Multiple conductance classes of mouse nicotinic acetylcholine receptors expressed in *Xenopus* oocytes

(receptor assembly/single channel recording)

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ABSTRACT Acetylcholine receptor (AcChoR) subunit mRNAs transcribed from mouse BC3H-1 cDNAs were injected into *Xenopus* oocytes and the expressed AcChoR channels were examined by single channel recording. Injection of α -, β -, γ -, and δ -subunit mRNAs produced two predominant channel classes with conductances of ≈ 50 and ≈ 12 pS, while infrequent openings of ≈ 25 -pS channels were also observed. Injection of α -, β -, and γ -subunit mRNAs produced a single class of ≈ 12 -pS AcChoR channels, which resembled the smallest conductance channels present in $\alpha\beta\gamma\delta$ -injected oocytes. Assembly of δ -less channels may thus explain the lowest conductance AcChoR channels in $\alpha\beta\gamma\delta$ -injected oocytes and might also account for similar channels that have been observed in vertebrate skeletal muscle.

The nicotinic acetylcholine receptor (AcChoR) of vertebrate skeletal muscle is a channel-forming pentamer thought to be composed of either $\alpha_2\beta\gamma\delta$ or $\alpha_2\beta\delta\epsilon$ subunits. The *Xenopus* oocyte expression system (1–3) has been used in recent years to explore the roles of different subunits in determining receptor properties. By this means it has been shown that two principal conductance classes (40 and 60 pS) of mammalian AcChoRs differ in subunit composition, the larger conductance channel having an ϵ subunit in place of γ (4). It has also been reported that functional channels can be assembled when one of the subunits is missing. In the case of *Torpedo* AcChoRs, weak responses to AcCho were observed in some oocytes that were lacking γ or δ subunits (5, 6). The single channel properties of receptors lacking γ or δ subunits have not been described. Although both kinds of receptors assemble less efficiently and the δ -less receptors have reduced agonist affinity (5), the weak responses to AcCho might also reflect a reduced conductance or a briefer open time. We were interested in examining the properties of these receptors because they might offer an explanation for the small conductance channels (10–25 pS) observed in developing amphibian muscle (7, 8). We injected mRNAs encoding the mouse α , β , γ , and δ subunits into *Xenopus* oocytes and found that multiple conductance classes of channels were expressed on the oocyte membrane, and one of these, the smallest, is due to deletion of the δ subunit.

MATERIALS AND METHODS

mRNA Preparation and Injection. The cDNA clones encoding mouse AcChoR subunits were generously provided by Jim Boulter (Salk Institute). mRNAs for α , β , γ , and δ subunits were individually transcribed with SP6 polymerase. The plasmids were linearized with *Hind*III (clones BMA 407, BMB 49, and BMD 451 encoding α , β , and δ subunits, respectively) or *Eco*RI (clone BMG 419, encoding γ subunit).

The transcription reactions were carried out under standard conditions (Promega) and typically contained 2–3 μ g of linearized cDNA template. The transcripts were resuspended in nuclease-free water at a concentration of 200 ng/ μ l (α -subunit transcript) or 100 ng/ μ l (β -, γ -, and δ -subunit transcripts). Samples were aliquoted and stored at -90°C until used. Oocytes were surgically removed from adult female *Xenopus laevis* frogs and were stored at 17°C in saline containing 115 mM NaCl, 1.5 mM KCl, 1.8 mM CaCl_2 , 10 mM Hepes (pH 7.4), and 50 μ g of gentamycin per ml (Sigma). The follicular envelope was mechanically removed from oocytes to be injected. The mRNA used for injection was premixed from equal volumes of stock solutions of the different subunits. Each oocyte was injected with 50 nl of mRNA mixture.

