can be attributed to differences in receptor subunit composition. In the best characterized example, a low conductance ACh receptor channel with slow kinetics is replaced by a channel type with 50% higher conductance and appreciably faster gating during differentiation of skeletal muscle (Brehm et al. 1984a,b; Leonard et al. 1984; Siegelbaum et al. 1984). Several different approaches have shown that this difference results from substitution in the receptor of a γ-subunit by an ϵ (Camacho et al. 1993; Criado et al. 1990; Gu et al. 1990; Mishina et al. 1986; Shepherd and Brehm 1994; Witzemann et al. 1987). Additional ACh receptor channel types, identified on the basis of different conductances, are also hypothesized to result from altered subunit composition. A 15 pS ACh receptor channel is observed in developing Xenopus myotomal muscle (Owens and Kullberg 1989b) and likely corresponds to an αβγ receptor type lacking the δ-subunit (Kullberg et al. 1990). The recent discovery, in embryonic chicken skeletal muscle, of mRNA coding for α4, α5, α7, and β4 subunits of the neuronal nicotinic receptor types (Corriveau et al. 1995) raises the possibility that functional diversity may also be achieved by hybrid receptors composed of muscle and neuronal type subunits.

Identification of different channel types is generally facilitated by the observed differences in single-channel conductance. However, some single-channel studies have suggested the presence of distinct types of ACh receptors that exhibit similar conductance but different gating. For example, the mean open time of the 45 pS ACh receptor channel decreases abruptly during development of Xenopus skeletal muscle (Leonard et al. 1984; Owens and Kullberg 1989a; Rohrbough and Kidokoro 1990) and during differentiation of mouse myotubes (Shepherd and Brehm 1994). The structural basis for a change in gating without a change in conductance has not been established. One possible explanation is provided by the observation that individual 45 pS ACh receptors exhibit transitions between a slow and fast gating mode in the absence of any change in conductance. Each mode can be identified on the basis of a characteristic mean open time and open probability (Auerbach and Lingle 1986; Naranjo and Brehm 1993). These observations raise the possibility that the developmental decrease in mean open time for the 45 pS channel type results from stabilization of the fast gating mode and a corresponding destabilization of the slow gating mode. An alternative possibility is that an altogether functionally distinct type of 45 pS ACh receptor appears during development of muscle. The experiments in this study were designed to explore aspects of heterogeneity in ACh receptor function observed after long-term culture of the mouse skeletal muscle cell line (C2). The cell line was found to recapitulate many of the time-dependent changes in receptor properties found during skeletal muscle development in vivo, including the appearance of 65 pS channels and the decrease in open time of 45 pS channels.

METHODS

Cell culture

Mouse C2 myoblasts were grown on collagen-coated tissue culture plates, in a Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum, 0.1 mM sodium pyruvate, 50 U/ml penicillin, and 50 μg/ml streptomycin. All tissue culture reagents were obtained from GIBCO BRL, Grand Island,
NY. To promote myoblast fusion, 10% calf serum was substituted for fetal bovine serum once the myoblasts were ~30% confluent. Cells were maintained in a humidified 5%-CO₂ atmosphere at 37°C. Once significant myoblast fusion had occurred, at ~70% confluence, β-d-arabinofuranoside was added to the culture medium at a concentration of 0.5 mg/ml to inhibit further cell proliferation. The culture medium was replenished every two days during the remaining period of culture.

Electrophysiology

Single-channel recordings were made from cell-attached patches of myotubes after a 4–15 day period of cell fusion. The extracellular recording solution contained (in mM) 120 KMeSO₄, 20 KCl, 1 NaCl, 10 N-2-hydroxyethylpiperazine-N'2-ethanesulfonic acid (HEPES), and 1 MnCl₂ (pH 7.4). Under these recording conditions, the resting membrane potential approximated 0 mV, minimizing the differences in channel kinetics that might result from membrane potential variability between cells. Patch electrodes were pulled from thick-walled borosilicate glass, and fire polished to a final tip diameter of ~2 µm. Pipettes were filled with an ACh-containing solution (0.1–50 µM) that contained (in mM) 140 NaCl, 1 KCl, 10 HEPES, and 1 CaCl₂ (pH 7.4), before being coated with Sigmacote (Sigma).

