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Agonist-activated ionic channels in acetylcholine receptor reconstituted into planar lipid bilayers

(reconstitution/single-channel current fluctuations/synaptic transmission/phase transition/*Torpedo marmorata*)

GÜNTHER BOHEIM†, WOLFGANG HANKE†, FRANCISCO J. BARRANTES‡, HANSJÖRG EIBL‡, BERT SAKMANN‡, GREGOR FELS§, AND ALFRED MAELICKE§

†Institut für Zellphysiologie, Ruhr-Universität Bochum, 4630 Bochum, Federal Republic of Germany; ‡Max-Planck-Institut für biophysikalische Chemie, 3400 Göttingen, Federal Republic of Germany; and §Max-Planck-Institut für Ernährungsphysiologie, 4600 Dortmund, Federal Republic of Germany

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ABSTRACT **Planar lipid bilayers were formed with the mixed chain phospholipid 1-stearoyl-3-myristoylglycero-2-phosphocholine. Acetylcholine receptor membrane fragments or the purified receptor protein was incorporated into these bilayers by fusing receptor-containing vesicles with the planar membranes a few degrees below the lipid phase transition temperature. Single-channel currents activated by nicotinic agonists in the reconstituted system resembled those observed in intact rat and frog muscle membrane as measured by the patch clamp technique. The observed channel characteristics did not depend on the degree of receptor purification. Thus, the receptor-enriched fragments and those depleted of nonreceptor peripheral peptides, the purified receptor monomer/dimer mixtures, and the isolated receptor monomer as defined by gel electrophoresis all shared similar electrochemical properties in the synthetic lipid bilayer. The agonist-activated ionic channel seems, therefore, to be contained within the receptor monomer.**

Reconstitution of a functional ion translocation system from the minimal number of components required to mimic a chemically activated membrane would certainly foster our understanding of membrane excitability in general.

Purified acetylcholine receptor (AcChoR) protein and receptor-enriched membrane preparations have been available for some time (1–3). Reliable methods of incorporating these into planar bilayers and quantitative electrochemical studies of the reconstituted system have to be developed. Part of this goal was recently achieved by Schindler and Quast (4), who formed a monolayer from AcChoR-rich membrane fragments and lipid and from these planar bilayers by using the method of Montal and Mueller (5). This reconstituted system produced single-channel current fluctuations in response to nicotinic agonists. Experiments with purified AcChoR should answer a crucial question of membrane excitability—i.e., whether the receptor protein contains not only the ligand binding site but also the ion channel. Expanding the work of Nelson *et al.* (6) to quantitative studies of single-channel currents, we can now answer this question affirmative.

By application of a recently developed method (7) we have fused protein-containing vesicles with preformed planar bilayer membranes of a pure mixed-chain lipid a few degrees below its phase-transition temperature. In this way we have incorporated AcChoR protein of different degrees of purification into lipid bilayers. Single-channel currents were recorded from these membrane systems. The currents were activated only by nicotinic agonists and were absent in the presence of saturating concentrations of antagonists. The single-channel characteristics were similar for AcChoR-rich membrane fragments before and after alkaline treatment and for purified AcChoR independent of whether monomer–dimer mixtures or pure monomers were used. Furthermore, the gating behavior was comparable to that of AcCho-activated channels of rat and frog muscle membranes measured with the patch-clamp technique (8).

MATERIALS AND METHODS

The synthesis of the mixed-chain lecithin 1-stearoyl-3-myristoylglycero-2-phosphocholine (1,3-SMPC) has been described (9). The temperature ranges for its phase transition (t_c) from fluid to solid and solid to fluid state are 30–27°C and 30–33°C, respectively, as determined by differential scanning calorimetry (10).

Torpedo marmorata electric organs were dissected from freshly killed specimens and the AcChoR-rich membrane fragments were prepared as described (11, 12). These showed sodium dodecyl sulfate gel electrophoresis patterns consistent with the subunit composition $\alpha_2\beta\gamma\delta$ and, in addition, had components with M_r 43,000 and M_r 90,000. Receptor concentrations of membrane fragments ranged between 2 and 4.5 μmol of α-toxin sites per g of protein. Alkaline extraction of AcChoR membranes (removal of the M_r 43,000 component) was performed as described (13). Vesicles were osmotically loaded by incubation in 1 M sucrose/1 M KCl (or 1 M NaCl)/4 mM Tris–Hepes, pH 7.4 (7).