Electrophysiology. During electrophysiology experiments, the oocytes were continuously perfused with a solution containing 120 mM NaCl, 1 mM KCl, 1.8 mM CaCl_2 , and 10 mM Hepes (pH 7.4). To elicit whole cell currents, the perfusing solution was switched to one containing AcCho (1–100 μM). Oocytes were screened for responses to AcCho by using a conventional two-electrode voltage clamp (Axioclamp 2A). In $\alpha\beta\gamma\delta$ -injected or $\alpha\beta\gamma$ -injected oocytes, responses to AcCho were usually large enough by 1 day after injection to begin single channel studies. All single-channel data were obtained within 3 days after injection. Endogenous muscarinic responses to AcCho were observed in some oocytes, but they could be distinguished from the nicotinic currents by their delayed onset and repetitive oscillation in the current amplitude. To prepare the oocytes for single channel recording, they were placed in a hypertonic solution containing 200 mM potassium aspartate, 10 mM KCl, 10 mM potassium EGTA, and 10 mM Hepes (pH 7.4) for ≈ 10 min prior to removal of the vitelline membrane (9). Patch electrodes were filled with a solution containing 80 mM KF, 20 mM KCl, 10 mM potassium EGTA, and 10 mM Hepes (pH 7.4) (9). All recordings were obtained from outside-out patches (10) at room temperature (20°C – 24°C). To activate AcChoR channels, the bath was perfused with a solution containing AcCho (10–100 nM). The calcium concentration of the AcCho-containing solution was lowered to 0.5 mM (other salts unchanged), thereby reducing the activation of chloride currents by calcium entering through AcChoR channels (3). Single channel events were recorded by a List EPC/7 patch clamp amplifier, and stored on FM tape for off-line analysis. The procedures for data analysis were similar to those described (7). Records from $\alpha\beta\gamma\delta$ -injected oocytes were low-pass Bessel filtered at 2 kHz. To improve the signal/noise ratio, records from $\alpha\beta\gamma$ -injected oocytes

Abbreviations: AcCho, acetylcholine; AcChoR, acetylcholine receptor.

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FIG. 1. Whole cell AcCho-induced current vs. membrane holding potential in voltage-clamped oocytes. The peak whole cell current in response to $1 \mu\text{M}$ AcCho is plotted against holding membrane potential for an $\alpha\beta\gamma\delta$ -injected oocyte (○) and an $\alpha\beta\gamma$ -injected oocyte (●). The oocytes were voltage clamped first at positive potentials and then shifted negatively in 10-mV steps. The same quantity of AcCho was applied in each trial.

were filtered at 1 kHz. The briefest openings accepted for kinetic analysis were either $300 \mu\text{s}$ (2-kHz records) or $500 \mu\text{s}$ (1-kHz records). Multiple events were excluded from the analysis; therefore, the proportion of longer events and the apparent mean channel open time may have been underestimated in some records. All data are reported as the means $\pm$ SD.

RESULTS

Injection of α -, β -, γ -, and δ -subunit mRNAs. Injection of oocytes with α -, β -, γ -, and δ -subunit mRNAs resulted in substantial expression of nicotinic AcChoRs within 24 hr. Bath-applied AcCho ($1 \mu\text{M}$) elicited prompt inward currents of up to several microamperes. From the current-voltage relationships, as shown in Fig. 1, the reversal potential of the

FIG. 2. Single channel events recorded from outside-out patches in response to AcCho. (A) Slow sweep records illustrate the onset of single channel activity after bath application of 100 nM AcCho at the arrows. (Left) Trace was obtained from an $\alpha\beta\gamma\delta$ -injected oocyte and the patch was held at -100 mV . (Right) Trace was taken from an $\alpha\beta\gamma$ -injected oocyte and the patch was held at -160 mV . (B) Faster sweep records illustrate single channel events recorded from patches of an $\alpha\beta\gamma\delta$ -injected oocyte (Left) and an $\alpha\beta\gamma$ -injected oocyte (Right). The patches were held at -180 mV for both sets of records and were exposed to 100 nM AcCho. Disregarding overlapping events, four discrete current levels can be distinguished in the $\alpha\beta\gamma\delta$ patch (see also Fig. 3), whereas only one current level is apparent in the $\alpha\beta\gamma$ patch, and it closely corresponds to that of the smallest events in the records on the Left.

