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Patch-recorded single-channel currents of the purified and reconstituted *Torpedo* acetylcholine receptor

(membrane channel/patch-clamp/liposomes/desensitization)

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ABSTRACT **Small unilamellar vesicles containing purified and reconstituted nicotinic acetylcholine receptors from *Torpedo* electroplax have been fused by a simple freeze-thaw procedure to form large liposomes. Giga-seal patch-recording techniques were used to form isolated patches of liposome-membrane and to measure single-channel properties of the reconstituted receptor–ion channel complex. The observed properties are quantitatively similar to those reported for vertebrate muscle nicotinic acetylcholine receptor species recorded *in situ*. The results demonstrate that the pentameric complex consisting of the $\alpha_2\beta\gamma\delta$ subunits is fully functional. The methods used in these experiments should be useful in studying the effects of chemical alterations on the properties of acetylcholine receptor channels as well as other types of purified and reconstituted ion channels.**

The nicotinic acetylcholine receptor from *Torpedo* electroplax is a chemically gated ion channel consisting of a pentameric complex of the five subunits $\alpha_2\beta\gamma\delta$ (for reviews, see refs. 1 and 2). It is reproducibly purified and, when reconstituted into small liposomes or planar lipid bilayers, it exhibits the basic agonist-induced cation transport properties anticipated from electrophysiological measurements *in vivo* (3–10). By applying recently developed methods (11, 12) for patch-recording from large liposomes containing reconstituted ion channels, we have now measured, with high resolution, single-channel properties of the purified and reconstituted *Torpedo* acetylcholine receptor. We report here that this pentameric complex showed an agonist-independent main conductance level, a subconductance state, saturation of the single-channel conductance at high Na^+ concentration, slow inactivation and burst-kinetics at desensitizing concentrations of agonists, and open channel lifetime distributions that are not described by single exponentials. The similarity of these properties with those reported for other vertebrate acetylcholine receptor species recorded *in situ* provides further evidence that the purified receptor is fully functional and that the well-characterized biochemical properties associated with this isolated receptor complex may apply to a wide class of nicotinic receptor ion channels.

MATERIALS AND METHODS

Preparation of Liposomes Containing Reconstituted Acetylcholine Receptor. Acetylcholine receptor was isolated, purified, and reconstituted as described (4). Briefly, postsynaptic membranes rich in acetylcholine receptor were prepared from freshly dissected electric organ of *Torpedo Californica*. The receptor was then purified from a Na cholate extract of the postsynaptic membranes by affinity chromatography on a choline carboxymethyl affinity gel as described by Huganir and Racker (4), except that the loaded column was washed with 20 column vol of "wash buffer" before elution. The purified and solubilized receptor was incorporated into asolectin (Associated Concentrates, Woodside, NY) small unilamellar vesicles by the detergent dialysis technique. The lipid-to-protein ratio was 100:1 to 50:1 by weight. Aliquots (50–100 μl) of reconstituted vesicles suspended at 25 mg of lipid per ml in 100 mM NaCl/50 mM KCl/0.1 mM EDTA/0.1 mM EGTA/10 mM NaP_i, pH 8.0, were frozen rapidly and stored in liquid nitrogen. A frozen sample was thawed on ice before an experiment, and the large liposomes produced by this "freeze-thaw" procedure were diluted (>2,000 fold) into a salt solution used for single channel recording. Except as noted in the text, this salt solution consisted of 150 mM NaCl/1 mM $CaCl_2$/1 mM $MgCl_2$/10 mM Hepes, pH 7.2 (buffer A).