The receptor protein from *T. marmorata* was solubilized and purified as described (14). Electric organs were homogenized in 160 mM NaCl/5 mM KCl/2 mM $MgCl_2$/10 mM $CaCl_2$, pH 7.4, and appropriate Na/K and Ca/Mg ratios were maintained throughout the purification procedure. As shown by gradient gel electrophoresis under nondenaturating conditions (14), these preparations contained various ratios of receptor monomer and dimer. The receptor protein had a subunit pattern consistent with the stoichiometry $\alpha_2\beta\gamma\delta$. Toxin binding capacties (15) were in the range of 8–10 μmol of mono-[^{3}H]pyridoxamine-labeled α-cobratoxin per g of protein. Acetylcholinesterase contamination was less than 0.05%. The receptor protein was freed from detergent and introduced into 1,3-SMPC by means of chromatography on hydroxylapatite. After binding of AcChoR to the crystals the column was first washed with 20 mM Tris·HCl, pH 7.0, and then with the same buffer saturated with 1,3-SMPC. Exchange of receptor-bound detergent with 1,3-SMPC in the course of receptor–lipid vesicle formation was monitored by both UV absorption and radioactivity. After the detergent concentration had dropped below the detection limit,

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Abbreviations: AcCho, acetylcholine; CbmCho, carbamoylcholine; $SubCho_2$ suberoyldicholine; AcChoR, acetylcholine receptor; 1,3-SMPC, 1-stearoyl-3-myristoylglycero-2-phosphocholine.

AcChoR was eluted with 5 mM KCl/200 mM sodium phosphate buffer, pH 7.0. The specific toxin binding capacities of this preparation were in the same range as those of receptor preparations in detergent.

Receptor–lipid vesicles containing only monomeric receptor were prepared as follows: Purified receptor protein was first introduced into 100 mM NaCl/0.1 mM $MgCl_2$/0.1 mM $CaCl_2$/5 mM KCl/10 mM Tris·HCl, pH 8.0, by using Sephadex G-25 and the centrifugation procedure of Neal and Florini (16). The buffer was made 20 mM in dithiothreitol and, after 1 hr at room temperature, was exchanged by centrifugation with the same buffer containing only 1 mM dithiothreitol. This solution was then made 10 mM in *N*-ethylmaleimide. After 30 min at room temperature, the receptor solution was applied onto a hydroxylapatite column and the lipid exchange was performed as described above. These preparations had the usual subunit pattern but, in addition, migrated only to the monomer position in polyacrylamide gradient gels run under nondenaturating conditions (14).

Virtually solvent-free planar bilayer membranes were formed according to the method of Montal and Mueller (5). Bilayers and electrochemical measurements were made within the temperature range 21–25°C (7, 10). The mechanical and electronic equipment used has been described (17). Polarity of the potential across the bilayer is defined as follows: A negative applied voltage refers to the potential of the rear compartment being more negative than that of the front compartment. Agonists were added to the front compartment. Current is designated negative and is downwards in all figures if cation transfer occurs from front to rear.

RESULTS

Single-Channel Current Fluctuations in Lipid Bilayers Containing AcChoR-Enriched Membrane Fragments and in Intact Cell Membranes. AcChoR-enriched vesicles from *T. marmorata* were osmotically loaded. After a virtually solvent-free bilayer of 1,3-SMPC had been formed (7), these vesicles were added to the front compartment of the system, where they fused spontaneously with the bilayer. Fig. 1 shows traces of single-channel current fluctuations in 1 M KCl at 21°C and −50 mV holding potential recorded 2 min (trace a), 6 min (trace b), and 15 min (trace c) after addition of 0.4 μM acetylcholine (AcCho). The traces shown represent the currents occurring with the highest probability (>50%) at the given times. These are labeled according to their pattern of activation: single-opening events (s-events, Fig. 1, trace a), multiple-opening events (m-events, Fig. 1, trace b), and persistent-opening events (p-events, Fig. 1, trace c). Thus m-events are defined by the mean lifetime of the closing gaps to be of the same order of magnitude as the mean open time. These different appearances of the AcCho-activated channels differ in their gating and lifetime properties, whereas the conductance amplitude remains unchanged. Transitions between these typical events have also been recorded. Good discrimination between them can be achieved only at a very low frequency of events—i.e., when the interval between two events is at least one order of magnitude larger than the duration of the event itself.

