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Myasthenic serum selectively blocks acetylcholine receptors with long channel open times at developing rat endplates

(synapse formation/channel kinetics/immunology/noise analysis/miniature endplate currents)

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ABSTRACT **We have examined the physiological effects of antibodies from a highly specific myasthenic serum on acetylcholine receptors at developing rat endplates. The antibodies reduced the amplitude of miniature endplate potentials by 60% in 3- to 6-day-old animals but had no effect after day 14. Between days 7 and 12 the antibodies had an intermediate effect. This is the same period during which acetylcholine receptors with long channel open times (slow channels) disappear and receptors with short open times (fast channels) increase in number. Therefore, we examined the effect of the antibodies at endplates with a mixture of channel types more carefully. At all times tested, both noise analysis and analysis of miniature endplate currents showed that the antibodies reduced slow channel activity selectively. Single-channel recordings indicated that acetylcholine receptors that remained active after antibody treatment had normal gating properties. Thus, the antibodies appeared to silence slow channels selectively.**

Acetylcholine receptors (AcChoRs) change in several ways during formation of the skeletal neuromuscular junction (reviewed in ref. 1). At developing rat endplates, a striking functional change occurs postnatally: the apparent mean channel open time decreases by a factor of 3–5, and the single-channel conductance increases by about 50%. In rat soleus (20) and diaphragm (3) muscles, these changes in gating properties occur over a 2-week period beginning several days after birth. The transition at any individual endplate probably occurs within a few days (3, 4). During the transition, individual endplates contain a mixture of slow, embryonic-like channels and fast, adult-like channels.

Several molecular properties of the AcChoR also change during muscle endplate development (reviewed in ref. 1). One of the molecular changes is detected by a class of antibodies (Abs) found in the serum of most patients with myasthenia gravis. These Abs recognize one or more determinants that are present on AcChoRs in the extrajunctional membrane of embryonic muscle but are not found on AcChoRs at adult endplates (5). Recently, a myasthenic serum that is highly specific for the embryonic type of AcChoR has been used to characterize AcChoRs at developing endplates (6). AcChoRs at endplates of newborn, but not adult, rats bind Abs in this serum. Endplate AcChoRs lose their reactivity to the Abs during the second and third postnatal weeks (7).

The parallel in time course of the loss of immunoreactivity and the transition in channel properties suggests that Abs in this serum might bind specifically to AcChoRs with slow, embryonic-like channels. Because these Abs block AcChoR function (21), we have been able to test this hypothesis explicitly by examining the physiological effects of the myasthenic serum at developing endplates that contain a mixture of channel types. We report here that at all times tested, the serum appeared to block the slow channels selectively. This suggests that Abs in this serum detect a structural change in the AcChoR that is responsible for the transition in channel properties.

MATERIALS AND METHODS

Myasthenic Abs. The Abs used in this study have been described elsewhere (6, 7). They were derived from a serum obtained at plasmapheresis by Peter Dau at Children's Hospital in San Francisco in February 1978. The 40% ammonium sulfate precipitate from this serum, after storage for 3 years, was dialyzed against 0.02 M ammonium bicarbonate, lyophilized, and stored at −20°C. For the experiments described here, the lyophilized protein was taken up in L-15 tissue culture medium (GIBCO) at 6.67 mg/ml, titrated to pH 7.4, and frozen in small aliquots at −80°C until further use. In control experiments, human IgG (Sigma) was used instead of the myasthenic Abs. The nonspecific IgG was prepared and stored in the same way as the lyophilized myasthenic protein.

Electrophysiology. One or both soleus muscles were dissected from Sprague–Dawley rats (Charles River Breeding Laboratories) between 1 and 22 days after birth. The muscles were pinned to a layer of cured Sylgard resin, bathed in L-15 medium, and placed on the stage of a Zeiss UEM microscope. When viewed at ×500 with differential interference contrast optics, endplates were visible and individual fibers could be distinguished from their neighbors. This permitted us to study the same endplates before and after treatment with Abs. All experiments were performed at 19–23°C.