Patch-Recording from Freeze-Thaw Liposomes. Single-channel currents of channels reconstituted into freeze-thaw liposomes were recorded by using patch-clamp techniques (13) as described (12). Briefly, a liposome was observed with a phase-contrast microscope (×400 magnification) and a giga-seal was formed on an exposed surface bilayer region by using a Sylgard-coated fire-polished patch pipette (TW150-6 borosilicate glass; W-P Instruments, Waltham, MA). The interior of the pipette always contained the same salt solution used to bathe the liposomes but was supplemented with a low concentration of nicotinic agonist. When this salt solution was buffer A, pre-seal electrode resistances were <10 MΩ and giga-seals typically showed resistances of 50 GΩ or more. After sealing, withdrawal of the pipette from the liposome surface and, if necessary, brief passage of the tip through the air/water interface resulted in an inside-out patch of liposome-derived bilayer spanning the orifice of the pipette. The isolated bilayer separated symmetrical salt solutions, and patches were stable for several hours. A patch-clamp system similar to that described by Hamill *et al.* (13) was used to apply a holding voltage (V_h) to the pipette interior (bath held at zero voltage) and to record single-channel currents. Data were stored on FM tape. Except as noted in the text, all experiments were done at room temperature (20–22°C).

RESULTS AND DISCUSSION

Nicotinic Agonist-Induced Single-Channel Currents. Rapid freezing of a suspension of asolectin small unilamellar vesicles containing reconstituted acetylcholine receptor, by immersion in liquid nitrogen and subsequent thawing, resulted in the formation of large (>10 μm in diameter) multilamellar liposomes. In previous reports (11, 12) it has been shown that giga-seal patch-recording techniques (13) can be applied to these freeze-thaw

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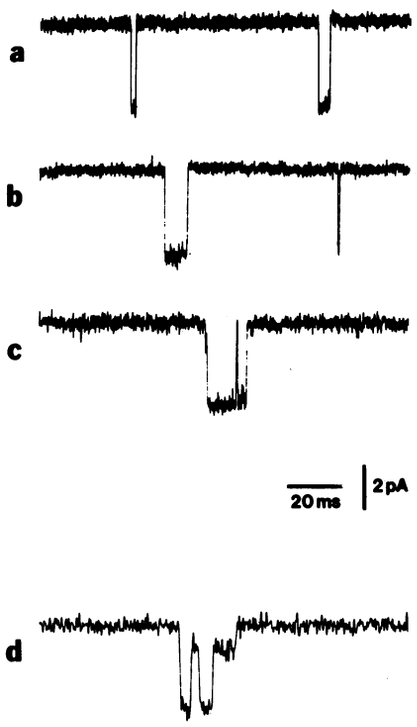

FIG. 1. Main conductance state (*a–c*) and a substate (*d*) of the purified and reconstituted *Torpedo* acetylcholine receptor. (*a*) Carbamoylcholine-activated single-channel currents. Pipette holding potential, +100 mV; 20 μM carbamoylcholine; 4-kHz bandwidth. (*b*) Acetylcholine-activated single-channel currents. Pipette holding potential, +100 mV; 1 μM acetylcholine; 4-kHz bandwidth. (*c*) Suberyldicholine-activated single-channel currents. Pipette holding potential, +100 mV; 0.5 μM suberyldicholine; 4-kHz bandwidth. (*d*) Two transitions from the main conductance state to a subconductance state during an acetylcholine-activated channel opening event. Pipette holding potential, +100 mV; 1 μM acetylcholine; 4-kHz bandwidth.

liposomes. Inside-out isolated patches (13, 14) of liposome-derived lipid bilayer, analogous to cell-membrane-derived isolated patches, can be formed, allowing single-channel currents to be recorded through reconstituted ion channels.

Using these methods, a low concentration of a nicotinic agonist in the pipette reproducibly induced discrete unit conductance changes (Fig. 1) in isolated patches. If no nicotinic agonist was present in the pipette, these single-channel currents were not observed in recordings lasting several minutes. [A rigorous search for "spontaneous" opening events (15) was not pursued.] We interpret these fluctuations as the opening and closing of individual reconstituted *Torpedo* acetylcholine receptor channels. When a holding potential of +100 mV was applied to the pipette interior, with the patch separating symmetrical salt solutions of buffer A, only one main current level, with a mean value of 4.2 pA, was observed for carbamoylcholine (Fig. 1*a*), acetylcholine (Fig. 1*b*), and suberyldicholine (Fig. 1*c*). The single-channel current–voltage relationship for this main conductance state in buffer A showed no obvious departure from linearity below 100-mV membrane potential and the reversal potential extrapolated close to 0 mV (data not shown). The slope conductance at 22°C was 42 pS.