AcCho-induced whole cell current was estimated to be $-7.4 \pm 4.3 \text{ mV}$ in four oocytes. The AcCho-induced current rectified inwardly at negative membrane potentials, as reported by others for mammalian AcChoRs expressed in oocytes (11, 12). Oocytes that responded to bath-applied AcCho with a substantial whole cell current were studied further by single channel recording using the outside-out patch configuration. In the absence of bath-applied AcCho, most patches exhibited little or no spontaneous channel activity. We routinely tested patches for the presence of stretch-activated channels by applying suction to the pipette. Patches that had stretch-activated channels or high levels of spontaneous activity were not used. In quiet patches, flooding the bath with 100 nM AcCho elicited channel activity, as illustrated in Fig. 2A, after a brief delay for fluid exchange.

Single channel records revealed two major classes of high and low current levels, which differed in amplitude by ≈ 4 -fold. The larger amplitude events were present in all patches studied. With strong hyperpolarization (-140 to -200 mV), the smaller amplitude events were also evident in most patches (9 of 13) and constituted $\approx 1/3$ rd of the observed openings in those patches. At a holding potential of -180 mV , the average amplitude of the large events was $-8.8 \pm 0.7 \text{ pA}$ (eight patches) as compared to $-1.9 \pm 0.5 \text{ pA}$ (seven patches) for the small events. Examination of records, such as shown in Fig. 2B, suggests that both the low and high amplitude classes may be composed of two or more closely spaced amplitude levels. This is reflected in multiple peaks of the amplitude histograms (Fig. 3). However, in only a few exceptional cases (at membrane potentials of -160 to -200

FIG. 3. Amplitude histograms of single channel events. Histograms were compiled from records obtained from outside-out patches of an $\alpha\beta\gamma\delta$ -injected oocyte (Upper) and an $\alpha\beta\gamma$ -injected oocyte (Lower). These histograms were taken from the same recordings shown in Fig. 2B. The patches were clamped at -180 mV . (Upper) Two small current levels are clearly resolved with mean amplitudes of -1.5 and -3.1 pA . The separation of small amplitude classes shown in this histogram was unusually clear, and this patch had relatively more small events than normal. The smallest group of events in the $\alpha\beta\gamma\delta$ histogram closely corresponded to the single class of events seen in the $\alpha\beta\gamma$ histogram, which had a mean amplitude of -1.5 pA . There is a cluster of larger events between -5 and -11 pA in the $\alpha\beta\gamma\delta$ histogram. The latter spread of events was typical and illustrates the difficulty of resolving closely spaced conductance classes.

mV) could we confidently resolve subclasses of the smallest channels, and none of the histograms revealed a definitive separation of subclasses of the 50-pS channels. Channel openings with closely spaced amplitudes were therefore grouped into two major classes of high and low current levels for the analysis of conductance and kinetics.

The current-voltage relationship of the large amplitude class exhibited a linear slope conductance of 47.3 pS with an estimated reversal potential of 4.4 mV (Fig. 4). The low amplitude class had a slope conductance of 11.7 pS, measured between -200 and -160 mV. The extrapolated reversal potential was -17.5 mV; however, there could be substantial error in this estimate because of the large extrapolation interval. These two classes are referred to as "50-pS" and "12-pS" channels.

The open duration histograms of the 50-pS channels displayed two components (Fig. 5). The slow component corresponded to a mean channel open time of 10.2 ± 4.9 ms (five patches) at -100 mV and was dependent on voltage, increasing *e*-fold for every 85 mV of hyperpolarization. On the average, the slow component comprised 54% of the total events in each histogram (corrected for missed events). The time constant of the fast component corresponded to a mean channel open time of 0.3 ± 0.1 ms (five patches) at -100 mV. We did not detect a significant voltage dependence of the fast component.