All recordings were made at 18–20°C by using an EPC-7 patch-clamp amplifier (List Medical Systems). Single-channel records were digitized at 20 kHz by using an ITC-16 A/D converter and Bessel filtered at 4 kHz before analysis. Single-channel currents were analyzed on an Atari computer using the TAC analysis program (Instrutech). A threshold corresponding to one-half amplitude was set for event detection, with the minimum duration for full resolution of events corresponding to ~90 µs. Infrequent openings by ACh receptors with characteristic single-channel amplitudes below those of the 45 pS channel types were not analyzed in this study (Shepherd and Brehm 1994). For events briefier than 90 µs, open time distributions were corrected according to the method described by Colquhoun and Sigworth (1983). This method utilizes the characteristics of actual filter bandwidth. Thus providing, in our case, theoretical corrections of open times for detected events as brief as 18 µs. In practice, however, we excluded all events briefier than 40 µs from the histograms before fitting. For construction of duration histograms, data containing superimposed openings were excluded. Amplitude histograms were fit by the sum of two Gaussian distributions and estimates of slope conductances for each amplitude class were obtained by linear-regression fit of the current-voltage relations. Single-channel reversal potentials were estimated by extrapolation of the current-voltage relations to zero current level, assuming no channel rectification.

To analyze channel kinetics, continuous recordings were performed either at low (0.1–0.3 µM) or at desensitizing (10–50 µM) ACh concentrations. To obtain time constants for channel opening and closing, frequency-duration histograms were fit by using log-likelihood methods (Sigworth and Sine 1987). The minimum number of exponential components required to fit the histograms was established by two criteria. First, because of the display provided by log-duration histograms, visual inspection generally indicated the obvious need for additional exponential components. Second, the reproducibility of time constants and areas from one patch to another further defined the minimum number of components necessary for systematic characterization. The presence of unnecessary components was signaled by inconsistencies in time constants and by the convergence of time constants corresponding to individual components. At low ACh concentrations kinetic analysis was restricted to fitting of log-open time histograms. At high ACh concentrations the channel openings occurred in bursts, reflecting consecutive openings by individual receptors. Kinetic analysis of individual receptors was made by measurements of open probability and by fitting of open duration and closed duration histograms measured within individual bursts. All kinetic analyses were performed at a pipette potential of 100 mV and data are presented as means ± SD. Statistical determinations were made with the use of the two-sample t-test with the null hypothesis that each population mean under test is the same. In all cases where differences are indicated as being significant, the P value is >0.25, which is taken to mean that the null hypothesis does not hold and that the means are significantly different.

RESULTS

Contrasting kinetics for two amplitude classes of receptor

Myotubes that have undergone differentiation for periods of 6–17 days in cell culture express two amplitude classes of ACh-activated single-channel currents corresponding to approximately ~4.7 and ~6.8 pA at ~100 mV (Fig. 1). Over a range of potentials from ~60 to ~120 mV, the current-voltage relations were reasonably linear allowing estimates of slope conductance and reversal potential to be made for the two amplitude classes (data not shown). The slope conductances measured 45 ± 3 (SD) pS (n = 7) for the smaller amplitude and 65 ± 4 pS (n = 7) for the larger amplitude channel class. No difference in average extrapolated reversal potential (~5 mV) was observed for the two amplitude classes.

Openings by the 65 pS class of channels were markedly briefer, on average, than those by the 45 pS type. At low concentrations of ACh (0.1–0.3 µM), this difference was reflected in the distribution of open times for the two amplitude classes (Fig. 1). The fitting of exponential components to open times histograms for the 65 pS channel class required the sum of slow and fast exponential components (Fig. 1B and Table 1). Channel openings of the 45 pS class were much more variable in duration than those of the 65 pS class. Some patches were characterized by frequent long-duration openings (Fig. 1A) and others lacked such openings (Fig. 1C). Long-duration openings by 45 pS channels were most frequently observed in recordings made from myotubes that had been maintained in culture for ~9 days (Shepherd and Brehm 1994). The presence of long-duration openings at early stages of differentiation resulted in widely varying time constants measured for the slowest component of the open duration histograms for overall openings by 45 pS channels. The distribution of lowest time constants for all 45 pS patches (Fig. 2A) ranged from 3 to 19 ms, reflecting greater variability than seen for the 65 pS channel type (Fig. 2B).

