Ion Channels Formed by a Highly Charged Peptide†

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Received March 29, 1990; Revised Manuscript Received January 10, 1991

ABSTRACT: A peptide (MA-β) corresponding to a segment of the nicotinic acetylcholine receptor (AChR) that has amphipathic α-helical periodicity forms ion channels in artificial phospholipid bilayers. The MA-β ion channels are very stable, comprise two discrete conductance states, and undergo rapid, flickering-type closings. The discrete-conductance ion channels formed by MA-β contrast with the continuous-conductance ion channels formed by a peptide (M2-δ) identical in sequence with M2 [Oiki, S., Danho, W., Madison, V., & Montal, M. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 8703–8707], a putative transmembrane segment of the AChR. Neither MA-β nor M2-δ sufficiently mimics the electrophysiological properties of the native AChR. We suggest that peptide ion channels can be classified into at least three general groups: discrete-conductance channels, such as MA-β; continuous-conductance channels, such as M2-δ; and membrane disruptors, such as those formed by short, amphipathic α-helical peptides.

Ion channel proteins mediate the transmembrane transduction of electrical and chemical signals between cells and between cellular compartments (Hille, 1984). A problem in understanding the mechanism of action of these proteins is in identifying the residues that constitute the ion-conducting channel. This problem is addressed for the nicotinic acetylcholine receptor (AChR), a well-studied ion channel which is found at the vertebrate neuromuscular junction. The AChR, a ligand-gated and cation-selective channel, is a heteropentamer of four homologous subunits ($\alpha\beta\alpha\gamma\delta$), which are arranged quasi-symmetrically around the ion channel. This 298-kDa protein complex possesses a 2.5-nm-wide, extracellular infundibulum, which presumably contains the entrance to the ion channel (Ross et al., 1977). The ion channel narrows from this large entrance to an apparent diameter of ~0.7 nm within the membrane (Mitra et al., 1989; Kistler et al., 1982).

The lining of the transmembrane portion of the ion channel is most likely formed by five quasi-equivalent segments, one from each subunit of the AChR, in pentameric arrangement. Each subunit of the AChR contains four primarily hydrophobic, putative α-helical transmembrane segments, M1 through M4, as well as a sequence having amphipathic α-helical periodicity, MA. Primary sequence analysis focused attention on MA as a possible ion channel forming segment because of its amphipathic α-helical periodicity (Finer-Moore & Stroud, 1984) (Figure 1a). However, recent experimental evidence indicates that the M2 region (Figure 1b) is a more likely candidate for the ion channel lining segment (Noda et al., 1983). Although generally hydrophobic, the M2 segment has three to five serines or threonines, depending on the subunit, which could contribute to the hydrophilic center of the ion channel. Mutagenesis of the M2 region (Imoto et al., 1988; Leonard et al., 1988) and photolabeling studies using channel blockers (Giraudat et al., 1987; Oberthür et al., 1986) have indicated a key role for this segment in ion channel function. The $GABA_A$ and glycine receptors, which are ligand-gated, anion-selective ion channels and are similar in sequence to the AChR, possess a segment resembling M2 but lack an MA-like segment (Grenningloh et al., 1987; Schofield et al., 1987).

A peptide (M2-δ) corresponding to the M2 region of the δ-subunit has been found to form ion channels in artificial phospholipid bilayers (Oiki et al., 1988a). To determine whether the MA region also possesses ion channel forming capability, a peptide (MA-β) corresponding to MA from the β-subunit of *Torpedo californica* AChR was synthesized, and its ion channel activity was assayed in artificial phospholipid bilayers. We present the electrophysiological properties of MA-β and discuss the relative properties of MA-β, M2-δ, and native AChR channels.

Experimental Procedures

Synthesis and Purification of Peptides. The 27 amino acid MA-β peptide (Figure 1a) and a 19-amino acid peptide (AL), which is unrelated to the AChR in sequence (Figure 1c), were synthesized by using solid-phase, stepwise *tert*-butoxycarbonyl (*t*-Boc)[1] chemistry and PAM resins on an Applied Biosystems 430A automated peptide synthesizer. They were purified by reverse-phase HPLC on a C_{18} column, using a 0–70% AcN gradient containing 0.1% TFA (Figure 2a). The purity and homogeneity of the peptides were verified by direct amino acid analysis. The identity and purity of the MA-β peptide were further determined by mass spectrometry (Figure 2b), which showed that no other peptide species besides MA-β was present. The MA-β and AL peptides are water-soluble to concentrations of at least 10 g/L.