A subconductance state of amplitude 1.0–1.2 pA at +100-mV membrane potential was occasionally observed (Fig. 1*d*). Not all patches showed channel opening events displaying transitions to this subconductance state, and in those patches that did, these transitions were relatively rare. Subconductance states of similar amplitude have been reported for nicotinic acetylcholine receptor channels recorded *in situ* in several different preparations of cultured muscle cells (16–18).

When the pipette interior was held at −100 mV and a low concentration of agonist was added to the bath, a channel opening event was only rarely observed. Previous experiments with isolated patches from freeze-thaw liposomes (11) have indicated that only a single bilayer spans the tip of the pipette. Therefore, it is unlikely that restricted access to the bilayer surface is responsible for this ineffectiveness of bath-applied agonist to induce channel opening events. Acetylcholine receptor reconstituted by detergent dialysis techniques into small unilamellar vesicles has a primarily unidirectional orientation (4). If this preference is conserved during the freeze-thaw step (as would be expected from simple fusion of small vesicles), then the agonist binding site would be expected to be on the pipette side of the "inside-out" isolated patch, consistent with our observations.

Ion Concentration and Temperature Affect the Single-Channel Conductance. To further characterize the permeation properties of single *Torpedo* acetylcholine receptor channels, we determined the dependence of the single-channel currents upon the concentration of NaCl in the recording salt solution. Freeze-thaw liposomes were diluted into a series of different salt solutions consisting of 1 mM $CaCl_2$/1 mM $MgCl_2$/10 mM Hepes, pH 7.2, and either 0.1, 0.15, 0.25, 0.3, or 0.5 M NaCl. The pipette always contained the same salt solution as the bath but was supplemented with 1 μM acetylcholine to activate single-channel currents. These currents were recorded with a pipette holding potential of +100 mV and mean channel amplitudes (± SEM) were determined from 30–50 opening events. As shown in Fig. 2, the size of the single-channel currents at +100 mV increased with the Na^+ activity ($[Na^+]$) of the recording salt solution. However, this increase was not linear. At a Na^+ concentration of 79 mM (0.1 M NaCl), the single-channel current at +100-mV holding potential was 3.4 pA. At greater than four times this activity (341 mM Na^+ concentration, corresponding to 0.5 M NaCl), the single-channel current was slightly less than twice as large (6.5 pA), indicating saturation of the channel conductance.

Horn and Patlak (14) have observed that for acetylcholine receptors present on cultured rat "myoballs," there is an apparent saturation of the single-channel conductance with increasing concentration of the permeant cation. In their study, the dependence of the single-channel current on $[Na^+]$ was ac-

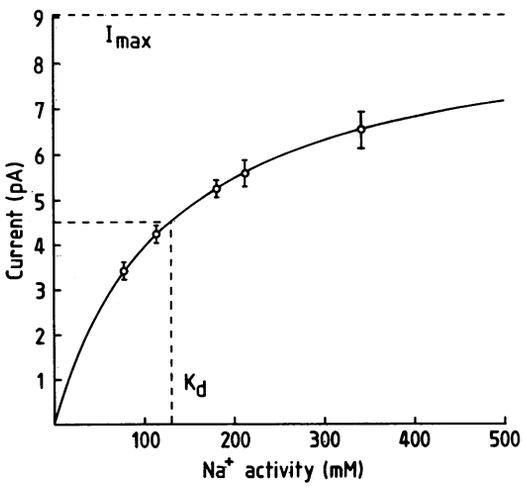

FIG. 2. Saturation of the single-channel main conductance level with increasing NaCl concentration. A curve of the form $I = I_{max}[Na^+]/(K_d + [Na^+])$ was fit to the data by using a least-squares fitting routine. The best-fit parameters were I_{max} = 9.1 pA and K_d = 129 mM $[Na^+]$.