In parallel with the change in channel gating characteristics, a strong reduction in the frequency of events is observed. Within 15 min the rate of event occurrence decreased at least

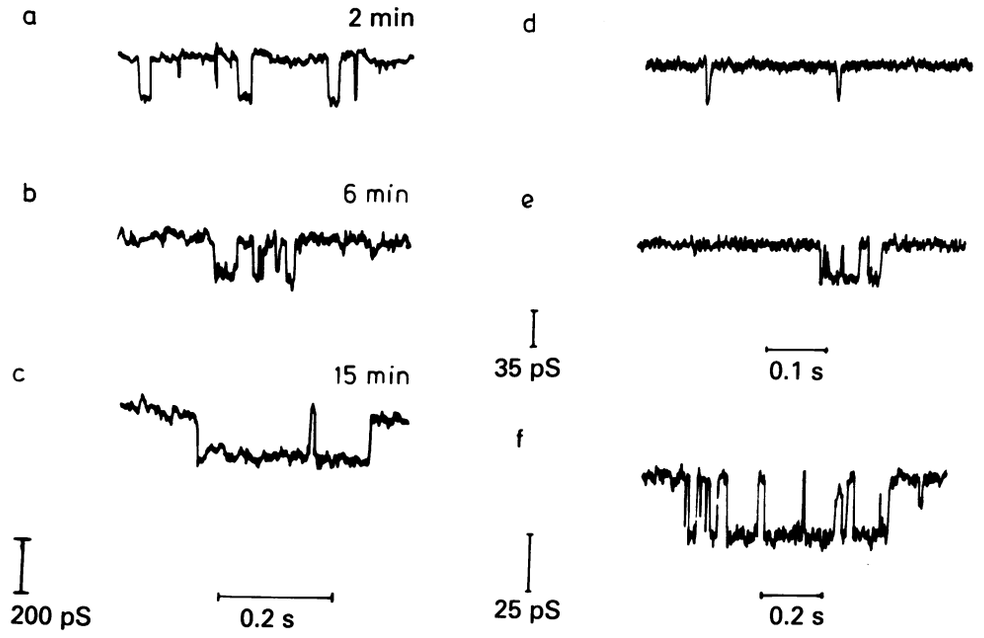

FIG. 1. Comparison of AcCho-induced single-channel fluctuations in biological and reconstituted membranes. The occurrence of three typical kinds of elementary events—i.e., single-opening events (a, d), multiple-opening events (b, e), and persistent-opening events (c, f)—is demonstrated in both cases. Traces a–c, AcCho-activated current patterns in planar 1,3-SMPC membranes with AcChoR-enriched membrane fragments. Five micrograms of protein (≈10 pmol of toxin binding sites) per 1 ml of solution was added to the front compartment. Aqueous solution in both compartments, 1 M KCl/4 mM Tris–Hepes, pH 7.4. AcCho concentration (front), 400 nM; applied voltage, −50 mV; temperature, 21°C. For details see text. Traces d–f, AcCho-activated single-channel currents recorded with the patch-clamp technique from the extrasynaptic membrane of chronically denervated rat (d, e) and frog (f) muscle fibers (8). Trace d, single-channel current steps occur at low frequency when the patch pipette contains 0.5 μM AcCho. Their mean lifetime was 4.6 ms in this recording, as determined from the decay time constant of a single exponential fitted to the distribution of step durations. Membrane potential, −120 mV; temperature, 21°C. Trace e, example of multiple openings from the same membrane patch. The average duration of closing gaps between current pulses was 1.4 ms, as determined from the distribution of channel closed times. Trace f, recording of an event of long duration in the presence of 0.5 μM AcCho. These current pulses are separated by brief closing gaps. The average duration of current pulses during such an event is much longer than the average pulse duration of 21 ms determined from a large number of single-channel current pulses of the same recording. The overall frequency of channel openings was $<1\ s^{-1}$. Membrane potential, −150 mV; temperature, 11°C.