Intracellular recording was performed by using a WPI-707 electrometer connected to the analog-to-digital converter of a DEC LSI-11/23 computer. Twenty to 30 miniature endplate potentials (MEPPs) were recorded from each of several endplates in each muscle, and the peak MEPP amplitudes were determined automatically. The resting potentials (*ca.* −70 mV) were also recorded. In some of the younger muscles, the MEPP frequency was increased by raising the osmolarity of the L-15 medium by 0.2 M with dextrose. The muscle was photographed, and the location of each intracellular recording site was marked on the Polaroid print.

After the initial series of recordings, the medium was removed and the muscle was incubated for 3 hr at room temperature in 100 μl of the Ab (or control IgG) solution described above (6.67 mg/ml in L-15 medium). Then the preparation was washed with L-15 medium and returned to the microscope stage. Each of the recording sites that had been studied initially was relocated, and 20–30 MEPPs were again recorded and analyzed as described above. We estimated

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Abbreviations: Ab, antibody; AcCho, acetylcholine; AcChoR, AcCho receptor; MEPC, miniature endplate current; MEPP, miniature endplate potential.

that the initial and final electrode penetrations in each fiber were at most 10 μm apart, thus minimizing differences in MEPP amplitudes due to different locations of the recording pipette. Variations in MEPP amplitude due to fluctuations in the resting potential were compensated by scaling each MEPP by the ratio of a standard resting potential (−70 mV) to the measured resting potential. This normalization procedure assumes a reversal potential of 0 mV and a constant input resistance.

Miniature endplate currents (MEPCs) and AcCho-induced current fluctuations were recorded extracellularly as described (8, 9), except that in each case the electrode was connected to a List LM EPC-5 patch-clamp amplifier rather than to a voltage amplifier. MEPCs or noise records were obtained from the same endplates before and after the preparation was incubated with Ab or control IgG. The analyses of the MEPCs and noise spectra will be described in detail elsewhere (20).

In a few experiments, single-channel records were obtained from perijunctional membrane patches after Ab treatment. These patch-clamp experiments were performed by using conventional techniques (10), except that the fibers were cleaned manually with a glass probe rather than with enzymes. The patch pipette contained 0.1–0.5 μM AcCho in L-15 medium or in rat Ringer solution (made up as in ref. 11).

RESULTS

Myasthenic Abs Reduce MEPP Amplitudes at Young Endplates. The myasthenic serum used in these experiments contains Abs that bind to AcChoRs in neonatal, but not mature, rat endplates (7). To see if Ab binding is associated with any change in AcChoR function, we recorded the amplitudes of spontaneous MEPPs from the same endplates before and after incubation with Ab. We did not study the effects of Abs on evoked endplate potentials because this would have required taking measures to reduce their amplitude to a subthreshold level, thus complicating the experiments.

We found that the Abs reduced the mean MEPP amplitude at postnatal day 6 but not at day 19 (Fig. 1). At the day 6 endplate, the mean MEPP amplitude (±SEM) fell from 6.4 ± 0.4 mV to 2.1 ± 0.2 mV after exposure to the Abs, while at the day 19 endplate the MEPPs were virtually unchanged (0.8 ± 0.1 mV before Abs; 0.9 ± 0.1 mV after Abs). The difference in MEPP amplitude at the two endplates *before* Ab treatment probably reflects both the lower input resistance of the larger day 19 fiber and the shorter channel open times of its AcChoRs (2, 3).

The Ab-mediated reduction in MEPP amplitude was largest at young (3–6 days) endplates (Fig. 2). The mean MEPP amplitude decreased an average of 60% ± 3% (mean ± SEM; n = 16 cells) following Ab incubation. This did not appear to be caused by nonspecific deterioration of the fibers. Following Ab incubation, the resting potentials were not changed (*ca.* −70 mV) and the fibers appeared healthy

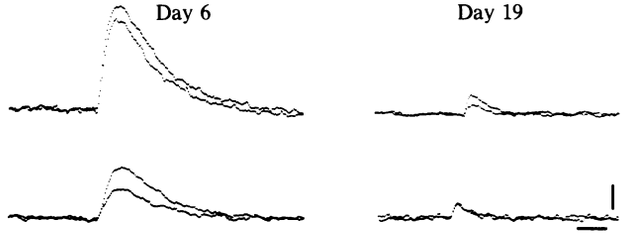

FIG. 1. Spontaneous MEPPs recorded from the same day 6 and day 19 soleus endplates before (*Upper*) and after (*Lower*) the muscles were incubated with Ab. The spontaneously occurring events have been aligned for illustration. Calibration bars = 2 mV, 10 msec.