After nine days in culture, a marked decrease in the frequency of long-duration openings by the 45 pS channel class was observed. To further investigate this change in kinetics, 45 pS patches were separated into two groups, fast and slow, on the basis of the slowest time constant of their fitted open time histograms. Patches were considered to be fast when the slowest time constant was ~8 ms. This cutoff was selected by identifying a minimum value in the distribution of slowest time constants measured for 45 pS channel patches at all stages of development (Fig. 2A). The fast patches represented myotubes that had been differentiated for over nine days in culture, whereas the slow patches represented myotubes cultured for shorter periods (Shepherd and Brehm 1994).
Comparison of open time histograms constructed for such slow and fast patches revealed qualitative differences in the distributions (Fig. 1). Analysis of 13 fast patches over the low ACh concentration range (0.1–0.3 μM) indicated that the distribution of open times could be fit by a single exponential distribution with an average time constant of 6.5 ms (Table 1). An additional eight patches identified as fast required an additional exponential component for proper fit. The time constant of this component averaged 0.3 ms and contributed an average of 18% of the total area.

By contrast, fitting of the open time histograms for slow patches typically required multiple exponential components. A very slow component corresponding to ~14 ms was present in all 27 slow patches analyzed (Table 1). This component was significantly different from both time constants measured for the fast 45 pS receptor. However, 17 of the patches required an additional brief component that averaged 0.4 ms. This time constant was not significantly different from that measured for the fast channel type. An additional intermediate component was required for proper fit in eight of the patches. The average time constant of this component was 3.9 ms and this component was also significantly different from all of the other time constants measured for both slow and fast channels.

Clustered openings by individual channels can distinguish two channel types

To test the idea that the observed differences in open time between fast and slow patches reflect two kinetically distinct types of 45 pS channel, data were collected with desensitizing concentrations of ACh (20 μM). At high concentrations of ACh, openings by both 65 pS and 45 pS channel types occurred in discrete bursts (Figs. 3 and 5). Each burst of openings is thought to represent the repetitive reopenings by an individual channel protein as it undergoes transitions into and out of the desensitized state (Sakmann et al. 1980). Therefore analysis of intraburst kinetics by either 65 or 45 pS channels is likely to reflect the behavior of an individual receptor.

To separate intraburst and interburst kinetics of channel openings for individual patch recordings, closed interval distributions were separately constructed for 65 pS channel and 45 pS channel openings. Figure 3 shows representative closed interval distributions for the 65 pS channel type that required the sum of six exponential components for proper fitting. The two longest time constants were assumed to represent closed periods between bursts and the briefer time constants representing closures occurring within bursts (Sine and Steinbach 1987). The major closed time constant for 65 pS channel events approximated 3 ms, which represented the principal closed interval within a burst.

To simplify the analysis of openings by the 45 pS channels, separate measurements were once again made for fast and slow patches. This approach provided an enriched population of either fast or slow channels, depending on the age of the cultured myotube. To distinguish between fast and slow patches overall open time histograms for 45 pS channel openings were constructed for each patch and the time constant of the slow component was measured, as previously

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<th>TABLE 1. Kinetic distinctions for three channel classes</th>
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<td>65 pS channel</td>
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<td>Low [ACh]</td>
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<tr>
<td>Fast 45 pS channel</td>
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<td>Low [ACh]</td>
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ing 6 ms. This was reflected in the recordings as longer gaps separating shorter duration events (Figs. 3 and 5).

Comparison of closed time histograms for 65 pS and all 45 pS channels suggested that a common $\tau_{\text{crit}}$ of 20 ms (Fig. 3, arrow) could be assigned as a conservative value to define and separate clustered openings by a single channel. Closures of a longer duration than 20 ms would therefore signal the end of an individual burst, allowing consecutive openings by a single receptor to be isolated.

Comparisons of individual bursts of 45 pS channel openings between fast and slow patches showed clear differences in mean open time values. The distribution of mean channel open times measured for individual bursts present in a single slow patch shown in Fig. 5 ranged from 3 to 14 ms and exhibited an overall mean value of \~10 ms. By contrast, the distribution of mean burst open times for a single fast patch shown in Fig. 5 ranged from 1 to 7 ms and exhibited an overall mean open time of 5 ms. Both values corresponded well to the slow components of the open time distributions obtained for slow and fast patches respectively at low ACh concentrations (Table 1). The overall individual mean burst open time for 23 different patches are shown in Fig. 6. The mean burst open time for all 45 pS patches ranged from 3 to 22 ms with an average value of \~12 ms. These values were also similar to those obtained from longest time constants derived from all 45 pS channel openings measured at low ACh concentration (Table 1).