Formation of Artificial Phospholipid Bilayers. Artificial phospholipid bilayers were formed on the tip of tight-seal pipets (Coronado & Latorre, 1983; Suarez-Isla et al., 1983). A Corning lead glass 1080 pipet, which had been pulled so that its tip resistance was 1–20 MΩ, was filled with a buffered salt solution and placed into a 2-mL bath containing the same salt solution. Five to ten microliters of a 1 g/L solution of soybean asolectin (Associated Concentrates), which had been purified (Kagawa & Racker, 1971) and solubilized in hexane, was spread on the approximately 100 mm^2 surface of the bath solution. Membrane-sealed tips were formed by allowing the hexane to evaporate for 10 min and then by removing and

† This work was supported by NIH Grant GM24485 and NSF Grant DMB8615712 (to R.M.S.) and partially by NIH Grants NS20429 and NS22389 (to A. J. Hudspeth). P.G. is a recipient of an NSF Predoctoral Fellowship and was partially supported by a grant from the Lucille P. Markey Charitable Trust.
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[1] Abbreviations: *t*-Boc, *tert*-butoxycarbonyl; PAM, phenylacetamidomethyl; AcN, acetonitrile; TFA, trifluoroacetic acid; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; EGTA, [ethylenebis(oxyethylenenitrilo)]tetraacetic acid; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid.

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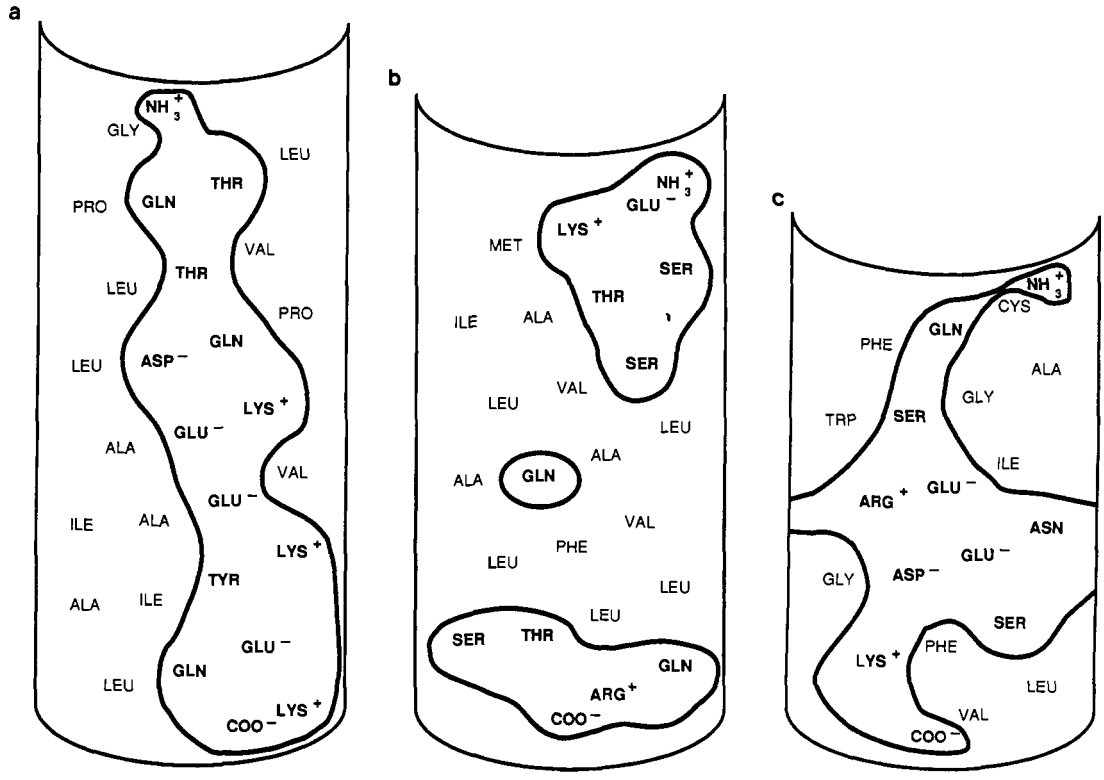

FIGURE 1: Sequences of the MA-β peptide (a), M2-δ (b), and AL peptide (c) are depicted on a helical net representation. The outlined areas correspond to polar amino acids, and the amino and carboxy terminii groups of each peptide. (a) The MA-β peptide sequence corresponds to residues 424–450 of the β-subunit of the *Torpedo californica* AChR, except at the ultimate carboxy-terminal residue, where a glutamate is replaced by a lysine. (b) The M2-δ sequence corresponds to residues 259–281 of the δ-subunit of the *Torpedo californica* AChR. (c) The AL peptide's sequence is not related to the AChR's.

reinserting the pipet in the salt solution. With a success rate of 80–90%, this procedure yielded electrically stable membrane-sealed tips with a resistance of 5–100 GΩ. The MA-β peptide was added to the unstirred bath solution to a final concentration ranging from 0.5 to 50 mg/L; the AL peptide was added to a final concentration ranging from 0.1 to 100 mg/L. Ion channels typically appeared 10–20 min after the introduction of the MA-β peptide into the bath solution. While lower concentrations of MA-β failed to yield ion channels within the 5–6 h duration of the experiment, higher concentrations tended to lyse the membrane seal.