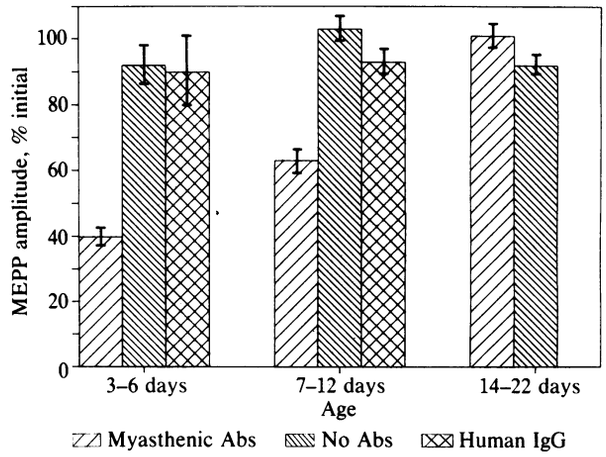

FIG. 2. Relative amplitudes of MEPPs recorded from the same endplates before and after incubation in L-15 medium plus myasthenic Ab, in L-15 medium alone, or in L-15 medium plus normal human IgG. Twenty to 30 MEPPs were recorded from each endplate both before and after incubation. Each bar represents the average MEPP amplitude after the incubation expressed as a percentage of the average MEPP amplitude measured at the same recording site before the incubation (data are presented as mean ± SEM). Within each age category, data were collected from 15–21 endplates incubated with myasthenic Abs, from 3–6 endplates incubated with L-15 medium alone, and from 8–17 endplates incubated with IgG.

when viewed with Nomarski optics at ×500. In control experiments, endplates were incubated either in medium supplemented with normal human IgG or in medium without Abs. In both cases, the decreases were small (8–10%) and not significantly different from zero. Thus, the reduction in MEPP amplitude appears to be a specific effect of the myasthenic Abs. The decrease was also much too large to be explained by increased turnover of the receptor during the brief period of incubation and therefore must represent a direct effect of the Abs on AcChoR function.

At endplates from rats between 7 and 12 days of age, the Abs again reduced mean MEPP amplitude, but the decrease averaged only 37% ± 4% (n = 21 cells), which was significantly smaller than at the younger endplates. The decreases in both control groups at this age were not significant. At still older endplates, the Abs had no effect on mean MEPP amplitude.

We conclude that the Abs effectively block AcChoR function at endplates in rats up to about 6 days of age. Over the next week, endplate AcChoRs gradually lose their susceptibility to the blocking effect of the Abs so that at some point between postnatal days 14 and 22 they apparently become completely resistant to Ab treatment.

Abs Block the Slow Component of Doubly Exponential MEPC Decays. The loss of susceptibility of rat endplate AcChoRs to inhibition by the myasthenic Abs parallels the developmental decrease in apparent mean channel open time. This suggests that the Abs may selectively block AcChoRs with slow, embryonic-type channels.

To test this explicitly, we examined the effects of Ab treatment on the decay phases of MEPCs. As described previously (12), the rate of MEPC decay depends on the channel properties of AcChoRs. In endplates that have a mixture of slow (embryonic) and fast (mature) channels, MEPCs have doubly exponential decay phases whose relative amplitudes reflect the relative numbers of slow and fast channels that are opened by neurally released transmitter (3, 4, 20). If the myasthenic Abs preferentially block either type of channel, they should shift the relative amplitudes of the slow and fast components of MEPC decays.

Treatment with Ab decreased the relative amplitude of the

slow component at endplates with doubly exponential MEPCs (Fig. 3). In the example shown, the slow component was initially 75% of total MEPC amplitude. After Ab treatment the slow component was only 25% of peak MEPC amplitude. This indicates that the Abs preferentially blocked the openings of slow channels.