Unlike the findings for 45 pS channels, measurements of open times during individual bursts for 65 pS channels described (Fig. 2). Patches with a slow time constant <8 ms were considered fast (as established for low ACh concentration) and those longer than 8 ms were considered slow. The open time histograms for fast and slow patch recordings showed the same trends as observed for low ACh concentration measurements (Fig. 4). The histograms for seven fast 45 pS channel patches were all fit by a single exponential component with a mean time constant of 6.5 ms (Table 1). The open time histograms for the slow 45 pS channel patches were also simplified by the absence of a brief exponential component to the distribution (Fig. 4). Of the 65 slow patches examined, 50 yielded distributions that were fit by a single long component averaging 14.7 ms. The remaining 15 patches required a second component with an average time constant of 3.4 ms that was similar to the intermediate time constant measured at low ACh concentration (Fig. 4).

Closed time histograms for fast and slow 45 pS channel patches indicated large differences. The major time constant for the slow 45 pS patch closed time histograms averaged 0.8 ms. This brief component reflected a large number of short-duration, partially resolved closures that interrupt long-duration openings (Figs. 3 and 5). By contrast, the major time constant for the fast 45 pS patches was longer, average-
indicated that little variability existed in recordings made from slow and fast patches (Table 1). In such cases the slow and fast patches were defined on the basis of 45 pS channel open time (Fig. 2). For 65 pS channel openings, the distribution of mean burst open times measured for a single patch was 1–4 ms with an average of 3 ms (Fig. 5). These values were similar to the overall range of mean burst open time measured for all patches (data not shown) and were comparable to the slowest component of open time histograms measured at low ACh concentrations for this channel type (Table 1).

Clearer distinction between slow and fast 45 pS channel types was obtained from measurements of open probability within individual bursts. The open probability distributions for slow 45 pS bursts had a dominant peak that ranged from 0.63 to 0.98. This high open probability reflects the long openings and short closures observed within bursts, examples of which are shown in Fig. 5. In addition to the major peak, the open probability distributions for slow 45 pS patches occasionally showed an additional component between 0.1 and 0.3 (Figs. 5A and 7A). By contrast, the open probability for bursts in the fast 45 pS patch shown in Fig. 5.

**FIG. 4.** Representative open time histograms for 3 different patches recorded at high ACh concentrations (20 μM). Histogram for 65 pS receptor (left) and fast 45 pS receptor (right) were fit to 1 exponential; slow 45 pS receptor histogram was fit to sum of 2 exponentials. Corresponding time constants for each histogram are indicated.

**FIG. 5.** Kinetics of slow 45 pS (top), fast 45 pS (middle), and 65 pS (bottom) receptors during application of 20 μM ACh. Representative bursts of single-channel activity (left) are shown for each patch along with frequency histograms for burst mean open times and burst open probability.
Slow 45 pS channels exhibit infrequent transitions to a fast gating mode

Additional heterogeneity was observed in the kinetics of the 45 pS channel class. Infrequently, measurements of bursts in slow patches suggested a second component in the open probability distribution (Figs. 5 and 7A). This component was too minor to measure accurately, but generally corresponded to a value of 0.1–0.3, which was in the range also measured for the 65 pS channel type. This component corresponded to very brief openings of channels within certain bursts and was only observed in slow patches. Such transitions were not apparent for the fast 45 pS channel. Transitions between high and low probability modes were observed within single bursts of slow 45 pS channel openings. In such cases no openings to a second level were observed, supporting the idea that these transitions occurred within a single receptor-channel. Such a transition between the high and low open probability modes is illustrated in Fig. 7A.

The converse transition, from low to high open probability, was observed for the 65 pS channel (Fig. 7B), but such transitions were so infrequent that they rarely appeared in plots of open probability. However, this observation raises the possibility that both slow 45 and 65 pS types may be capable of switching between shared modes of gating as previously suggested by studies on mammalian ACh receptors expressed in Xenopus oocytes (Naranjo and Brehm 1993).