one order of magnitude. With carbamoylcholine (CbmCho) instead of AcCho under otherwise identical conditions much less significant changes in channel characteristics with time were observed. Right from the beginning mainly s-events (>80%) mixed with a few m-events, with only one or two closing gaps were observed. The amplitude of AcCho-activated currents increased linearly with membrane voltage. In 1 M KCl solution the underlying conductance change was 185 pS (Fig. 2*A*), whereas in 1 M NaCl this value was 95 pS. The average duration of the s-type event was ≈8 ms in both solutions (Fig. 2*B*) and was not voltage dependent.

The observed activity of channels depended on the physical state of the lipid bilayer. With systems that showed agonist-induced current fluctuations at 25°C no such effects were observed when the temperature was increased to 38–40°C—i.e., to well above the phase transition temperature (t_c) of 1,3-SMPC. The functioning reconstituted systems—i.e. below t_c—did not show any sidedness of response. This was shown by applying the membrane vesicles from the front and the ligand from the rear side of the preformed lipid bilayer. Current fluctuations were identical with those observed when both vesicles and ligand were added to the same side.

We compared the properties of AcCho-activated current fluctuations in the reconstituted system with single-channel currents in the sarcolemmal membrane of denervated rat fibers. Experimental conditions were comparable except that the transmembrane ion concentration was higher in the bilayer experiments. The predominant type of AcCho-activated channel in rat muscle has a conductance of $\bar{\Lambda}$ = 34 pS in physiological conditions as well as in symmetrical transmembrane concentrations of 0.15 M NaCl measured in isolated membrane patches (18). In the presence of 0.5–1 μM AcCho, single-channel current steps occur mostly at random intervals, as illustrated in Fig. 1, trace d. The average duration of these current steps increases as the membrane potential is made more negative. At 20°C and at −80 to −120 mV membrane potential their average duration is $\bar{\tau}$ = 4–7 ms. In many membrane patches the distribution of intervals between current steps is not described by a single exponential as expected for random activation of single channels. It is characterized by a fast component in the time range <2 ms. This reflects the occurrence of current steps interrupted by brief closing gaps as illustrated in Fig. 1, trace e. Thus, the s- and m-type events in the reconstituted membrane reflect a gating pattern that resembles that of the channels in the intact cell. However, no time-dependent changes were observed in the single-channel current pattern. Currents resembling the p-type events are extremely rare. An example is illustrated in Fig. 1, trace f.

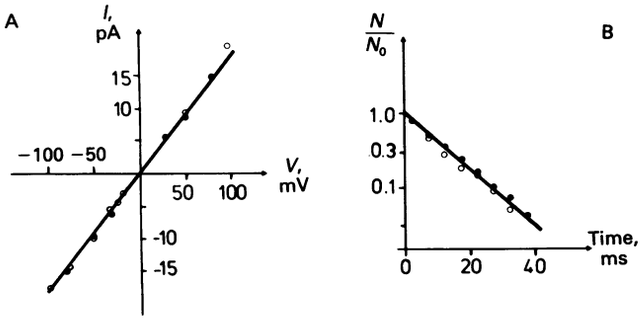

FIG. 2. Single-channel characteristics of AcCho-induced channels in reconstituted planar bilayer membranes after incorporation of AcChoR-rich membrane fragments (●) or purified AcChoR in 1,3-SMPC vesicles (○). Experimental conditions are the same as for Fig. 1, traces a–c. (*A*) Current–voltage (*I–V*) curve characterized by $\bar{\Lambda}$ = 185 pS. (*B*) Lifetime distribution for s-events characterized by $\bar{\tau}$ = 8.8 ms; number of evaluated events N_0 was 216 (●) and 166 (○).