A more detailed analysis better indicates the magnitude of the block. Assume that 1000 channels were open at the time of peak MEPC amplitude in the untreated endplate (4) and that fast channels have a 50% greater unitary conductance than slow channels (13, 20). Then "75% slow component" corresponds to 820 slow and 180 fast channels open during the MEPC peak. After Ab treatment, the slow component was only 25% of peak MEPC amplitude. If fast channels were completely unaffected, then there were 90 slow and 180 fast channels open during the MEPC peak—that is, the Abs blocked about 90% of the slow channels. If the Abs also blocked some fast channels, then the percentage of blocked slow channels must have been greater still.

We cannot exclude the possibility that the Abs blocked some fast channels in this experiment. The lack of an Ab effect on intracellular MEPPs at older endplates argues against this possibility, but fast channels at young endplates may differ from those at older endplates. Although it was clear that Ab treatment reduced total MEPC amplitude at endplates that contained substantial numbers of slow channels, we cannot quantitate the magnitude of the decrease in each of the two components. In extracellular recordings, total MEPC amplitude depends strongly on the distance between the pipette tip and the transmitter release site (14), and we cannot be sure that the electrode position was precisely the same before and after Ab treatment. As a result, we can compare only the relative magnitudes of the fast and slow MEPC components in the presence and absence of Abs. Nevertheless, the fact that we could record MEPCs at all argues against the possibility that the Abs blocked a substantial fraction of fast channels at these younger endplates.

Other experiments showed that there is apparent channel conversion at endplates in soleus muscles maintained in organ culture (unpublished data)—that is, at endplates that contain a mixture of fast and slow channels, the relative size of the slow MEPC component decreases over time even in the absence of Ab. Since the muscles were incubated with Ab for 3 hr, it was important to demonstrate that the decrease of the slow component in the presence of Ab was significantly greater than what would be expected from spontaneous channel conversion.

MEPCs were recorded from 27 endplates before and after Ab treatment and from 11 other endplates before and after treatment with normal human IgG. All of the endplates were from 6- to 12-day muscles and appeared to have a mixture of fast and slow channels. In both cases, the rate of change in the slow component was determined by calculating (% slow before Abs − % slow after Abs)/(time between measurements), yielding a value in units of "change in % slow component per hour." The rate of change with Ab treatment was double that observed with normal IgG (Table 1). The IgG value was similar to that observed in the absence of any Ab. Thus, the reduction of the slow component of the MEPC after Ab treatment cannot be attributed to channel conversion alone.

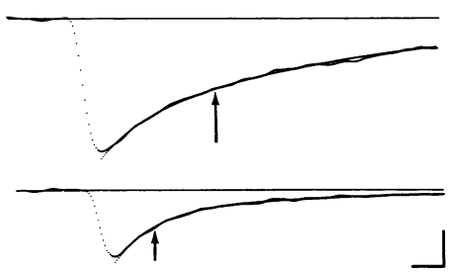

FIG. 3. Digitized MEPCs recorded extracellularly from the same day 8 endplate before (*Upper*) and after (*Lower*) incubation with myasthenic Abs. Superimposed on each MEPC is the best-fit sum of two exponential curves. In each case the two individual exponentials had time constants of 7.5 msec and 1.2 msec. The percentage of peak MEPC amplitude contributed by the slow exponential component fell from 75% to 25% after Ab treatment, thus shortening MEPC decays. This is evidenced by a shift in the half-decay time point (arrows). The thin solid lines indicate the baseline current levels. Calibration bars = 50 pA, 1 msec.

The effects of the Abs were also tested at 8 endplates of 3- to 5-day-old rats at which the slow component was initially about 90% of total MEPC amplitude. In these cases, we found that MEPCs were small and difficult to record after Ab treatment, presumably because of extensive block of AcChoR function. This is in agreement with the effects of Ab on intracellularly recorded MEPPs. However, even though the Abs reduced *total* MEPC amplitude, their effect on the *relative* size of the slow decay component was smaller at these endplates (2.75%/hr ± 0.67%/hr) than at the 6- to 12-day endplates described above (Table 1).

We attribute the reduced effect of Abs on the shape of MEPC decays at young endplates to the relative scarcity of fast channels. Apparently, at most of these endplates only a minority of functional channels was fast even after many of the slow channels had been silenced by Ab. Thus, the slow MEPC component still dominated after Ab treatment despite its reduction in absolute amplitude.