curately fit by a rectangular hyperbola characterized by a maximal current of 8.3 pA and an apparent dissociation constant (the $[Na^+]$ at which the single-channel current is half-maximal) of K_d = 104 mM $[Na^+]$. This dependence is predicted if ions move through the channel in a single file (19). Our measured dependence of reconstituted *Torpedo* acetylcholine receptor single-channel currents upon Na^+ activity was consistent with the same kinetic model (Fig. 2). The least-squares best-fit curve was characterized by I_{max} = 9.1 pA and K_d = 129 mM $[Na^+]$ (corresponding to 180 mM NaCl), in good agreement with the data on cultured rat myoballs. Hence, this permeation property of nicotinic acetylcholine receptor channels is similar in two distantly related vertebrate species and is conserved during the purification and reconstitution procedures used in the present study.

The temperature dependence of the single-channel conductance in buffer A was measured by recording single-channel currents activated by 1 μM acetylcholine with the bath temperature held constant at 22°C, 14°C, and 9°C. The pipette holding potential was +100 mV and mean single-channel currents (±SEM) were calculated by averaging 30–50 opening events. The amplitude of single-channel current decreased monotonically with decreasing temperature. An Arrhenius plot of the data is shown in Fig. 3. The solid line represents a linear least-squares fit to the data and corresponds to an apparent entropy of activation of 9.1 kcal mol^{-1}.

Desensitization and Burst-Kinetics. Even at low agonist concentrations, our initial attempts at recording single reconstituted *Torpedo* acetylcholine receptor channels (11) were hampered by the transience of the period during which opening events were observed. However, further work showed that many channel openings could be recorded if patch isolation, application of the pipette holding potential, and data acquisition occur quickly after sealing of the agonist-containing pipette to the liposome surface. Nevertheless, the frequency of channel opening always decayed eventually to an extremely low equilibrium level. An example of this time dependence in a patch is shown in Fig. 4 *a–c*. In this experiment, the isolated patch separated symmetrical buffer A salt solutions and 1 μM acetylcholine was present in the pipette. The arrow in Fig. 4*a* indicates the time at which the holding potential of the pipette was changed from a continuous train of small (200 μV) voltage

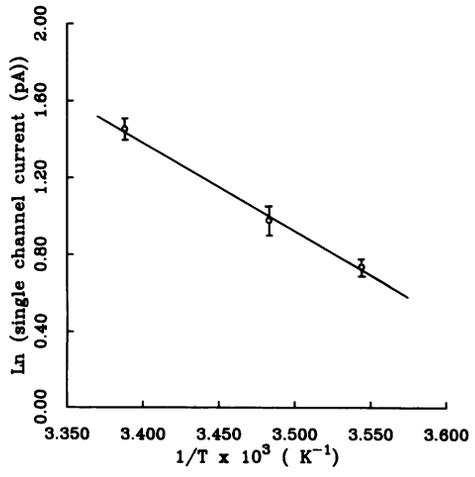

FIG. 3. Arrhenius plot of the single-channel conductance. A temperature-controlled microscope stage was used to cool the pipette bathing solution below room temperature. Bath temperature was held constant to within 1°C. Channels were activated by 1 μM acetylcholine and the isolated patches separated symmetrical buffer A salt solutions.