Pharmacological Specificity of Channel Properties. The observed current fluctuations were specific in that they were induced only by nicotinic agonists and were blocked by antagonists. Preincubation with 500 nM α-bungarotoxin led to a complete block of membrane currents. As shown in Fig. 3, traces a–c, single-channel characteristics depended on the agonist employed. The mean lifetime of s-events increased in the sequence CbmCho, AcCho, $SubCho_2$, whereas the mean channel conductance remained constant. As a further test of specificity, the mode of action of the lidocaine derivative QX-222 (19) was investigated. Addition of QX-222 changed the appearance of CbmCho-activated channels (Fig. 3, trace d, right). A burst of unresolved spikes with a mean lifetime of the open state of $\bar{\tau} < 1$ ms is apparent. The burst-like behavior and the longer duration of these bursts is consistent with previous findings concerning the mode of action of this channel-blocking substance (19).

Influence of Ca^{2+} on the Agonist-Induced Current Fluctuations. The effect of 4 mM Ca^{2+} and 2 mM Mg^{2+} added to both compartments on the mean open channel conductance $\bar{\Lambda}$ in the presence of 1 M KCl is shown in Fig. 4, traces a–c. Whereas no change in $\bar{\Lambda}$ was observed with AcCho as ligand, $\bar{\Lambda}$ changed by a factor of more than 2 in the presence of CbmCho. As shown in Table 1, $\bar{\Lambda}$ depended on the ionic composition of the compartment solutions, whereas the channel lifetime $\bar{\tau}$ remained unchanged within the limits of experimental reproducibility ($\bar{\tau} \pm 0.3\bar{\tau}$). The permeability ratios P_{Alk}/P_{Cl} indicated that the effect is apparently due to the coincidental change of two channel properties, the channel selectivity and the open-channel conductance for Na^+ and K^+. This may be understood by assuming two channel conformations, one having a permeability

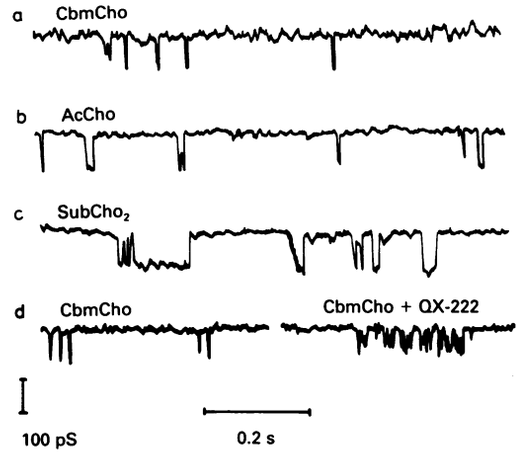

FIG. 3. Pharmacological specificity of single-channel conductance fluctuations in reconstituted planar bilayers after fusion of AcChoR-enriched membrane fragments. With CbmCho (100 nM), AcCho (10 nM), and suberoyldicholine ($SubCho_2$) (2 nM) single channels with identical conductances ($\bar{\Lambda}$ = 95 pS) but different mean lifetimes (3.5 ms, 7.6 ms, and 16 ms, respectively) were observed. Agonists were given to the front compartment; all other experimental conditions were identical. Aqueous solution in both compartments, 1 M NaCl/4 mM Tris·HCl, pH 7.2; applied voltage, −100 mV; temperature, 21°C. Modulation of the CbmCho-induced current fluctuation pattern from s-events (for data see Table 1) to burst-like sequences of spikes by the blocking action of QX-222 is shown in trace d. Left trace, 100 nM CbmCho; right trace, 100 nM CbmCho + 20 μM QX-222. The drugs were added successively with a time difference of 5 min to the front compartment. Aqueous solution in both compartments, 1 M KCl/4 mM $CaCl_2$/2 mM $MgCl_2$/2 mM K,Na phosphate buffer, pH 7.3; applied voltage, −100 mV; temperature, 25°C.