Finally, we tested the effects of Ab treatment on MEPCs recorded from five 17- to 21-day endplates. In agreement with the intracellular MEPP experiments, the Abs had no apparent effect on total MEPC amplitude in these more mature muscles. Analysis of MEPC decays indicated a decrease in the relative size of the slow component from *ca.* 10% of total MEPC amplitude before Ab treatment to *ca.* 2% of total MEPC amplitude after Ab treatment, but this decrease was at the limits of our experimental resolution and may not be significant. The relative lack of an Ab effect at endplates with virtually all fast channels supports the view that the Abs are selective for slow channels.

Ab Blocks Slow Channel Openings in AcCho Noise Spectra. The effect of the myasthenic Ab on MEPC decay phases indicated that Ab preferentially silenced slow channels. This view was confirmed in noise analysis experiments. Power spectra of AcCho-induced current fluctuations were determined at the same soleus endplates before and after Ab treatment. For these experiments we chose 8- to 9-day endplates at which the initial spectra indicated that there were substantial numbers of both fast and slow channels.

The myasthenic Ab significantly reduced the relative size of the slow Lorentzian component in every one of 11 cells tested. Using methods that will be described elsewhere (20), we estimated the relative amount of fast and slow channel

Table 1. Rate of change of slow MEPC and AcCho noise components

Treatment	Rate of change in relative size of slow component, %/hr	
	MEPCs	AcCho noise spectra
Myasthenic Abs	9.0 ± 0.8 (27)	10.3 ± 1.0 (11)
No Abs	4.2 ± 0.5 (41)	1.9 ± 0.5 (22)
Human IgG	4.5 ± 0.9 (11)	—

MEPCs were recorded at 6- to 12-day endplates and AcCho noise records were obtained from 8- to 9-day endplates. Data are presented as mean ± SEM. *n* (shown in parentheses) represents the number of endplates tested before and after each treatment.

activity during the noise recordings. On the average, the Abs reduced the percentage of total channel openings due to slow channels from 64% ± 3.6% to 18% ± 1.7% (mean ± SEM). A comparison of these results with those of normal cells shows that they cannot be accounted for solely by channel conversion during the Ab incubation period (Table 1).

It should be noted that in these double Lorentzian fits, the two corner frequencies were similar before and after Ab incubation (Fig. 4). This indicates that the Abs did not affect the apparent mean channel open times of either population of AcChoRs.

Ab Does Not Change the Single-Channel Conductance of Fast or Slow Channels. The experiments described above show that the myasthenic Abs preferentially block slow channels. This could come about in two ways. The Abs either could reduce the unitary conductance of slow channels or could decrease (perhaps completely) the frequency of slow channel openings. This was tested by recording single-channel currents (10) in perijunctional regions of soleus fibers following Ab treatment. Although our records were too short for us to determine the effect of the Abs on channel opening frequencies, we were successful in testing their effect on channel conductances.

Single-channel records obtained after Ab treatment (Fig. 5) were not noticeably different from those of control cells. In both cases, we observed the smaller currents characteristic of embryonic-type channels and the larger currents characteristic of adult-type channels. The smaller currents seen after Ab treatment presumably were due to embryonic-type channels that escaped Ab-mediated inhibition (see *Discussion*).

Single-channel current amplitudes were measured in five experiments. The data fell into two groups corresponding to