Channel Recordings. A voltage-clamp amplifier (EPC-5, List Electronic) was used to set the transmembrane potential and to measure ionic currents. Ground potential was defined as that of the bath, so the reported potentials represent those of the trans compartment; the cis compartment is defined as the compartment to which peptide was added. The sign convention is that for recording from cell-attached or outside-out membrane patches: current into the pipet is defined as positive and shown as an upward deflection. Current and voltage records were filtered at 1.0 kHz with an 8-pole Butterworth filter and stored on FM recording tape. The tapes were digitized at 100 μs per point for analysis. Because of the time resolution of filtering, only events of duration 1 ms or greater were examined.

Results

MA-β Peptide Forms Very Stable Ion Channels. The 27 amino acid MA-β peptide has the potential to form an amphipathic α-helix, having 4 negatively and 3 positively charged residues on one side of the α-helix (Figure 1a; Table I). The peptide is identical with the MA segment of the β-subunit of *Torpedo californica* AChR (Stroud & Finer-Moore, 1985) except at position 27, where a lysine is substituted for a glutamate. The glutamate at position 27 is not conserved in other AChR subunits and in β-subunits from other species, and is generally replaced by a positively charged amino acid. This residue was altered to compensate for the extra negative charge present on the carboxy terminus of the peptide, and to promote α-helix formation by placing a positive charge at the negative end of the helix dipole.

The MA-β peptide forms very stable ion channels in artificial phospholipid bilayers (Figure 3a). The majority of recordings resulted in multiple, open ion channels (Figure 3c), each of which appeared sequentially. On the basis of three separate recordings showing the presence of a single channel, the open time of the MA-β peptide channel was estimated to be on the order of many seconds to minutes. These three recordings also showed that an opening is eventually followed by closure to a quiescent, fully closed state whose lifetime is many seconds to minutes. This closure is differentiable from flickering-type closings, which occur throughout an opening and result in partial or full closure of the channel for several milliseconds (Figure 3b). The channel remains in these brief, partially or fully closed states 4% of the total open time and in the fully open state 96% of the total open time, indicating that the flickering-type closings are a minor fraction of the peptide channel's activity. Neither the latency of appearance nor the open lifetimes of MA-β ion channels appeared to be dependent on the transmembrane potential.

To determine whether these ion channels resulted uniquely from MA-β peptide's action, channel recordings were carried out for a second peptide, AL, which was synthesized and purified in a manner parallel to that of MA-β. AL peptide's sequence, which is unrelated to the AChR's and eight amino acids shorter than MA-β's, contains five charged residues and possesses amphipathic α-helical periodicity, although less pronounced than MA-β's (Figure 1c, Table I). The AL

Table I: Some Ion Channel Forming Peptides

peptide	no. of amino acids	α-helical amphipathicity[a]	max power[b]	frequency of max power[c]	conductance class
MA-β	27	7.66	9.40	1/3.4	discrete
$(LSSLLSL)_3$[d]	21	6.43	6.43	1/3.5	discrete
alamethicin[e]	20	3.11	4.39	1/2.8	discrete
M2-δ[f]	23	2.21	7.65	1/10.0	continuous
sodium channel peptide[g]	22	2.17	7.68	1/2.8	continuous
AL	19	4.01	6.55	1/3.8	disruptor
$(LSSLLSL)_2$[d]	14	6.43	6.43	1/3.5	disruptor

[a] The Fourier transform with respect to the hydrophobicities of the amino acids in the peptide (Finer-Moore & Stroud, 1984) were calculated according to $I(\nu) = 100[(1/n)\sum_{j=1}^{n} H_j \exp(2\pi ij\nu)]^2$, where $I(\nu)$ is the power spectrum of hydrophobicities, n is the number of amino acids in the peptide, H_j is the hydrophobicity of residue j in kilocalories per mole (Eisenberg et al., 1982), and ν is the frequency. The value reported is the power at a frequency of 1/3.5 residues^{-1}, corresponding to the periodicity of an α-helix. The larger the value, the more marked is the amphipathic α-helical periodicity. [b] The maximum value in the Fourier transform of hydrophobicities, which was calculated for frequencies between 0.0 and 0.5. [c] The frequency at which the maximum value in the Fourier transform occurs, reported in units of residues^{-1}. [d] Lear et al. (1988). [e] The sequence is Ac-XPXAXAQXVXGLXPVXXEQY (Cascio & Wallace, 1988), in which Ac represents an acetyl group, X represents α-aminoisobutyric acid, and Y represents phenylalaninol. For the Fourier-transform analysis, the hydrophobicity of the α-aminoisobutyric acid residues was approximated by the hydrophobicity of glutamic acid, and the hydrophobicity of phenylalaninol was approximated by the hydrophobicity of phenylalanine. [f] Oiki et al. (1988a). [g] The sequence of this peptide is DPWNWLDFTVITFAYVTEFVDL (Oiki et al., 1988b).