Two components were also observed in the open durations of the 12-pS channel (Fig. 5). Kinetic analysis of this channel was problematic because of the low amplitudes and the tendency of events to be broken up by noise. Sample sizes from individual patches were small, due partly to the exclusion of overlapping events in our analysis. We therefore pooled the open durations from all patches into a single histogram for the purpose of estimating open time. At an applied membrane potential of -180 mV, we estimated open times of 0.4 and 20.9 ms, corresponding to the fast and slow components of the open duration histogram. About half of the

FIG. 4. Mean single channel current vs. membrane potential. Each point represents the grand mean current ($\pm$ SD) obtained for a given amplitude class from all patches at a single membrane potential. $\circ$, Δ , and ∇ , Data from $\alpha\beta\gamma\delta$ -injected oocytes; $\bullet$, data from $\alpha\beta\gamma$ -injected oocytes. The mean current of each amplitude class was first calculated for each patch at a given potential, and those values were then used to compute the grand means plotted here. The average number of patches sampled for each data point was 7 (range, 2–14). The data points are fitted by linear regression lines for the purpose of estimating slope conductances and reversal potentials. Because of apparent rectification, the solid circles were fitted between -200 and -100 mV, and the open circles were fitted between -200 and -160 mV. The other symbols were fitted by using all data points. The estimated slope conductances are 43.7 ± 1.3 pS (Δ), 25.8 ± 4.1 pS (∇), 11.7 ± 2.2 pS ($\circ$), and 12.7 ± 0.4 pS ($\bullet$).

FIG. 5. Open duration histograms of single channel events. The histograms are all fitted by the sum of two exponential functions. (Top) Compiled from 50-pS events recorded from a single outside-out patch at -100 mV and fitted best with two time constants of 0.3 and 10.1 ms. (Middle) Composed of the pooled sample of 12-pS events recorded from all patches of $\alpha\beta\gamma\delta$ -injected oocytes at -180 mV. It was fitted best by two time constants of 0.4 and 20.9 ms. (Bottom) Obtained from a single patch of $\alpha\beta\gamma$ -injected oocyte membrane at -180 mV and fitted best with time constants of 0.5 and 14.9 ms.

events in the histogram (54%) belonged to the slow component.

In addition to the two major amplitude classes, occasional intermediate events were seen that had a mean current of -4.8 ± 1.8 pA (five patches) at -180 mV. They were observed in 10 of 18 patches and constituted $\approx 15\%$ of the events in those patches. The intermediate channels had a slope conductance of 25.8 pS with an extrapolated reversal potential of -6.9 mV (Fig. 4). We did not analyze their kinetic properties. The conductance of these events is similar to that reported for stretch-activated channels in oocytes (9). It is thus conceivable that the 25-pS events represent spontaneous openings of stretch-activated channels, which were not detected by application of suction to the pipette.

Effects of Subunit Deletions. Injection of oocytes with α -, γ -, and δ -subunit mRNAs (deleting β) resulted in little or no detectable AcChoR activity. The largest whole cell current recorded was <10 nA at -90 mV in response to bath-applied AcCho (100 μ M), and in most cases there was no measurable response to AcCho. Weak responses to AcCho were fre-

quently seen after injection of α -, β -, and δ -subunit mRNAs (deleting γ). The maximum current seen was 75 nA at -90 mV in response to $100 \mu\text{M}$ AcCho. We attempted to record the activity of single AcChoR channels in these oocytes but were unable to detect any activity that could be attributed unambiguously to AcChoR channels.

The results of deleting β - or γ -subunit mRNAs contrasted markedly with those in which δ -subunit mRNA was deleted. Injection of α -, β -, and γ -subunit mRNAs resulted in abundant expression of functional AcChoRs. However, the peak whole cell currents induced by $1 \mu\text{M}$ AcCho were lower in δ -less oocytes than in those injected with the full complement of subunit mRNAs (Fig. 1). The reversal potential as measured from the peak currents in response to bath-applied AcCho was -7.0 ± 5.1 mV (five oocytes), which was comparable to that of the $\alpha\beta\gamma\delta$ -injected oocytes. The whole cell current-voltage relationships exhibited a strong inward rectification at negative membrane potentials (Fig. 1). Inward rectification of whole cell currents has also been observed in δ -less *Torpedo* AcChoRs expressed in oocytes (13).