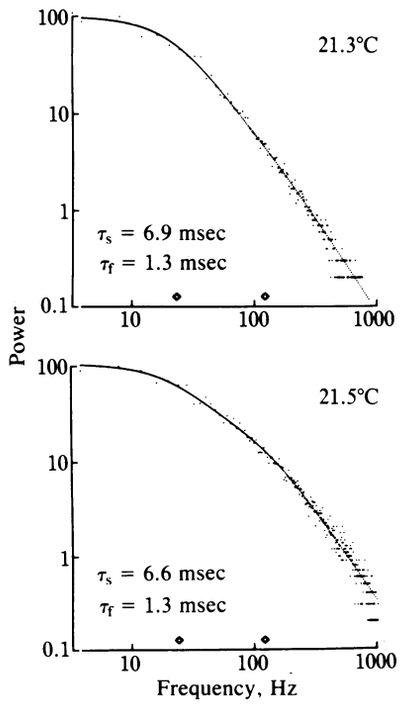

FIG. 4. Power spectra of AcCho-induced current fluctuations recorded from the same day 8 endplate before (*Upper*) and after (*Lower*) incubation with myasthenic Abs. Superimposed on each spectrum is the best-fit sum of two Lorentzian curves. The diamonds indicate the corner frequencies of the Lorentzians, and the corresponding apparent mean channel open times (τ) are printed within each spectrum. Analyses of these spectra indicate that the percentage of total channel activity contributed by slow channel openings fell from 74% to 26% after Ab treatment. This experiment and that illustrated in Fig. 3 were performed on different endplates.

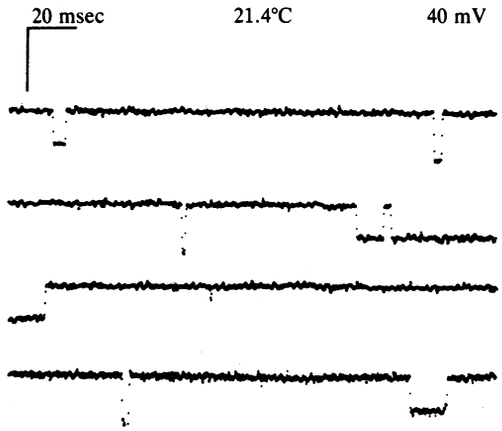

FIG. 5. Digitized single-channel recordings obtained from a perijunctional cell-attached patch on a day 11 soleus muscle fiber that was treated with myasthenic Abs. The traces are nonconsecutive and were chosen to illustrate the two amplitude classes that were observed. The membrane patch was exposed to 500 nM AcCho in rat Ringer solution and was hyperpolarized by 40 mV. The patch current was low-pass filtered at 1.5 kHz. Vertical bar = 7.5 pA.

single-channel conductances of 35 ± 2 pS and 51 ± 3 pS (mean ± SD). Both types of channels were observed after Ab treatment at days 4, 5, 9, and 11. At day 21 only the high-conductance channels were seen. These values are not significantly different from those observed in untreated cells (35 ± 2 pS and 48 ± 3 pS; n = 15 cells), indicating that the Abs act by reducing the frequency of slow channel opening.

DISCUSSION

Although AcChoRs undergo striking physiological changes during endplate development, embryonic and mature AcChoRs are almost indistinguishable biochemically. One of the few ways in which they can be distinguished is by a class of Abs found in the serum of patients with myasthenia gravis. We have investigated the relationship of this immunological difference to the developmental changes in AcChoR channel properties by using a myasthenic serum of unusual specificity. When incubated with AcChoRs derived from extrajunctional membranes, these Abs block the binding of α-bungarotoxin to one of the two toxin-binding sites associated with each receptor molecule (6). Because preincubating AcChoRs with toxin blocks virtually all Ab binding, Abs directed against the toxin-binding site are thought to represent the major species (7).

We have shown that these Abs reduce MEPP amplitudes by 60% at neonatal endplates that have mostly slow, embryonic-type channels but not at all at mature endplates that have mostly fast, adult-type channels. During the second postnatal week, when endplates have a mixture of channel types, the Abs have an intermediate effect on MEPP amplitude. Immunocytochemistry experiments show a parallel developmental decrease in the ability of endplate AcChoRs to bind Ab (6, 7). Taken together, these experiments suggest that the myasthenic Abs selectively bind to, and block, AcChoRs with slow channels.

We tested this hypothesis by examining the effects of the Abs on the decay phases of MEPCs. Many developing endplates have MEPCs with doubly exponential components reflecting the mixture of slow and fast channels within the same membrane. At such endplates, the Abs selectively reduced the slow decay phase generated by the embryonic-type AcChoRs. This was true at every 6- to 11-day endplate tested; the Abs appeared equally effective throughout this period. In part this change may have reflected apparent

channel conversion *in vitro* (unpublished data). However, the reduction of the slow MEPC component after Ab treatment was twice that observed at endplates exposed to normal human IgG or to Ab-free medium.