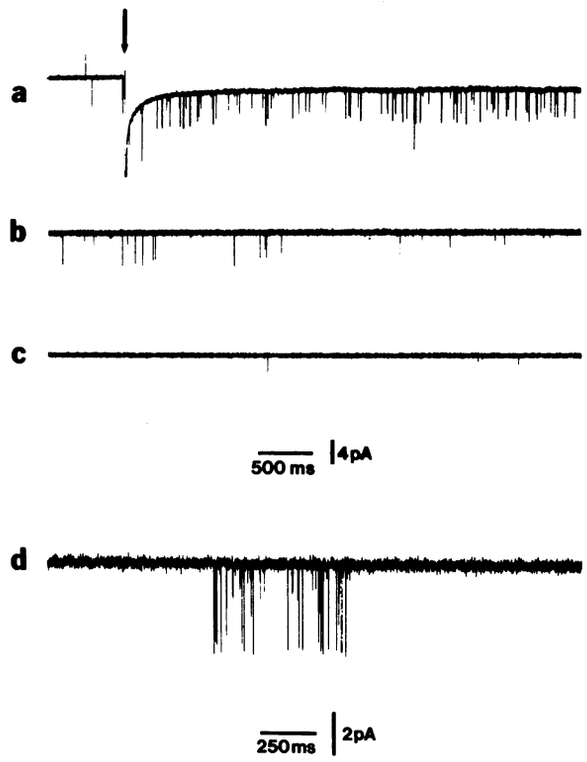

FIG. 4. Slow inactivation (*a–c*) and burst-kinetics (*d*) of the reconstituted *Torpedo* acetylcholine receptor. Shown in *a–c* are 10-s segments, at 1-kHz bandwidth, taken from a continuous record of channel opening events in a single patch. Channels were activated by 1 μM acetylcholine present in the pipette. In *a*, at the time point indicated by the arrow, the pipette holding potential was changed to +150 mV. The segments in *b* and *c* began 2.5 min and 5 min, respectively, after giga-seal formation. Shown in *d* is an example of burst-kinetics of channel opening with 10 μM acetylcholine present in the pipette. Pipette holding potential, +100 mV; 2-kHz bandwidth. All opening events that did not reach the main conductance level of 4.2 pA are bandwidth limited.

pulses used to test for giga-seal formation (note that, to the left of the arrow, only a pair of capacitative transients were induced by this voltage pulse, indicating a GΩ seal) to the pipette holding potential of +150 mV, which then remained on for the rest of the recording. This change, which allowed immediate observation of channel opening events, occurred 14 s after the pipette touched the liposome surface and first exposed the acetylcholine receptors in the isolated patch to agonist. Note the initial high rate of single-channel opening in Fig. 4*a*, which resulted in several instances of transitions in the current record to a level corresponding to two simultaneously open channels. The segments in Fig. 4 *b* and *c* began 2.5 and 5 min after initial giga-seal formation; there was a slow decay in channel opening events to an extremely low equilibrium level.

By counting the number of opening events per unit time at different intervals after the establishment of the giga-seal, we could quantitate this decay. As shown in Fig. 5, it was consistent with an exponential decay in the frequency of opening events; with 1 μM acetylcholine and a holding potential of +150 mV, the single time constant of the decay was 92 s. At higher concentrations of acetylcholine there was a more rapid rate of inactivation, and opening events that were observed occurred in infrequent bursts. One such burst in the presence of 10 μM acetylcholine is shown in Fig. 4*d*; long quiet periods were interrupted by brief periods (0.1–1 s) of rapid opening and closing events.

We attribute the slow decay and the burst-kinetics to different steps in the inactivation (desensitization) of the receptor.

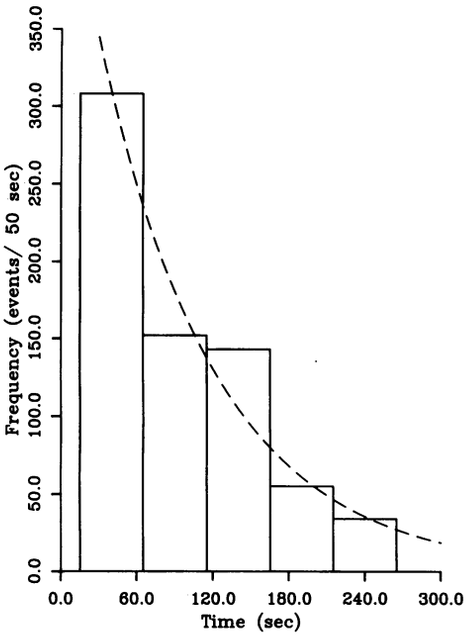

FIG. 5. Exponential decay with time in the frequency of channel opening events. Data were taken from a single continuous record, segments of which are shown in Fig. 4 *a–c*. The number of channel opening events in successive 50-s intervals after the application of the pipette holding potential was determined and plotted against the mid-time of these intervals, measured relative to the time of giga-seal formation. Least-squares linear regression was used to calculate the best-fit straight line through a semilogarithmic plot of the same data. This fitted exponential is shown as the dashed curve. The time constant of decay was 92 s.