Bath application of AcCho elicited single channel activity with a delay comparable to that of $\alpha\beta\gamma\delta$ -injected oocytes (Fig. 2A). Single channel records exhibited a single amplitude class of events (Figs. 2B and 3), with a mean current of -1.4 ± 0.2 pA (eight patches) at -180 mV. The current-voltage relationship of these channels was parallel to that found for the 12-pS class of channels in $\alpha\beta\gamma\delta$ -injected oocytes (Fig. 4). The channels had a slope conductance of 12.7 pS (between -200 and -100 mV) and an extrapolated reversal potential of -70.0 mV. We noticed a systematic tendency of the current amplitudes of the δ -less channels to be slightly lower than those of the low-conductance channels in oocytes injected with all subunits. Comparison of the histograms in Fig. 3 suggests that the δ -less channels may represent the smaller of two closely spaced small conductance classes of AcChoR channels present in oocytes injected with all subunits.

As found for channels in $\alpha\beta\gamma\delta$ -injected oocytes, open duration histograms of the δ -less channels revealed two kinetic components, which were well described by the sum of two exponential functions (Fig. 5). At a membrane potential -200 mV, the fast component corresponded to a mean channel open time of 0.5 ± 0.2 ms (three patches), and the slow component corresponded to 11.3 ± 4.2 ms (three patches). On the average, about a third of the events in each histogram (31%) belonged to the slow component. We could not rigorously compare the kinetics of these channels with those of the 12-pS channels in $\alpha\beta\gamma\delta$ -injected oocytes because of the small sample sizes; however, the histograms do not suggest major differences in open state kinetics.

DISCUSSION

Injection of synthetic mRNAs encoding mouse α -, β -, δ -, and γ AcChoR subunits into *Xenopus* oocytes resulted in the expression of AcChoR channels with multiple conductance levels. We saw one major class with a conductance of 50 pS and another at 12 pS, and, in most patches, there was evidence of conductance heterogeneity within each of these

classes. We also detected infrequent events with an intermediate conductance of ≈ 25 pS. These observations differ from previous single channel studies of both *Torpedo* and bovine nicotinic AcChoRs expressed in oocytes, which have identified only one conductance class associated with this combination of subunits (4, 11).

The 50-pS channels presumably represent assembly of all four subunits since injection of α -, β -, γ -, and δ -subunit mRNAs was required to express these channels in significant amounts. At least one of the small conductance levels (12 pS), which we observed in $\alpha\beta\gamma\delta$ -injected oocytes, can be explained by the assembly of channels lacking the δ subunit. Injection of oocytes with α -, β -, and γ -subunit mRNAs yielded a single, homogeneous class of openings with a conductance comparable to the smallest event class in the recordings from $\alpha\beta\gamma\delta$ -injected oocytes. A plausible subunit stoichiometry of this channel is suggested by a recent study of *Torpedo* AcChoRs, indicating that the channel conductance is determined by negative charges positioned on either side of the M2 membrane spanning region (ref. 14; reviewed in ref. 15). Replacement of negatively charged residues by neutral and positive residues at these positions was found to reduce channel conductance. Alignment of the *Torpedo* subunit sequences suggests that these charges form three rings of glutamine and negatively charged amino acids, two located on the cytoplasmic side of the pore and one on the extracellular side. A similar alignment of charged amino acids from mouse BC3H-1 subunit sequences is presented in Fig. 6. Assuming (i) that the δ -less channel assembles as a pentamer, (ii) that at least one α subunit is necessary for gating of the channel by AcCho, and (iii) that at least one β subunit is required (based on our observation that β -less oocytes exhibited little or no response to AcCho), we propose putative structures for the δ -less channel. The most likely stoichiometries are either $\alpha_2\beta\gamma_2$ or $\alpha\beta_2\gamma_2$. Compared to the wild-type $\alpha_2\beta\gamma\delta$, both stoichiometries would have reduced negative charge in the inner rings, without altered negative charge in the outer ring. This could account for the lower conductance observed for δ -less channels. The stoichiometry $\alpha\beta\gamma_3$ is unlikely to result in a cation-conductive channel, since it would have net positive charges in both the innermost and the outer rings. All other δ -less combinations of subunits with a single γ subunit (namely, $\alpha_2\beta_2\gamma$, $\alpha_3\beta\gamma$, and $\alpha\beta_3\gamma$) are unlikely to account for a small conductance channel. Such channels would have more net negative charge in the outer ring than the wild-type channel and would therefore be expected to have a higher conductance than that associated with $\alpha_2\beta\gamma\delta$.

