Steep concentration dependence and fast desensitization of nicotinic channel currents elicited by acetylcholine pulses, studied in adult vertebrate muscle

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Abstract. Skeletal muscles of adult mice and frogs were dissociated enzymatically and prepared for patch-clamping within less than 6 h. Outside-out patches were superfused with repetitive pulses of acetylcholine (ACh) with switching times of about 0.2 ms. Peak responses were reached within 1 ms. In mouse muscle the average channel conductance was 65 pS and the average open time 1 ms (20°C). Between 1 and 10 μM ACh, the peak responses increased proportional to the second to third power of the ACh concentration, and less steeply between 10 and 1000 μM ACh. The apparent Km of the dose-response curve was about 100 μM. After the peak, channel opening probability declined with time constants decreasing from about 1 s with 1 μM ACh to 15—50 ms with 1 mM ACh. After 100 ms desensitization the channel opening had decreased to less than 1/300 peak value. The rate of desensitization increased with rising temperature, with Q10 values of 1.7—2.5 between 10 and 30°C. The desensitization characteristics of channels from frog muscle were similar to that from mice. With pulses of 100 μM ACh the channels opened with a probability of 0.55, the open probability declining with a time constant of about 60 ms and dropping to less than 0.001 after 300 ms. The results support the view that three binding steps of ACh are necessary for opening of the channel. Desensitization in the presence of high ACh concentrations is slower than the decay of synaptic currents.

Key words: Nicotinic channels — Desensitization — Dose-response curve — Acetylcholine binding steps — Frog muscle — Mouse muscle

Introduction

Most studies of membrane channels activated by chemical ligands have been done in the steady state, i.e. in the continuous presence of the chemical agonist. In many classes of synapses, activation of the synaptic current is mediated by a rapidly rising and falling agonist concentration, which for instance is supposed to be present for less than 1 ms in the acetylcholinergic (nicotinic) endplates of vertebrate muscle (see Parnas et al. 1989). In addition to steady-state measurements, therefore, channels operating in fast synaptic systems should be activated by rapidly rising and falling pulses of agonist concentration. Such measurements may show the activation and deactivation kinetics of the channels, but also desensitization, the decline of activation in the presence of agonist.

In one class of fast synapses, the glutamatergic neuromuscular systems of crayfish and locusts, desensitization of channels was demonstrated to proceed with time constants of 1—7 ms (Franke et al. 1987; Dudel et al. 1988, 1990 a—c). Similarly rapid desensitizations were also observed in some glutamatergic channel types of vertebrate central neurons (Trussell et al. 1988; Hatt et al. 1989; Mayer and Vyklicky 1989; Tang et al. 1989; Trussell and Fischbach 1989; Dudel et al. 1990d). In the most extensively studied synaptic system, the neuromuscular endplates of vertebrates, the nicotinic channels were shown to desensitize with “short” time constants of a few seconds and long ones in the range of minutes (Katz and Thesleff 1957; Feldtz and Trautmann 1982; Penmfather and Quastel 1982; Connor et al. 1984; Adams 1987; Cachelin and Colquhoun 1989). In these studies the application of acetylcholine (ACh) or other agonists was relatively slow, and rapid desensitization processes may have been undetectable. Fast applications are possible only to superfused outside-out patches, and such methods were applied to nicotinic channels from cell lines of mouse muscle (Bekkers 1986; Brett et al. 1986; Dilger and Brett 1990; Maconochie and Knight 1989), resulting in time constants of desensitization of some 10 ms. Similar short time constants of desensitization were also reported for purified Torpedo receptor preparations (Heidmann and Changeux 1984; Cox et al. 1985). At present, however, it seems at least unclear whether such rapid desensitizations occur at nicotinic receptors of adult vertebrate muscle (Cachelin and Colquhoun 1989). Rapid desensitization might also limit the maximal responses and consequently
the maximal slope of the dose-response curve, unless very rapid ACh applications are achieved. We have succeeded in studying nicotinic channels in excised patches of muscles of mice and frogs, and have applied ACh with the rapid liquid-filament switch (Franke et al. 1987).

Materials and methods

Interossal muscles from the feet of adult mice or from the fore-feet of adult frogs were excised and placed in a dish, and were treated further as described by Allen et al. (1984). They were dissociated enzymatically by superfusing them for 3 h with physiological solution containing 0.6 mg/ml collagenase (Sigma, type I A, München), and then for 30 min with 0.5 mg/ml trypsin in CaCl²-free solution (1:250, Sigma), both at 37°C. Finally, the dissociated muscles were plated into culture dishes, which were kept at 4°C. At this temperature, the cells did not change their properties for up to 10 h. When plated into culture dishes, which were kept at 4°C, the dissociated muscles were superfused for 3 h with physiological solution containing 0.6 mg/ml collagenase (Sigma, type I A, München), and then for 30 min with 0.5 mg/ml trypsin in CaCl²-free solution (1:250, Sigma), both at 37°C. Finally, the dissociated muscles were plated into culture dishes, which were kept at 4°C. At this temperature, the cells did not change their properties for up to 10 h. When taken out for experiments at room temperature, the cells in the dishes could be used for patch-clamping for 1–2 h. Most data were obtained within less than 6 h after excising the muscles. Data were evaluated from 23 outside-out patches obtained from 18 preparations of mouse muscles, and from 4 cell-attached patches from mouse muscle. Data from 4 patches from frog muscle were included in addition.