Single-channel burst-kinetics are observed with frog perisynaptic (20) and rat "myoball" (16) acetylcholine receptors. Bursts are interpreted as representing rapid opening and closing of a single acetylcholine receptor molecule during its transient return from a desensitized to an active state. Also, bursts occur in clusters in these preparations suggesting that there are at least two distinguishable kinetic processes in desensitization (16). We have also observed that bursts of the purified and reconstituted acetylcholine receptor can occur in clusters (not shown). Two inactivation rates have also been observed by using rapid-mixing techniques to measure radiolabeled cation flux in native membrane vesicles from *Torpedo* (21) and in reconstituted liposomes containing *Torpedo* acetylcholine receptor (22). In these experiments, a fast inactivation occurs in the range of 0.1–1 s and a slow inactivation occurs on the minute time scale. The rates of both the fast and slow inactivation processes were increased with increasing concentrations of agonist. The second, slower, inactivation leads to an equilibrium level that has undetectable agonist-induced ion flux. The durations of individual bursts that we have observed in our patches at 10 μM acetylcholine are consistent with the fast inactivation rate measured in the rapid-mixing experiments with *Torpedo* acetylcholine receptor. Likewise, the slow inactivations of the channel opening rate that we have observed after exposure to 10 or 1 μM acetylcholine are consistent with the reported time constants for slow inactivation to a very small equilibrium population of active acetylcholine receptors.

Distribution of Open-Channel Lifetimes. Typical open channel lifetimes (see Fig. 1) were in the range of <1.0–10 ms. This time scale is consistent with the time course of the decay of focally recorded spontaneous miniature endplate potentials at *Torpedo* electroplax neuromuscular junctions (see, for example, ref. 23). By using a high density of acetylcholine receptors per unit area of vesicle surface (corresponding to about 100–200 receptors per square micron) or pooling data from several patches (or both) we could record a sufficient number of channel openings, despite desensitization, to study the statistical distribution of open times. One such distribution for channels activated by 20 μM carbamoylcholine (buffer A salt solution) with a holding potential of +100 mV is shown in Fig.