The reversal potential of δ -less channels was estimated to be -7 mV from whole cell current-voltage data and -70 mV from extrapolation of the single channel amplitudes (between -200 and -100 mV). These different estimates of the reversal potential could be reconciled by inward rectification of the δ -less channels. Such rectification would be consistent with the $\alpha_2\beta\gamma_2$ or $\alpha\beta_2\gamma_2$ structures proposed above. Both of these stoichiometries predict reduced negativity on the inner rings of charge, and in mutated *Torpedo* receptors such changes generally resulted in inward rectification of channel conduc-

α	T	D-S G -	E-K M T L S I S V L L S L T V F L L V I V	E-L I P S T S	288
β	P	D-A G -	E-K M G L S I F A L L T L T V F L L L L A	D-K V P E T S	302
γ	A	K-A G G	Q K C T V A T N V L L A Q T V F L F L V A	K-K V P E T S	399
δ	G	D-C G -	E-K T S V A I S V L L A Q S V F L L L I S	K-R L P A T S	306
		1	2	3	
			M2		

FIG. 6. Amino acid sequences (single-letter code) of the M2 and neighboring regions of mouse α -, β -, γ -, and δ subunits (from refs. 16–19). Boxes 1 and 2 enclose the postulated cytoplasmic rings of negative charge, and box 3 encloses the extracellular ring. The γ subunit is unusual in that it contains a lysine (K+) rather than aspartate (D-) at position 1 and it contains a polar glutamine (Q) rather than glutamate (E-) at position 2. It also differs from α and β subunits in having lysine (K+) rather than glutamate (E-) or aspartate (D-) at position 3.

tance. Unmodified *Torpedo* receptors, like the 50-pS channels described here, did not rectify appreciably (14).

In addition to subunit stoichiometry, there are other possible sources of conductance heterogeneity. Native AChRs are reported to have one or more subconductance states (20–24), and a recent study of γ -aminobutyric acid type A channels expressed in oocytes (25) showed that a homomeric channel can have multiple conductance states. In such cases, the channels invariably exhibit direct transitions between different conductance levels. Since we saw no evidence in our records of direct transitions between conductance levels, our data do not support this explanation of conductance heterogeneity. It might also be argued that additional conductance levels could result from second messenger actions. However the outside-out patch configuration would be expected to wash out cytoplasmic second messengers, and the extracellular and pipette solutions were adjusted to minimize increases in calcium concentration inside the patch due to the opening of AChR channels. A further consideration in explaining conductance heterogeneity is posttranslational processing. AChRs undergo glycosylation, acylation, and phosphorylation (26); however, the effects, if any, of these modifications on channel conductance are presently unknown.

Recordings from BC3H-1 cells (27) have not revealed multiple conductance levels of AChRs such as we observed in oocytes; however, AChR channels with conductances lower than 40 pS have been reported in *Xenopus* muscle *in vivo* (7, 8, 28) and in denervated mouse muscle (29). One of these, a 25-pS channel, has been well characterized in developing *Xenopus* muscle (8). In some recordings, this is the predominant channel type, suggesting that it may play a significant role in neurotransmission. There is also evidence for a smaller conductance class in *Xenopus* muscle, which corresponds to the 12-pS channels reported here (28). These observations suggest that the multiple classes of AChR channels expressed in oocytes represent variations in receptor properties that occur normally in muscle.

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1. Barnard, E. A., Miledi, R. & Sumikawa, K. (1982) *Proc. R. Soc. London Ser. B* **215**, 214–246.
2. Miledi, R., Parker, I. & Sumikawa, K. (1982) *EMBO J.* **1**, 1307–1312.
3. Dascal, N. (1987) *CRC Crit. Rev. Biochem.* **22**, 317–386.