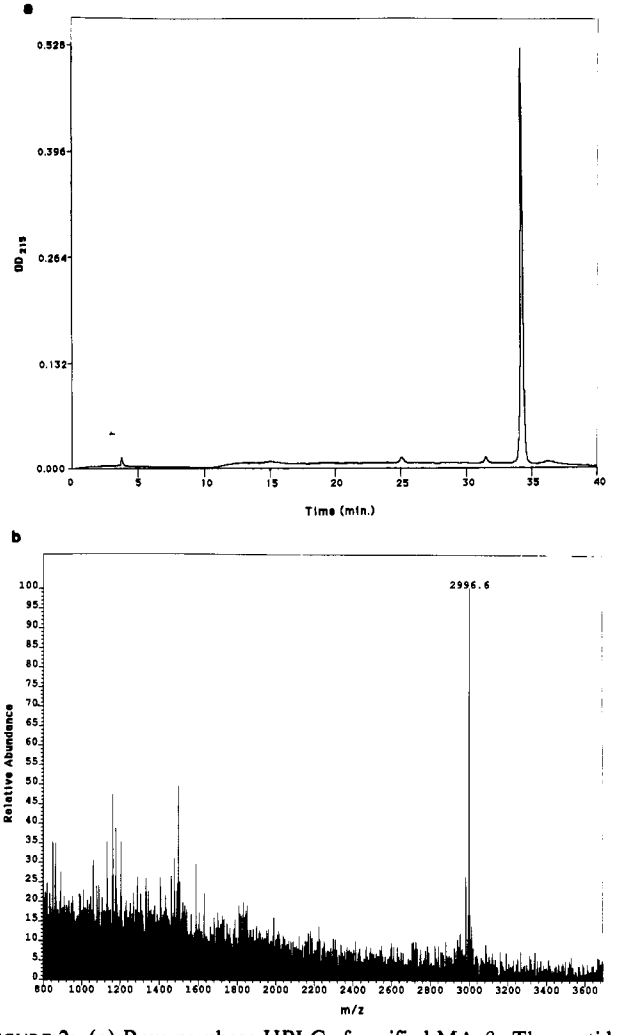

FIGURE 2: (a) Reverse-phase HPLC of purified MA-β. The peptide was applied to a C_{18} column and was eluted with a 0–70% AcN linear gradient containing 0.1% TFA. The gradient reached 70% AcN in 35 min, and the flow rate was 1 mL/min. (b) Liquid secondary ion mass spectrum (LSIMS) of purified MA-β. The single peak present corresponds to MA-β, whose calculated m/z is 2996.7 (for the MH^+ species). The experimentally measured mass is noted above the peak.

peptide did not produce square-waveform transitions in ionic current; instead, it infrequently gave rise to irregular increases in membrane conductance (Figure 3d). The difference between the action of the two peptides suggests that discrete-conductance ion channels, such as those resulting from MA-β, can be distinguished from membrane-disrupting events, such as those caused by AL.

MA Peptide Has Primarily Two Open-Channel Conductance Levels. The MA peptide primarily forms channels of two types, a high-conductance channel of 12.5 pS (±0.2) and a low-conductance channel of 8.6 pS (±0.2) in 500 mM NaCl (Figure 4). For both conductance-type channels, the ionic current is directly proportional to the applied transmembrane voltage and is equal in magnitude at positive and negative transmembrane voltages. The high-conductance type channel is observed more frequently, constituting 69% of all openings, while the low-conductance type constitutes 30% of all openings. Channels of even lower conductance, 4–6 pS in 500 mM NaCl, were observed infrequently, representing 1% of all openings. Both high- and low-conductance channels display similar long-lived openings and flickering-type closings.

To determine whether the high- and low-conductance channels constitute independent states, the magnitude of transitions between conductance levels was analyzed (Figure 5). Both 8.5- and 12.5-pS transitions, corresponding to steps between fully closed and fully open states for the low- and high-conductance channels, respectively, are found. However, the 4.0-pS step which would account for a transition between the low-conductance state and the high-conductance state is absent, indicating that the low- and high-conductance channels are independent and do not interconvert.

Between 140 and 1000 mM NaCl, the conductance of the two channel types increases linearly as a function of salt concentration and does not saturate. In this range, the rate of increase in the conductance of the high-conductance channel is 5.9 pS/M NaCl and of the low-conductance channel 4.9 pS/M. Although the linear relationship accounts for the behavior of the two channel types at concentrations of NaCl greater than 140 mM, it is not valid at lower salt concentrations. Linear extrapolation of the conductance to a salt concentration of zero yields a conductance much greater than the expected value of zero. The extrapolated zero salt concentration is 9.4 pS for the high-conductance-type channel and 6.2 pS for the low-conductance-type channel. Therefore, the relationship of conductance to salt concentration appears to be nonlinear at salt concentrations below 140 mM.