DISCUSSION

Many studies have noted variability in the open-state and shut-state kinetics of skeletal muscle nicotinic ACh receptors (for review see Steinbach 1989). In particular, the kinetics of the lower conductance channel type, corresponding to the 45 pS channel in this study, have been noted to vary widely. This variability is reflected in the channel open and closed time distributions measured for receptors on different cell types (Auerbach and Lingle 1986; Brehm et al. 1988; Gibb et al. 1990; Igusa and Kidodoro 1987), within the same cell (Young and Poo 1983) and even within individual patches (Auerbach and Lingle 1986; Naranjo and Brehm 1993; Sine and Steinbach 1987). The complexity in kinetics of the 45 pS channel type is further reflected in the requirement, typically, of three exponential components for fit of the open-state lifetime distributions. One or two exponentials are adequate to fit the distributions for the 65 pS channel (Steinbach 1989). The additional exponential components required for fit of the 45 pS open state lifetime distributions reflect a greater variability in the time constants of the longest component, when compared with the 65 pS time constants (see Fig. 2). This difference exists in spite of the fact that 45 and 65 pS channel types are each thought to reflect a single receptor type corresponding to αβγ and αβδ, respectively.

Our findings with ACh receptors on C2 muscle demonstrate that a major source of variability in overall 45 pS channel kinetics is the presence of kinetically distinct channel types within this conductance class of the receptor. Two lines of evidence point to the existence of distinct fast and slow 45 pS channel types: 1) a switch from long- to short-
The major component of the histogram had a mean time constant of 6 ms, significantly shorter than the 14 ms time constant seen at earlier stages of development.

The second line of evidence for two functionally distinct 45 pS channel types is provided by analysis of individual ACh receptors within patches. Under desensitizing conditions the bursts of ACh receptor openings are likely to reflect the transient recovery of individual receptors from the desensitized state (Sakmann et al. 1980), a behavior that allows for comparison of the kinetics of individual receptors within a patch (Auerbach and Lingle 1986; Sine and Steinbach 1987). Slow patches were characterized by bursts that adopted either of two kinetic patterns. The predominant burst pattern for 45 pS channels was composed of long-duration events that contained frequent unresolved closures. The second and less frequently observed burst pattern was characterized by a series of very brief events interspersed with relatively long-duration closures. This kinetic pattern in slow patches consistently resulted in bimodal burst open probability distributions with a major peak at ~0.9 and a minor peak at ~0.3, similar to that previously described for the low conductance channel type in embryonic Xenopus myotomal muscle (Auerbach and Lingle 1986). This behavior reflects, in large part, the transition of the slow channel type between gating modes (Auerbach and Lingle 1986; Naranjo and Brehm 1993). During an individual burst, transitions from the slow mode to a faster mode can be observed, supporting the idea that the receptor can adopt one of two kinetically distinct gating modes. The slower mode corresponds to the high open probability bursts and the less frequently observed faster mode accounts for the low open probability bursts.

A third distinct burst pattern for 45 pS channels, which differed from either burst pattern seen in slow patches, was observed on fast patches from C2 muscle cells that had differentiated longer than eight days. Analysis of such patches revealed a bursting pattern that was characterized by the total absence of the long-duration openings observed for slow patches. Fast patches exhibited shorter mean open time (averaging 5 ms) during the burst and a single open probability distribution with a mean corresponding to 0.6. Both the open time and open probabilities in such patches were consistently intermediate to those observed for the slow channel bursts. Thus the openings by this fast channel do not correspond to either the fast or slow gating mode seen in slow 45 pS channels and reflect a distinct type of opening by a 45 pS channel.

The coexistence of two kinetically distinct channel types with similar conductances renders separation within a single patch very difficult. Only at advanced stages of muscle development, where slow channel kinetics are less frequently observed, do the overall kinetics appear reasonably simplified. The coexistence of multiple 45 pS channel types in muscle with differing open state lifetimes was originally proposed by Jackson et al. (1983). This proposal was based on the observation that successive openings by ACh receptors on muscle are correlated in channel open time. Subsequent studies have supported these earlier observations by demonstrating that short closed intervals are correlated with successive long-duration openings and long intervals correlated with successive short-duration openings (Colquhoun et al. 1983).