4. Mishina, M., Takai, T., Imoto, K., Noda, M., Takahashi, T., Numa, S., Methfessel, C. & Sakmann, B. (1986) *Nature (London)* **321**, 406–411.
5. Mishina, M., Kurosaki, T., Tobimatsu, T., Morimoto, Y., Noda, M., Yamamoto, T., Terao, M., Lindstrom, J., Takahashi, T., Kuno, M. & Numa, S. (1984) *Nature (London)* **307**, 604–608.
6. White, M. M., Mayne, K. M., Lester, H. A. & Davidson, N. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 4852–4856.
7. Owens, J. L. & Kullberg, R. W. (1989) *J. Neurosci.* **9**, 1018–1028.
8. Owens, J. L. & Kullberg, R. (1989) *J. Neurosci.* **9**, 2575–2580.
9. Methfessel, C., Witzemann, V., Takahashi, T., Mishina, M., Numa, S. & Sakmann, B. (1986) *Pflügers Arch.* **407**, 577–588.
10. Hamill, O. P., Marty, A., Neher, E., Sakmann, B. & Sigworth, F. (1981) *Pflügers Arch.* **391**, 85–100.
11. Sakmann, B., Methfessel, C., Mishina, M., Takahashi, T., Takai, T., Kurasaki, M., Fukuda, K. & Numa, S. (1985) *Nature (London)* **318**, 538–543.
12. Yoshii, K., Yu, L., Mayne, K. M., Davidson, N. & Lester, H. A. (1987) *J. Gen. Physiol.* **90**, 553–573.
13. White, M. M. (1987) *Soc. Neurosci. Abstr.* **13**, 798.
14. Imoto, K., Busch, C., Sakmann, B., Mishina, M., Konno, T., Nakai, J., Hideaki, B., Mori, Y., Fakuda, K. & Numa, S. (1988) *Nature (London)* **335**, 645–648.
15. Dani, J. A. (1989) *Trends Neurosci.* **12**, 125–128.
16. Boulter, J., Luyten, W., Evans, K., Mason, P., Ballivet, M., Goldman, D., Stengelin, S., Martin, G., Heinemann, S. & Patrick, J. (1987) *J. Neurosci.* **5**, 2545–2552.
17. Buonanno, A., Mudd, J., Shah, V. & Merlie, J. P. (1986) *J. Biol. Chem.* **261**, 16451–16458.
18. Yu, L., LaPolla, R. J. & Davidson, N. (1986) *Nucleic Acids Res.* **14**, 3539–3555.
19. LaPolla, R. J., Mayne, K. M. & Davidson, N. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 7970–7974.
20. Hamill, O. P. & Sakmann, B. (1981) *Nature (London)* **294**, 462–464.
21. Auerbach, A. & Sachs, F. (1983) *Biophys. J.* **42**, 1–11.
22. Takeda, K. & Trautmann, A. (1984) *J. Physiol. (London)* **349**, 353–374.
23. Colquhoun, D. & Sakmann, B. (1985) *J. Physiol. (London)* **369**, 501–557.
24. Morris, C. E. & Montpetit, M. (1986) *Can. J. Pharmacol.* **64**, 347–355.
25. Blair, L. A. C., Levitan, E. S., Marshall, J., Dionne, V. E. & Barnard, E. A. (1988) *Science* **242**, 577–579.
26. Anderson, D. J. (1987) in *The Vertebrate Neuromuscular Junction*, ed. Salpeter, M. M. (Liss, New York), pp. 285–315.
27. Sine, S. M. & Steinbach, J. H. (1984) *Biophys. J.* **45**, 175–185.
28. Owens, J. L., Hartig, G. S., Laitinen, F. A. & Kullberg, R. (1987) *Soc. Neurosci. Abstr.* **13**, 98.
29. Henderson, L. P., Lechleiter, J. D. & Brehm, P. (1987) *J. Gen. Physiol.* **89**, 999–1014.