Flickering-Type Closings Are Short-Lived. Flickering-type closings punctuate the long-lived openings of both the low- and high-conductance channels (Figure 3b). Since the duration of flickering-type closings tends to increase with the amplitude of these events (Figure 6), the closings are most likely unresolved by our recording system. The distribution of flicker-

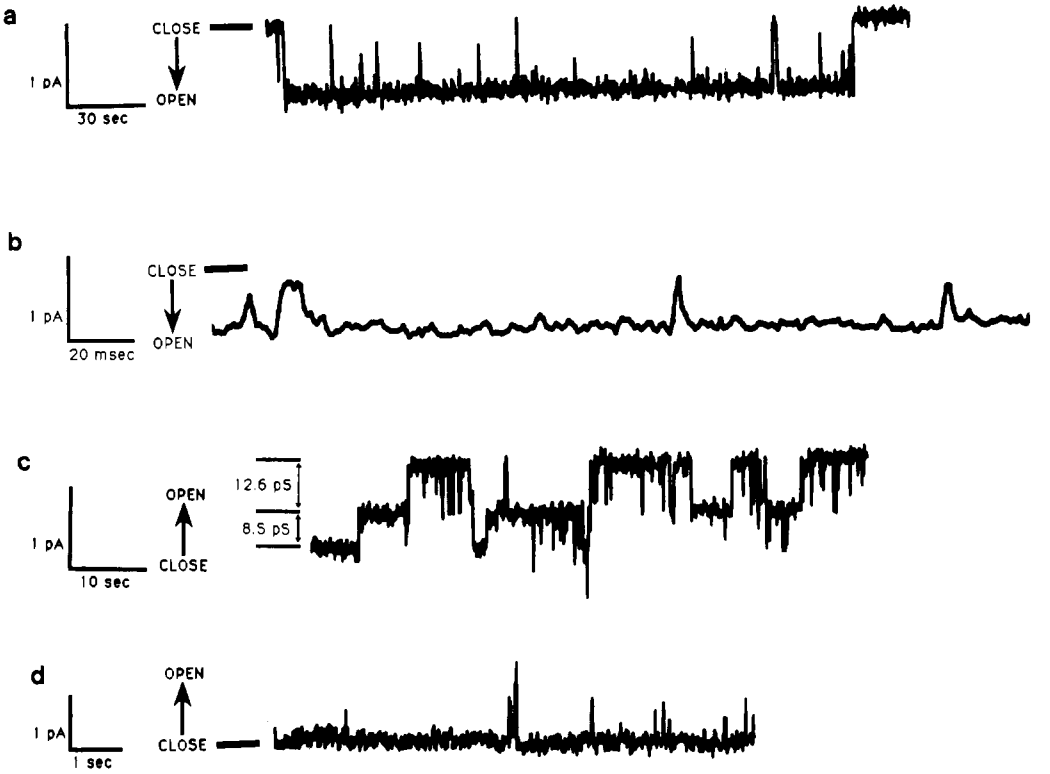

FIGURE 3: (a) Single-channel currents resulting from the MA-β peptide in symmetrical 500 mM NaCl, 1 mM $CaCl_2$, and 10 mM HEPES, pH 7.2, at a holding potential of +94 mV. The downward deflection represents an opening of a low-conductance channel which remains open for approximately 4 min. Flickering-type closings are evident throughout the opening as spiky, upward deflections. The horizontal bar indicates the current level without any channels present in the membrane. For purposes of display, the trace was digitized and plotted at 10 ms/point. (b) Portion of the single-channel current from (a) is shown at a greater time resolution. The horizontal bar indicates the current level of the fully closed state. Four flickering-type closings are evident. For purposes of display, the trace was digitized and plotted at 1 ms/point. (c) Multiple open channels formed by the MA-β peptide in symmetrical 500 mM NaCl, 1 mM $CaCl_2$, and 10 mM HEPES, pH 7.2, at a holding potential of −50 mV. Upward deflections correspond to opening events, and spiky, downward deflections correspond to flickering-type closings. The patch, which contains at least three channels, contains both a high-conductance (12.5 pS) and a low-conductance channel (8.6 pS). The current level without any channels present is not shown. For purposes of display, the trace was digitized and plotted at 5 ms/point. (d) Erratic increases in current caused by the AL peptide in symmetrical 1000 mM NaCl, 1 mM $CaCl_2$, and 10 mM HEPES, pH 7.3, at a holding potential of −124 mV. Upward deflections indicate opening events. The bar indicates the current level without any channels present in the membrane. For purposes of display, the trace was digitized and plotted at 1 ms/point.

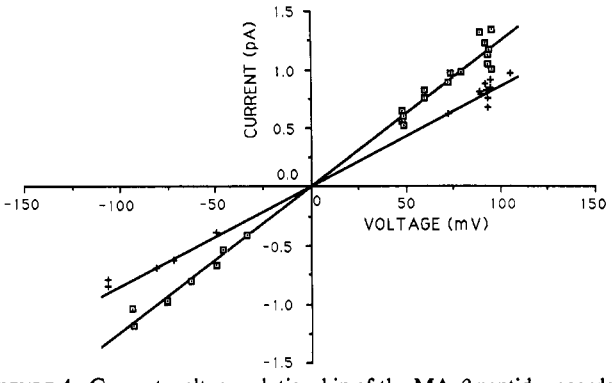

FIGURE 4: Current–voltage relationship of the MA-β peptide recorded in symmetrical 500 mM NaCl, 1 mM $CaCl_2$, and 10 mM HEPES, pH 7.2. (⊡) corresponds to the high-conductance channel, and (+) corresponds to the low-conductance channel. Each point represents an observation of a single open channel, and current amplitudes were determined from amplitude histograms. The plot includes data from nine different membranes.