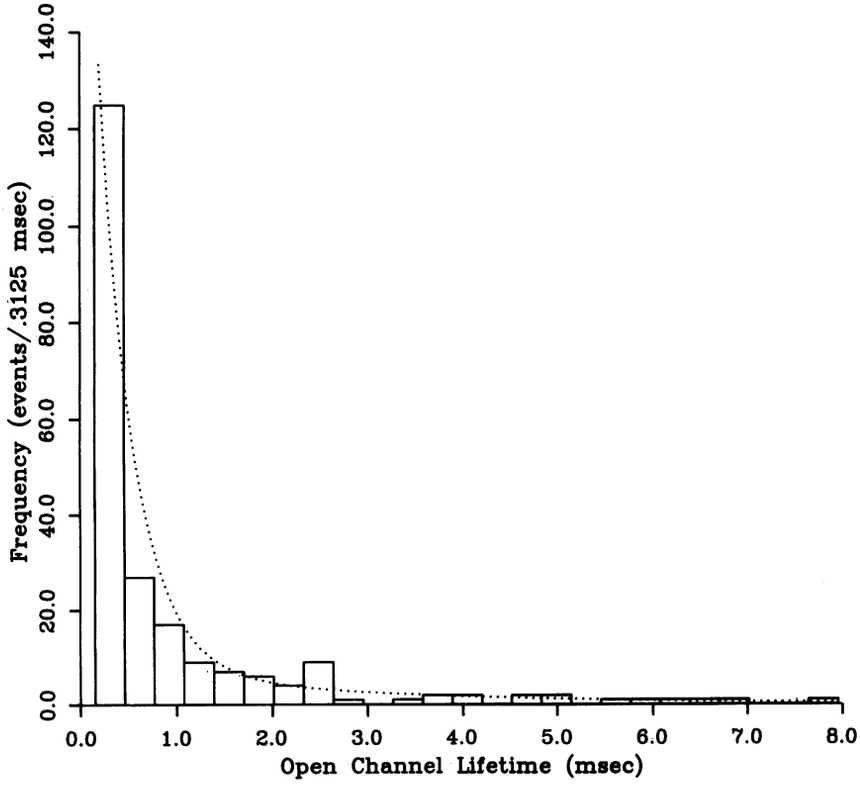

FIG. 6. Open-channel lifetime distribution of *Torpedo* acetylcholine receptors in a single patch. The patch separated symmetrical buffer A salt solutions and 20 μM carbamoylcholine was present in the pipette. The holding potential was +100 mV and channels were recorded on an FM tape at 5-kHz bandwidth. Data recording started ≈10 s after sealing of the pipette to the liposome surface (an isolated patch was produced in this interim period) and 250 events were observed in the subsequent 5-min period. The tape record was subsequently replayed at decreased speed and open-channel lifetimes were obtained by direct measurement on a storage oscilloscope of all opening events. For each opening the time between the point at which the channel opened above one-fourth of the full open channel amplitude and the point at which it dropped below this level were recorded. These times were subsequently corrected to correspond to the lifetime of idealized full-amplitude current pulses that would give the same one-fourth amplitude duration when injected into the patch-clamp headstage amplifier (24). These data were used to generate a histogram of the number of observed lifetimes falling within successive lifetime intervals of 0.3125 ms. Openings with lifetimes less than 0.160 ms were not reliably detected and hence not included in the histogram. The dotted curve shows a double-exponential distribution given by $A_{fast}\exp(-t/\tau_{fast}) + A_{slow}\exp(-t/\tau_{slow})$ with $A_{fast} = 216$, $A_{slow} = 2.0$, $\tau_{fast} = 0.38$ ms, and $\tau_{slow} = 2.8$ ms.

6. This distribution could not be fit by a single exponential. However, a good fit was obtained to the sum of two exponentials with characteristic mean times of $\tau_{fast} = 0.38$ ms and $\tau_{slow} = 2.8$ ms. A double-exponential distribution of open times is also observed for frog perisynaptic acetylcholine receptors (24) and for acetylcholine receptors on several species of cultured muscle cells (25, 26). Two exponentials could result from two different open molecular states of a single population of acetylcholine receptors with indistinguishable conductance, from two populations of acetylcholine receptors with the same conductance, or from more complicated kinetic schemes. Although a study of the correlation between fast and slow opening events has ruled out several kinetic models (26), further analysis is needed to provide an unambiguous association with one of the remaining models.

CONCLUSIONS

Using methods for patch-recording from ion channels reconstituted into large liposomes, we have shown that the purified and reconstituted *Torpedo* acetylcholine receptor, the most biochemically characterized chemically gated ion channel, shares single-channel permeation and gating properties with acetylcholine receptors recorded *in situ* from vertebrate muscle preparations. The results demonstrate unambiguously that the purified receptor complex, consisting of five subunits with a stoichiometry of $\alpha_2\beta\gamma\delta$, contains all of the necessary components for complete function. The biochemical characterization of vertebrate muscle receptors is far less complete than for the *Torpedo* acetylcholine receptor, but interesting similarities in subunit stoichiometry and sequence homology have recently been reported (27). The similarity in channel properties further suggests that biochemical information accumulated for the *Torpedo* receptor may apply to the general class of vertebrate muscle nicotinic acetylcholine receptors.