Similar results were obtained with noise analysis. At endplates with a mixture of slow and fast channels, AcCho power spectra are best fit with the sum of two Lorentzian curves, one for each channel population. Ab treatment selectively reduced the Lorentzian component contributed by the activity of slow, embryonic-type channels. Although there was some decrease in the slow Lorentzian component with time even at control endplates, the change after Ab treatment was five times greater.

The simplest interpretation of these observations is that the Abs selectively reduce the efficacy of slow, embryonic-type channels at all stages of endplate development. Fast, adult-type channels apparently are unaffected. The MEPP experiments show that virtually no fast channels at 14- to 22-day endplates are blocked by the Abs. We cannot rule out the possibility that some fast channels at younger endplates are susceptible, but our data show that the Abs have a clear preference for slow channels at all ages.

In principle, the Abs could reduce the efficacy of slow channels by decreasing their open time, by lowering their conductance, or by blocking their opening. Our results favor the last possibility. It is unlikely that the Abs reduce the mean open time of slow channels. In both MEPCs and AcCho power spectra, Abs do not affect the time constants of the slow and fast components. Although this leaves open the possibility that the Abs might decrease the mean open time of the slow channels to match that of the fast ones, this is ruled out by the finding that the Abs do not significantly change the shape of MEPCs at very young endplates in which the vast majority of the channels are slow. If the Abs decrease the open time of slow channels, they should generate a large fast MEPC component at newborn endplates.

It also seems unlikely that the Abs reduce the unitary conductance of slow channels. Both large and small AcChoR currents appear normal after Ab treatment. Although it is possible that blocked AcChoRs may have a single-channel conductance of up to several pS (the approximate resolution of our patch-clamp recordings), it seems more likely that the Abs block the opening of slow channels. A similar conclusion was reached in studies of C2 myotubes. In these experiments, the Abs reduced carbachol-induced ^{22}Na uptake but did not affect single-channel conductance or the distributions of open and closed intervals (21).

A consistent finding in our experiments was the incomplete block of slow channel activity, an effect also seen in C2 myotubes. One explanation is that we were unable to obtain saturating Ab concentrations because of the low titer of the serum. There are several other possibilities. (*i*) The Abs may have limited accessibility to some endplate AcChoRs. This cannot be the only explanation, however, because incomplete block also occurs in perijunctional regions (see Fig. 5) and in C2 myotubes (21). (*ii*) There may be a subpopulation of slow channels that is resistant to these Abs. If so, the fractional size of this subpopulation must be roughly constant throughout development. (*iii*) The Abs may reduce the opening frequency of slow channels rather than silence them completely. If so, it is unlikely that this is due to a decrease in the opening rate constant (see ref. 15) because the intraburst behavior of slow channels appears normal (data not shown). (*iv*) Some antibodies may bind to AcChoRs without inhibiting the opening of their channels.

The close correspondence between channel properties and immunological reactivity suggests that there is a structural difference between AcChoRs with embryonic-type channels and those with adult-type channels that is recognized by these Abs. The recent observation that AcChoRs at individual endplates change their properties more rapidly than they are replaced (unpublished data) implies that such a structural change must occur *in situ*. Because the alteration is detected on the external surface by Ab, the most likely explanation is that there is an enzymatic conversion of the AcChoR catalyzed by a protein in the membrane or in the extracellular matrix. This conversion may involve oligosaccharides associated with the AcChoR (16).

A final question concerns the possible role of these Abs in the pathophysiology of myasthenia gravis. Myasthenic endplates show a reduced response to AcCho (17, 18) and a decreased number of ^{125}I-labeled α-bungarotoxin-binding sites (19). Both effects could result from binding of blocking Abs to endplate AcChoRs. However, the Abs described here would not be expected to bind to adult endplate AcChoRs, assuming that adult and embryonic AcChoRs differ in humans as they do in rats. Binding would occur if myasthenic endplates accumulate embryonic-type AcChoRs, but Cull-Candy *et al.* (18) have found that AcChoRs in normal and myasthenic human endplates are physiologically identical. An interesting alternative is that even at adult endplates, AcChoRs newly incorporated into the surface membrane might have slow channels that are subsequently converted into fast ones. If so, the myasthenic Abs might interfere with conversion.

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