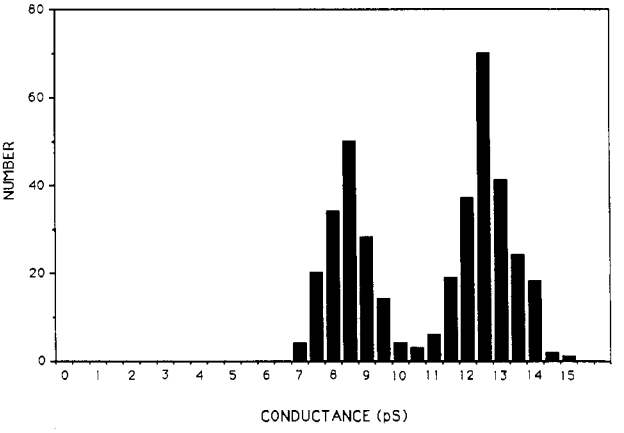

FIGURE 5: Histogram of the magnitude of opening and closing transitions between conductance levels. The step size of transitions was determined from current amplitude histograms. Sixteen membrane patches contributed to this analysis, at potentials ranging from +105 to −105 mV. All recordings were carried out with symmetrical 500 mM NaCl, 1 mM $CaCl_2$, and 10 mM HEPES, pH 7.2.

ing-type closing durations can be fitted by a single exponential with a time constant of 1.4 ms (Figure 7a), which is at the limit of our recording system and probably represents this limit rather than the kinetics of the channel. As indicated by the resolution limit of our recording system, the lifetime of the flickering-type closings appears to be 1 ms or less. The duration that the channel exists in the fully open state uninterrupted by flickering-type closings can be fitted by a single exponential with a much longer time constant of 225 ms (Figure 7b). Therefore, the fully open state is more stable than the flickering-type closed states by a factor of at least 160.

Since the flickering-type closings may have arisen from a blockade of the ion channel caused by Ca^{2+} present throughout these experiments, the concentration of Ca^{2+} was varied from 0 to 50 mM while the NaCl concentration was held constant

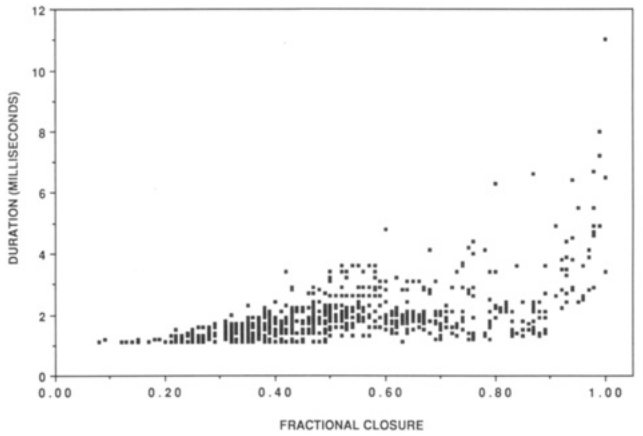

FIGURE 6: Scatter plot of the amplitude of flickering-type closings versus the duration of those events. The amplitude of closing is expressed as a fraction of full closure with 1.0 representing full closure. Flickering-type closing events were identified and analyzed by using the programs C-Clamp and C-Crunch (Indec, Sunnyvale, CA). Only flickering-type closings whose duration was 1 ms or greater and whose amplitude was at least twice the root-mean-square deviation of the full open channel current were analyzed. One low- and two high-conductance channels, which were recorded in symmetrical 500 mM NaCl, 1 mM $CaCl_2$, and 10 mM HEPES, pH 7.2, at voltages between +95 and −85 mV, were used for this analysis.

at 500 mM. The number and frequency of the flickering-type closures were independent of Ca^{2+} concentration (data not shown). When EDTA and EGTA were added to the bath to remove trace amounts of Ca^{2+}, the membrane seal failed to form. Changes in buffer from HEPES to Tris or dimethylglutarate and changes in pH from 7.2 to 4.0 did not alter the number or frequency of partial or full closures (data not shown). These changes in Ca^{2+} concentration, buffer, and pH did not affect the conductance of the two types of channel.

Discussion

A peptide corresponding to the MA segment of the β-subunit of *Torpedo californica* AChR forms discrete-conductance ion channels. The β-subunit's MA segment was selected since it has the most pronounced amphipathic α-helical periodicity of any of the other subunit's MA segments (Finer-Moore & Stroud, 1984). The MA-β channels do not mimic the electrophysiological properties of the native AChR. While the *T. californica* AChR has a conductance of 47 pS in 500 mM NaCl and 0.5 mM $CaCl_2$ (Montal et al., 1986), the MA-β peptide forms two discrete-conductance channels of 8.6 and 12.5 pS in 500 mM NaCl and 1.0 mM $CaCl_2$. In addition, the native AChR is not observed to undergo the flickering-type closings found for MA-β. The most striking difference between the native AChR and MA-β is that the open time for the AChR is a few milliseconds and for MA-β is on the order of minutes. Thus, the ion flux through the MA-β peptide channel is lower than through the native AChR, but the open peptide channel is much more stable than the open native AChR channel.