Our experimental methods should be of general use in the attempt to correlate ion channel structure with transport properties. In particular, the ability to chemically modify a protein complex under controlled conditions before reconstitution should allow study of the regulation of channel properties by covalent protein modification. For example, the *Torpedo* acetylcholine receptor is phosphorylated by a cAMP-dependent protein kinase (28), and the state of phosphorylation of the receptor appears to change during development from neonatal to adult (29). It will be interesting to see if changes in the state of phosphorylation of the purified and reconstituted channel correlate with changes in single-channel properties that have been observed during synaptogenesis at vertebrate neuromuscular junctions (see refs. in ref. 30). The basic properties of the adult unmodified *Torpedo* acetylcholine receptor that we have presented here provide a starting point for that investigation.

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1. Changeux, J.-P. (1981) *Harvey Lect.* **75,** 85–254.
2. Conti-Tranconi, B. M. & Raftery, M. A. (1982) *Annu. Rev. Biochem.* **51,** 491–530.
3. Huganir, R. L., Schell, M. A. & Racker, E. (1979) *FEBS Lett.* **108,** 155–160.
4. Huganir, R. L. & Racker, E. (1982) *J. Biol. Chem.* **257,** 9372–9378.
5. Changeux, J.-P., Heidmann, T., Popot, J. L. & Sobel, A. (1979) *FEBS Lett.* **105,** 181–187.
6. Lindstrom, J., Anholt, R., Einarson, B., Engel, A., Osame, M. & Montal, M. (1980) *J. Biol. Chem.* **255,** 8340–8350.
7. Wu, W. C. S. & Raftery, M. A. (1979) *Biochem. Biophys. Res. Commun.* **89,** 26–35.
8. Nelson, N., Anholt, R., Lindstrom, J. & Montal, M. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 3057–3061.
9. Boheim, G., Hanke, W., Barrantes, F. J., Eibl, H., Sakmann, B., Fels, G. & Maelicke, A. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 3586–3590.
10. Schindler, H. & Quast, U. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 3052–3056.
11. Tank, D. W., Miller, C. & Webb, W. W. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 7749–7753.
12. Tank, D. W. & Miller, C. (1983) in *Single Channel Recording*, eds. Neher, E. & Sakmann, B. (Plenum, New York), in press.
13. Hamill, O. P., Marty, A., Neher, E., Sakmann, B. & Sigworth, F. J. (1981) *Pflügers Arch.* **391,** 85–100.
14. Horn, R. & Patlak, J. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 6930–6934.
15. Sanchez, J. A., Dani, J. A., Siemen, D. & Hille, B. (1983) *Biophys. J.* **41,** 65a (abstr.).
16. Hamill, O. P. & Sakmann, B. (1981) *Nature (London)* **294,** 462–464.
17. Auerbach, A. & Sachs, F. (1983) *Biophys. J.* **42,** 1–10.
18. Trautmann, A. (1982) *Nature (London)* **298,** 272–275.
19. Lauger, P. (1973) *Biochim. Biophys. Acta* **311,** 423–441.
20. Sakmann, B., Patlak, J. & Neher, E. (1980) *Nature (London)* **286,** 71–73.
21. Walker, J. W., McNamee, M. G., Pasquale, E., Cash, D. J. & Hess, G. P. (1981) *Biochem. Biophys. Res. Commun.* **100,** 86–90.
22. Walker, J. W., Takeyasu, K. & McNamee, M. G. (1982) *Biochemistry* **21,** 5384–5389.
23. Miledi, R., Molinoff, P. & Potter, L. T. (1971) *Nature (London)* **229,** 554–557.
24. Colquhoun, D. & Sakmann, B. (1981) *Nature (London)* **294,** 464–466.
25. Jackson, M. P. & Lecar, H. (1982) *Biophys. J.* **37,** 310a (abstr.).
26. Jackson, M. B., Wong, B. S., Morris, C. E., Lecar, H. & Christian, C. N. (1983) *Biophys. J.* **42,** 109–114.
27. Conti-Tranconi, B. M., Gotti, C. M., Hunkapiller, M. W. & Raftery, M. A. (1982) *Science* **218,** 1227–1229.
28. Huganir, R. L. & Greengard, P. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 1130–1134.
29. Saitoh, T. & Changeux, J.-P. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 4430–4434.
30. Fambrough, D. (1979) *Physiol. Rev.* **59,** 165–227.