**FIG. 7.** Single-channel ACh receptor channels exhibiting abrupt shifts in kinetics. *A:* 3 examples of bursting activity by individual 45 pS ACh receptor channels are shown in response to 20-μM ACh. *Top:* slow modal 45 pS activity. *Middle:* less prominent fast modal 45 pS channel activity. *Bottom:* mode shift from slow to fast activity during a single burst of activity. Distribution of mean burst open probabilities is shown for all bursts recorded from the patch. *B:* 4 nonconsecutive current traces showing different kinetic modes of 65 pS channel. *Top:* high and low open probability modes. *Bottom:* transitions between high open probability mode and low open probability mode. Occurrence of high open probability modes are infrequently observed for this channel type.
and Sakmann 1985; Igusa and Kidokoro 1987; Sine and Steinbach 1987). A second line of independent evidence pointing to the existence of two kinetically distinct types of the 45 pS channel was provided by studies on developing Xenopus (Carlson and Leonard 1989; Leonard et al. 1984; Owens and Kullberg 1989a; Rohrbough and Kidokoro 1990) and mouse (Shepherd and Brehm 1994; Steele and Steinbach 1986) skeletal muscle. In both preparations a progressive change in the 45 pS channel kinetics was observed during maturation such that the openings by 45 pS channels decreased significantly in average duration.

The basis for differences in fast and slow 45 pS channel function is still unclear. Expression studies on cDNAs encoding nicotinic ACh receptor subunits have shown that altered subunit composition leads to altered function. For example, expression of αβδε receptors reproduces the conductance and simplified open lifetime distributions observed for the 65 pS channel type in skeletal muscle. Similarly, the expression of αβδγ receptors in Xenopus oocytes results in mixed kinetics, similar to those described here for the 45 pS channel function seen in newly differentiated skeletal muscle. Some of the functional variability after expression of cDNAs coding for αβδγ subunits has been shown to arise from alternative subunit combinations such as αβδ or αγδ that are expressed in the presence of all four subunits (Charnet et al. 1992; Jackson et al. 1990; Kullberg et al. 1990; Kurosaki et al. 1987; Liu and Brehm 1993; Lo et al. 1990). In fact αβδ receptors exhibit a conductance only slightly lower than αβδγ receptors, but with a longer open time (Liu and Brehm 1993). Additional structural combinations that may result in 45 pS channels are suggested by the fact that αβδε receptors, in addition to giving rise to 65 pS channels, also express a lower conductance channel type with fast kinetics (Camacho 1993; Gu et al. 1990). Finally, additional endogenous ACh receptor subunits have also been identified in both Xenopus oocytes (Buller and White 1990; Hartman and Claudio 1990) and in developing skeletal muscle (Corriveau et al. 1995; Mileo et al. 1995), which may account for functionally distinct types of ACh receptors in muscle. An alternative form of the γ subunit, lacking 52 N-terminal amino acids, has recently been identified in developing mammalian muscle and in C2 muscle cells (Mileo et al. 1995). Expression of a cDNA encoding this newly described γ short-loop subunit leads to open times that are significantly longer than the previously identified mammalian gamma subunit.

Indirect evidence exists for the idea that ACh receptor channel kinetics can be altered through mechanisms other than subunit composition. Single-channel studies suggested that modulatory factors such as glycosylation, phosphorylation (Covarrubias et al. 1989; Eusebi et al. 1987; Zani et al. 1986), or membrane environment (Gibb et al. 1990; Lo et al. 1990; Young and Poo 1983) may contribute to ACh receptor kinetics through posttranslational alterations. Specifically, in the case of the 45 pS receptor, the change in mean channel open time during skeletal muscle development (Carlson and Leonard 1989) and denervation that follows (Rozental 1991) was proposed to occur via posttranslational modification. In both studies the developmental change in kinetics for the 45 pS channel type occurred in the absence of protein synthesis. Regardless of mechanisms underlying the functional distinctions, our studies indicate that two functionally distinct receptor types with similar conductances are likely to coexist. Together with the appearance of the higher conductance 65 pS channel, this shift toward fast 45 pS channel function during development contributes to the fast synaptic kinetics characteristic of mature neuromuscular synapses.

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