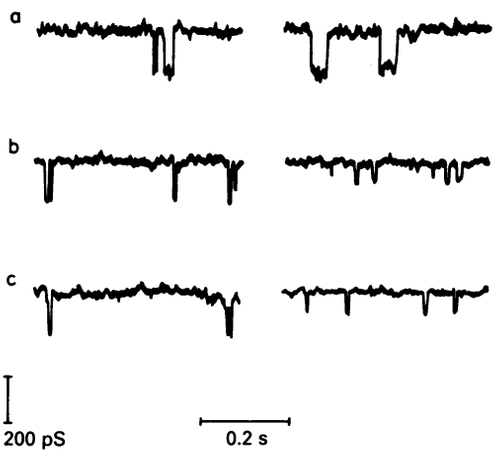

FIG. 4. Effect of Ca^{2+} on the mean open-state conductance $\bar{\Lambda}$ of AcCho- and CbmCho-induced channels in the presence of K^+. Traces on the left were obtained with Ca^{2+},Mg^{2+}-free aqueous solutions, whereas traces on the right were recorded with the same solutions supplemented with 4 mM $CaCl_2$ and 2 mM $MgCl_2$ in both compartments. Ca^{2+} had no effect on $\bar{\Lambda}$ of the AcCho-induced channel (trace a, $\bar{\Lambda}$ = 185 pS). In contrast, Ca^{2+} altered the CbmCho-induced channels (traces b and c) from $\bar{\Lambda}$ = 190 pS to $\bar{\Lambda}$ = 79 pS (see Table 1). This effect was observed with all three vesicle preparations. Trace a, AcChoR-rich membrane fragments, 40 nM AcCho; trace b, alkali-treated fragments, 100 nM CbmCho; trace c, purified AcChoR in 1,3-SMPC vesicles, 100 nM CbmCho. Vesicles and agonists were given to the front compartment in either sequence. Aqueous solution in both compartments (regardless of Ca^{2+} and Mg^{2+}), 1 M KCl/2 mM K,Na phosphate buffer, pH 7.3; temperature, 25°C; applied voltage, −50 mV (a, b) or −100 mV (c).

ratio for K^+ and Na^+ of 1:1, the other one with a ratio of 2:1. These two states of the channel were observed with CbmCho but not with AcCho.

Preservation of Single-Channel Current Properties Upon AcChoR Purification. Alkaline extraction of nonreceptor peripheral peptides results in further enrichment of the receptor protein while preserving its membrane-bound nature (ref. 13 and references therein). Upon fusion with the planar lipid bilayer, current patterns qualitatively similar to the pattern seen with untreated membrane fragments were observed in the presence of agonists (Fig. 5, traces a–c). m-Events occurred more frequently (≈60–80% of the number of observed events) immediately after addition of AcCho and p-events had a lower probability of occurrence (<5%) even after 30 min of agonist application. Alkali-treated membranes were sensitive to the ionic species present during purification. When a constant ratio of Na^+ and K^+ concentrations was maintained throughout the preparation (12) (C_{Na+}/C_{K+} = 20) primarily s-events (70–80%) were observed instead of the otherwise dominating m-events.

As a further stage of purification, affinity chromatography-purified AcChoR was applied in the reconstitution studies. As shown in Fig. 5, traces d–f, the current fluctuations recorded from reconstituted purified AcChoR were closely similar to those recorded in bilayers containing membrane fragments. A higher probability of m- and p-events (adding to ≈50%) was observed shortly after AcCho addition when the receptor was purified in the absence of K^+ or when higher concentrations of AcCho were employed. When a constant ratio of Na^+ to K^+ concentration (≈20) was maintained, the fraction of s-events increased to ≈80%. Finally, the 9S monomeric receptor species (20–23) was employed. We observed similar channel characteristics as described for the less purified preparations. The current–voltage characteristic (*I–V* curve) and lifetime distribution of AcCho-induced channels from AcChoR-rich membrane fragments and purified AcChoR in 1,3-SMPC vesicles under conditions of Figs. 1 and 5 are shown in Fig. 2 *A* and *B*. Whereas for the *I–V* curve (Fig. 2*A*) all types of events were taken into consideration, the lifetime distribution (Fig. 2*B*) was obtained only from s-events occurring during the first 3 min after AcCho application.