A 23 amino acid peptide (M2-δ) corresponding to the M2 segment of the δ-subunit of *T. californica* AChR has also been found to form ion channels (Figure 1b, Table I) (Oiki et al., 1988a). We synthesized a peptide (M2-α) corresponding to the M2 segment of the α-subunit of *T. californica* AChR (residues 256–283), but it proved to be insoluble in a variety of solvents and could not be purified or reliably assayed for ion channel formation. M2-α's insolubility may result from the fact that 18 of its 27 amino acids are hydrophobic, while only 13 of the soluble M2-δ peptide's 23 amino acids are hydrophobic. In artificial phospholipid bilayers, the M2-δ

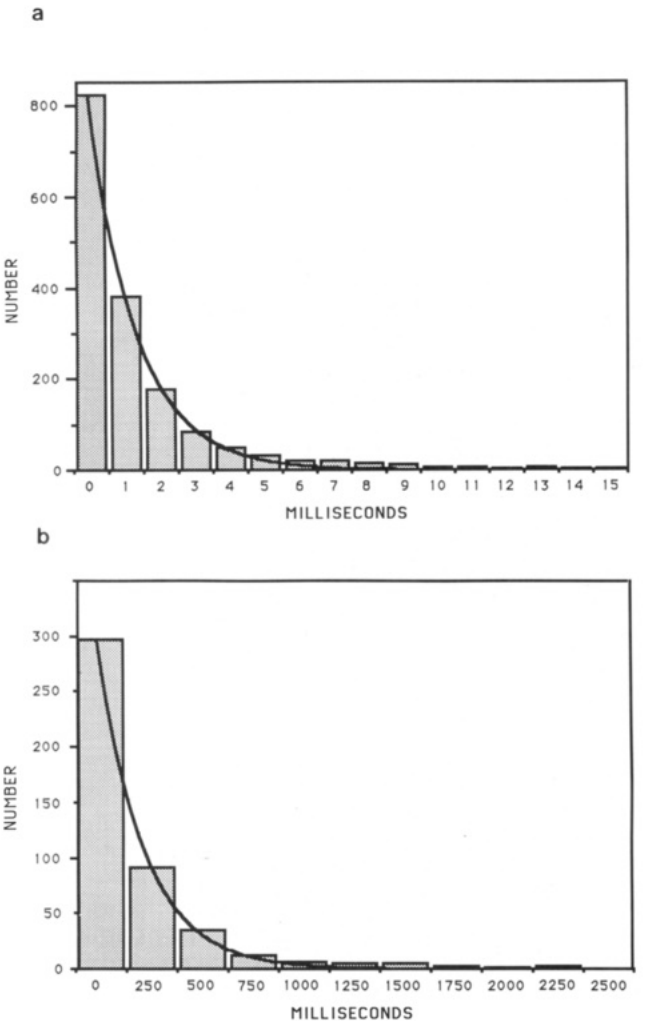

FIGURE 7: (a) Histogram of the duration of flickering-type closings is fitted with a single exponential with a time constant equal to 1.4 ms. Twelve independent channels, which were recorded in symmetrical 500 mM NaCl, 1 mM $CaCl_2$, and 10 mM HEPES, pH 7.2, at voltages between +105 and −105 mV, were used for this analysis. (b) Histogram of the duration that the channel remains in the fully open state uninterrupted by flickering-type closings is fitted with a single exponential with a time constant equal to 225 ms. One low- and two high-conductance channels, which were recorded in symmetrical 500 mM NaCl, 1 mM $CaCl_2$, and 10 mM HEPES, pH 7.2, at voltages between +94 and −85 mV, were used for this analysis.

peptide forms a continuum of conducting species that range in conductance from 5 to 65 pS; the most frequently observed conducting species has a conductance of 10 pS. Somewhat greater than 10% of all openings have a conductance of 40 pS, which is similar to the conductance of the native AChR. The mean open time of these channles is on the order of milliseconds, within the same range as the native AChR. The continuum of conductance states found for M2-δ contrast with the discrete conductance states found for MA-β.

The M2-δ ion channels are functionally similar to ion channels formed by a 22 amino acid peptide derived from a segment of the voltage-gated sodium channel (Table I) (Oiki et al., 1988b). Like M2-δ peptide, the sodium-channel peptide produces a continuum of conducting species. These range from 5 to 60 pS, and the major species has a conductance of 15–25 pS, which includes the 20-pS conductance of the native voltage-gated sodium channel. However, the sodium channel peptide produces channels which mimic the conductance of the native AChR as frequently as the AChR-derived M2-δ peptide. Thus, neither M2-δ nor the sodium channel peptide exclusively mimics the functional properties of the protein ion

channel from which it is derived.