The physiological salt solution superfusing the preparations and also the outside of patches contained, in case of mouse muscle (mM): 162 NaCl, 5.3 KCl, 2 CaCl₂, 0.67 NaH₂PO₄, 0.22 KH₂PO₄, 15 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 5.6 glucose; pH 7.4; and in case of frog muscle: 115 NaCl, 2.5 KCl, 1.8 CaCl₂, 5 Tris-maleate buffer, 5 glucose; pH 7.2. Temperature during recording was generally 20°C. Outside-out patches were prepared as described by Hamill et al. (1981). The solution inside the patch contained (mM) in case of mouse muscle: 140 KCl, 1 CaCl₂, 2 MgCl₂, 11 ethylenebis(oxoniitrito)tetraacetate (EGTA), 10 HEPES buffer, 10 glucose, pH 7.2; and in case of frog muscle: 115 KCl, 2 NaCl, 1 CaCl₂, 10 EGTA, 2 MgCl₂, 10 Tris-maleate, pH 7.2.5.

While approaching the muscle cells with the electrode in order to form a patch, endplate regions were visible as densities on the plated muscle fibres. This identification was confirmed by specific fluorescent staining (D. Koeltgen, C. Franke and H. Hatt, unpublished data). Patches were formed within about 100 μm from the endplate. Patches apparently from the centre of the endplate and patches 100 μm from the endplate yielded channel recordings with no obvious kinetic differences.

The outside-out patches were placed in the liquid-filament switch in which the solution superfusing the patch can be changed within about 0.2 ms (Franke et al. 1987; Dudel et al. 1990a). Channel currents were recorded with a Neher-Sigworth (List EPC 7) or a Dagan 3900 patch-clamp amplifier. Records were stored on video tape (modified Sony PCM-501 ES). They were digitized for processing at 20–50 kHz and evaluated on a series 300 Hewlett-Packard microcomputer (Franke et al. 1986; Dudel and Franke 1987).

Results

Channels from mouse muscle

When the channels were exposed to 550-ms pulses of ACh, openings were elicited within 0.5–1 ms (Fig. 1). As shown in the recordings with high time resolution (Fig. 1, D₂, E₂), the rise times (from 10% to 90% amplitude) of the summed single channel currents were 0.4–0.5 ms with 100 μM ACh and 0.5–0.6 ms with 10 μM ACh. It cannot be decided, at present, whether the relatively small increase in the rate of activation of the patch from 10 μM to 100 μM ACh is a real kinetic property of the system or due to lags in the diffusion of ACh. The shortest rise time observed in this study after application of 100 μM ACh was 0.3 ms (averaged record).

After the initial peak the rate of channel opening declined, eventually to a very low level. Only the isolated clusters of single channel openings were observed 200 ms after application of ACh, which are characteristic for steady-state recordings (Sakmann et al. 1980; Colquhoun and Sakmann 1985; Siné and Steinbach 1987; Colquhoun and Ogden 1988). Figure 1F shows a distribution of the current amplitudes seen in the patch of Fig. 1 A–C. The peaks of the distribution (arrows) correspond to the superposition of up to four single channel currents and are spaced at 2.9-pA intervals, corresponding to a single channel conductance of 65 pS. Single openings of the channels have an average duration of about 1 ms (Fig. 2A). Short interruptions of the openings which are characteristic of recordings in frog muscle (Colquhoun and Sakmann 1985) were seen in less than 1/10 of the openings, in recordings with 10 kHz filtering. In order to control a possible change in channel characteristics due to the outside-out excised state, we also performed cell-attached recordings. The average open time of 1 ms and rare short closings were also observed (Fig. 2B), the pipette containing 0.1–10 μM ACh.

The cell cultures of embryonic rat muscle cells investigated by Jaramillo and Schuetze (1988) should be close relatives of the preparation studied by us. The cell cultures contain a 23- and a 34-pS "adult" channel type. The "high conductance" channel shows two components of the burst length of about 0.1 and 1.6 ms duration, with an average 1–2 short (0.1 ms) gaps. In preliminary experiments on channels from mouse myotubes, we have also observed relatively frequent short gaps within the bursts. These channels differ in their conductance and kinetics from the ones seen by us in adult mouse muscle.

Concentration dependence of activation and desensitization of channels from mouse muscle

In the data shown in Fig. 1 A–C and 1 D–E the peak response and the rate of desensitization increased on raising the ACh concentration from 5 to 100 μM. The average response of another patch to a wider range of ACh concentrations is shown in Fig. 3 A–D. The peak response, Iₘₐₓ, increased very steeply between 2 and 10 μM ACh, and much less above 10 μM ACh. The double-logarithmic plot in Fig. 3E shows a slope of 2.3–2.8 below 10 μM ACh. In five experiments of this type, double-logarithmic slopes of 2.0–2.8 were observed in the range of 1–5 μM ACh, with a mean value of 2.4. The measured slopes are lower limits for the power law describing the dependence of channel opening on ACh concentration. Above 10 μM ACh concentration, the response to ACh pulses approached saturation. The responses still rose above 100 μM ACh; in four experiments the peak current increased by factors of 1.3–2.0, on average 1.7-fold,