Table 1. Mean of pooled single-channel data of fused AcChoR-rich membrane fragments in the presence of CbmCho

Ionic composition		$\bar{\Lambda}$, pS	$\bar{\tau}$, ms	E^*_{Alk}, mV	P_{Alk}/P_{Cl}
No Ca^{2+},Mg^{2+}	1 M KCl	190	2.8	22	5.1
	1 M NaCl	95	2.7	17	3.3
With Ca^{2+},Mg^{2+}	1 M KCl	79	3.1	22	5.1
	1 M NaCl	90	3.8	23	5.7

Aqueous solutions in both compartments for $\bar{\Lambda}$ and $\bar{\tau}$ determination: 1 M KCl or 1 M NaCl, without or with 4 mM $CaCl_2$, 2 mM $MgCl_2$, respectively, 2–4 mM buffer solution, pH 7.2–7.4; 40–400 nM CbmCho in the front compartment; applied voltage, −50 to −100 mV; temperature, 25°C. Inversion potentials E^*_{Alk} of single channels were measured in a 1.0–0.25 M alkali chloride concentration gradient. The permeability ratios P_{Alk}/P_{Cl} were calculated by means of the Goldman equation, using E^*_{Alk}.

DISCUSSION

We present here a comparison of AcCho-activated currents measured in an intact cell membrane with those obtained with *Torpedo* AcChoR incorporated into 1,3-SMPC bilayers. The similar current patterns observed establish that these originate from the same kind of protein complex, the AcChoR. A quantitative comparison of the reconstituted AcChoR system with the intact membrane current patterns must take into account differences in species and experimental conditions. Conductance and mean lifetime of s-events in the *in vitro* systems fall within the same order of magnitude as those reported in rat muscle membrane (Fig. 1). At a concentration of 1 M NaCl in the bilayer system a single channel conductance of ≈95 pS is found. This compares with ≈34 pS in the rat cell membrane in the presence of 0.15 M NaCl. Comparable data for both membrane systems are available in the case of the pore-forming antibiotic alamethicin. The values of the second conductance state (24) are 140 pS in 1,3-SMPC bilayers with 1 M NaCl (unpublished results) and 27 pS in rat sarcolemmal membranes under physiological conditions of ≈0.15 M NaCl (25). Thus, single-channel conductances for the reconstituted and cell membrane systems presented in this paper are comparable within a factor of 2. The mean lifetime of s-events in the reconstituted systems is longer than in rat muscle membrane and longer than would be expected from the decay of miniature endplate currents measured at the intact *Torpedo* electroplaque (26). This might be accounted for by the higher ionic strength (27) in the case of bilayer experiments and by the differences in lipid composition of artificial and biological membranes. The fact that channel lifetimes are independent of membrane voltage in the reconstituted systems may be a property of ionic channels in those species in which an end-plate potential does not trigger an action potential (28).

The strongest evidence for the similarity of AcCho-activated currents in the *in vitro* system and the patch clamp recordings in the cell membrane derives from their common pharmacological properties. In both membranes nicotinic AcCho agonists activate single-channel currents of similar size but different average duration (8), and α-bungarotoxin and QX-222 abolish or modify AcCho-activated currents in the same manner (19). A more quantitative comparison of AcChoR channel properties in reconstituted systems and intact cell membranes, however,