A peptide composed of a repeat of leucines and serines has also been found to form ion channels in artificial phospholipid bilayers (Lear et al., 1988) (Table I). This 21 amino acid peptide, composed of 3 repeats of the heptamer LSSLLSL, is designed to form an amphipathic α-helix capable of spanning the phospholipid bilayer and is not mimetic of any known native channel. Like the MA-β peptide channel, the leucine-serine repeat peptide forms a discrete-conductance ion channel, having a conductance of 70 pS in 500 mM KCl and an open time in the millisecond range. In contrast to the 21 amino acid triad repeat peptide, a 14-amino acid peptide composed of 2 of the same heptamer repeats does not form ion channels but gives rise to erratic increases in membrane conductance. It is probable that the dyad repeat peptide is not of sufficient length to span the bilayer as an α-helix and therefore acts as a chaotropic agent, as does the AL peptide.

These synthetic peptides are reminiscent of the naturally occurring peptides that insert into membranes and form ion channels, such as alamethicin (Hall et al., 1984) and gramicidin A (Finkelstein & Andersen, 1981). Alamethicin, a 20 amino acid antibiotic which contains the unusual amino acid α-aminoisobutyric acid, forms discrete, voltage-dependent ion channels (Table I). These channels have a number of conductance states in the nanosiemen range and open lifetimes in the millisecond range (Vodyanoy et al., 1982). On the basis of structural and functional studies, the various conductance states of the alamethicin channel are thought to be formed by various aggregates of mostly α-helical monomers (Fox & Richards, 1982; Cascio & Wallace, 1988). The number of alamethicin monomers per channel seems to vary between 6 and 12. Gramicidin A, a 15 amino acid antibiotic, bears less resemblance to the synthetic peptides than alamethicin since it is composed of alternating L- and D-amino acids. Gramicidin A forms discrete ion channels, which have a conductance of approximately 10 pS in 500 mM NaCl and open times in the millisecond to second range (Finkelstein & Andersen, 1981). The gramicidin A channels are not formed by α-helices but instead by two $\beta^{6.3}$-helical monomers arranged amino terminus to amino terminus (Arseniev et al., 1985), a structure not likely to be found in proteins.

By analogy to alamethicin, the MA-β peptide ion channel is most probably formed by a multimeric association of α-helical peptides. The MA-β sequence is predicted to form an α-helix (Finer-Moore & Stroud, 1984) and as such would be 4.0 nm in length and capable of spanning the lipid bilayer. A pentamer of these α-helices would create a channel with a radius of 0.4 nm, a size sufficient to allow the passage of a Na^+ ion with one shell of water, which in total has a radius of 0.32 nm (Stroud & Finer-Moore, 1985). If the channel were indeed formed by multimers, then the two conductance types may reflect a difference in the multimeric state of the peptide. For example, the high-conductance channel may be formed by a hexamer and the low-conductance channel by a pentamer of peptides. The association state of the peptide could not be easily evaluated in our recording system, because only a narrow range of peptide concentrations could be successfully tested for ion channel formation. The long open times of the channel, which range from seconds to minutes, may result from favorable electrostatic interactions among the large number of charges located within the channel's interior. Among the aforementioned peptides capable of forming ion channels, MA-β peptide possesses the greatest number of charged residues: three lysines and four aspartates or glutamates. Closure of the ion channel may result from the exit of the peptide multimers from the membrane, conformational change within the multimers to a nonconducting state, or dissociation of the multimers into nonconducting monomers or oligomers.

Our data suggest that ion channel forming peptides can be grouped into at least three general classes: discrete-conductance channels, such as MA-β, the triad leucine-serine repeat peptide, and alamethicin; continuous-conductance channels, such as M2-δ and the sodium channel peptide; and membrane disruptors, such as AL and the dyad leucine-serine repeat peptide (Table I). The discrete-conductance channels, such as MA-β and the triad leucine-serine repeat peptide, have in common marked amphipathic α-helical periodicities while the two continuous-conductance channels have the weakest amphipathic α-helical periodicities of the peptides examined. Alamethicin, a discrete-conductance channel, has an intermediate amphipathic α-helical periodicity, perhaps because it is not α-helical for its entire length (Fox & Richards, 1982). Like the discrete-conductance channels, the two membrane disruptors possess notable amphipathic α-helical periodicity but appear to be too short to span the membrane as α-helices.

Although peptide ion channels fail to mimic the action of the protein ion channels from which they are derived, they are among the best examples of easily alterable ion channel formers. Along with alamethicin (Menestrina et al., 1986) and gramicidin A (Durkin et al., 1990), they present a tractable system in which to examine the relationship between sequence composition and ion channel properties.

ACKNOWLEDGMENTS

We thank Dr. A. James Hudspeth and Dr. Juan Korenbrot for their assistance and the generous use of their laboratories. We also thank Dr. William M. Roberts and Dr. Fernán Jaramillo for the use of their computer programs, Richard A. Jacobs for technical assistance, and Dr. A. J. Hudspeth, Dr. Michael J. Shuster, Dr. Alexander Kamb, and Stephanie Mel for critical reading of the manuscript.

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