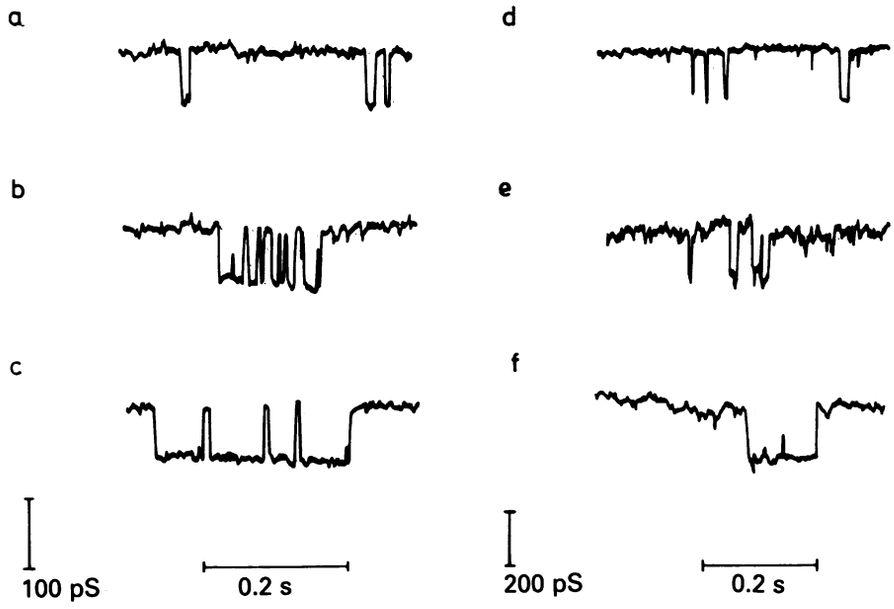

FIG. 5. Single-channel fluctuations from lipid bilayers containing (a–c) alkali-treated AcChoR-rich membrane fragments and (d–f) purified AcChoR (two-component system). Traces a–c, 5 μg of protein ($\approx$15 pmol of toxin-binding sites) per 1 ml of solution was given to the front compartment. The aqueous solution was the same as for Fig. 1, traces a–c, but 1 M NaCl instead of 1 M KCl. AcCho concentration (front), 40 nM; applied voltage, −80 mV; temperature, 21°C. Traces d–f, 2.5 μg of protein ($\approx$10 pmol of toxin-binding sites) per 1 ml of solution was added to the front compartment. Experimental conditions were the same as for Fig. 1, traces a–c. Traces a and d, s-events; traces b and e, m-events; traces c and f, p-events. For details see text.

must await measurements under identical transmembrane ion concentrations. Another problem to be solved in further *in vitro* experiments is the change with time of the gating behavior from s-type events to the m-type and p-type of gating.

Our studies suggest that the ionic channel resides in the protein moiety of the AcChoR: (*i*) Alkaline extraction of the non-receptor M_r 43,000 peptide from AcChoR-rich membrane fragments had only minor effects on the gating and conductance properties of the reconstituted system. (*ii*) Successful reconstitution was also achieved with AcChoR solubilized with nonionic detergents and purified by affinity chromatography on α-cobratoxin-Sepharose columns. In sodium dodecyl sulfate/polyacrylamide gel electrophoresis this receptor had a subunit pattern consistent with a stoichiometry of $\alpha_2\beta\gamma\delta$ (20, 21) and was made up of a mixture of monomers, dimers and some higher oligomers. (*iii*) A preparation of receptor monomers¶ obtained by treatment of purified receptor with dithiothreitol and *N*-ethylmaleimide had channel properties similar to those of the monomer/dimer mixture. We do not know yet whether dimers also display analogous channel properties, but we can certainly define the receptor monomer as the structure in which the ion channel resides. This channel may be composed of one or more subunits of the receptor or it may be formed by parts of the surfaces of receptor subunits. The involvement of minor amounts of endogenous lipids cannot yet be excluded, should they bind tightly enough to the receptor protein to endure the solubilization and purification procedures. However, bulk quantities of endogenous lipids do not seem to be required. It has to be emphasized in this context that the observed current fluctuations occurred below the phase transition temperature of 1,3-SMPC—i.e., in the frozen state of the membrane matrix (10).

The successful reconstitution of an excitable membrane from a synthetic phospholipid and a receptor protein offers the possibility to analyze synchronously major functional properties of membrane-receptor systems, ligand binding, and channel formation. The similarity in channel properties of this artificial system and intact muscle membrane strongly suggests that the chemically activated conductance increase in nicotinic synaptic membranes is an intrinsic property of the AcChoR proteins.

¶ "Dimer" refers to two monomers linked covalently by a disulfide bridge between their δ subunits (22, 23). The formation of noncovalently linked dimers or oligomers in the membrane-bound state is not excluded.

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