J. Membrane Biol. 98, 101–115 (1987)

The Journal of
Membrane Biology

Topical Review

Reconstitution of Channel Proteins from Excitable Cells in Planar Lipid Bilayer Membranes

Mauricio Montal
Department of Neurosciences, Roche Institute of Molecular Biology, Nutley, New Jersey 07110

Introduction and Scope of the Review

The field of ion channel reconstitution has exploded with the availability of mg quantities of purified channel proteins and with the refinement of the techniques necessary to incorporate these proteins in planar lipid bilayers that readily allow the detailed characterization of the activity of the protein at the level of single molecular events. This period of rapid growth in the biophysical, biochemical, and molecular biological characterization of channel proteins is asserted by the recent publication of several reviews as well as monographs detailing the techniques for the characterization of membrane proteins (Ragan & Cherry, 1986) as well as for the study of reconstituted ion channels (Miller, 1986). Comprehensive reviews of the field (Montal, 1976; Montal, Darszon & Schindler, 1981; Miller, 1984; Etemadi, 1985) and more specialized reviews specifically considering channel proteins have appeared (Goldin, Moczydlowski & Papazian, 1983; Hanke, 1985, 1986; Latorre et al., 1985; Agnew, Rosenberg & Tomiko, 1986; Coronado, 1986; Coronado & Affolter, 1986; Darszon, 1986; Hartshorne et al., 1986*b*; Latorre, 1986; Montal, 1986; Montal, Anholt & Labarca, 1986; Tanaka, Furman & Barchi, 1986). The reader is referred to this literature to consult the specific details concerning the solubilization of membrane proteins from native membranes, the purification from other membrane constituents, the recombination with specific phospholipids and the reconstitution properly into planar lipid bilayer membranes. In this brief review, I will address three central questions: (1) Which channel proteins have been genuinely reconstituted into planar lipid bilayers? (2) What has been learned from the reconstituted channel proteins? and (3) What can be learned from the reconstituted system?

Key Words reconstitution · membranes · channels · ion transport · excitability · lipid bilayers

Which Channel Proteins have been Reconstituted in Planar Lipid Bilayer Membranes?

In keeping with the biochemical tradition, reconstitution into planar lipid bilayers is explicitly defined as the condition where a specific channel protein is solubilized from its native membrane, purified from other membrane constituents, recombined with specific phospholipids and then reassembled into a well-defined planar lipid bilayer membrane that exhibits functional activity (*cf.* Montal, 1986). At present, the channel proteins from eukaryotic cells that appear to fulfill these criteria are the following: (a) The nicotonic cholinergic receptor from the electric fishes *Torpedo californica* and *Torpedo marmorata* as well as a putative acetylcholine receptor from neuronal membranes of locusts; (b) The voltage-dependent sodium channel from mammalian brain and skeletal muscle and from electrical eel; (c) A voltage-dependent calcium channel from the transverse tubule of skeletal muscles. Bacterial porins, the channel proteins present in the outer membrane of enteric bacteria and the mitochondrial "VDAC"—voltage-dependent anion channel—have been purified and reconstituted. The amino acid sequence of VDAC was recently elucidated (Forte, Guy & Mannella, 1987). The literature was recently reviewed by Benz (1986) and by Colombini (1986), respectively, and will not be further discussed here, for the sake of brevity. I now turn to describe in more detail the composition of the purified channel proteins from excitable membranes.

A) The acetylcholine receptor from *Torpedo californica, marmorata* and the acetylcholine receptor from *Locust migratoria*. The nicotinic acetylcholine

receptor (AChR) is the postsynaptic membrane protein that converts the binding of the ligand acetylcholine (ACh) into the transient opening of a cation-selective channel. This signal transduction event is the fundamental process in the transfer of information across synaptic junctions in excitable cells. The best characterized nicotinic AChRs are those present in the peripheral postsynaptic membranes of the fish electromotor synapse, especially of *T. californica* and *T. marmorata*. The AChR molecule has an M_r of approximately 270,000 and is composed of five transmembrane glycoproteins with a subunit stoichiometry of $\alpha_2\beta\gamma\delta$ (Reynolds & Karlin, 1978; Lindstrom, Merlie & Yogeeswaran, 1979; Raftery et al., 1980). The entire amino acid sequence of all its subunits was elucidated and specific models for the arrangement of the polypeptide chains across the membrane bilayer were suggested and tested (Noda et al., 1982, 1983; Claudio et al., 1983; Devilliers-Thiery et al., 1983; Karlin, Kao & DiPaola, 1986; Lindstrom, 1986). The AChR has two ACh binding sites which were mapped to the primary sequence of the α subunits at CYS 192 and 193. The sequences of α, β, γ, and δ subunits are about 40% homologous; however, CYS 192 and CYS 193 are unique to α (Kao et al., 1984; Kao & Karlin, 1986). The AChRs from *T. californica* or *marmorata* are usually solubilized from electric organ membranes with a cholate-lipid mixture (Epstein & Racker, 1978; Changeux et al., 1979; Huganir, Schell & Racker, 1979; Lindstrom et al., 1980; Anholt et al., 1981, 1982; *cf.* McNamee, Jones & Fong, 1986; Montal et al., 1986). In contrast, the nicotinic AChR from *Locust migratoria*, was solubilized from insect ganglionic membrane preparations using sodium deoxycholate and it was further purified by sucrose density gradient centrifugation and affinity chromatography techniques. The purified AChR appears on SDS-polyacrylamide gels as a single band with an $M_r \simeq 250{,}000$–$300{,}000$ and, under denaturing conditions (reduction and boiling), it migrates as a single band with an apparent $M_r \simeq 65{,}000$. These proteins cross-react with monoclonal antibodies raised against the *Torpedo* AChR. It was suggested that this insect neuronal receptor represents an homopolymeric complex of four identical or similar subunits (Breer, Kleene & Hinz, 1985). Caution must be exercised as to the conclusiveness of the existence in insect neuronal receptors of a single subunit, due to the potential contribution of proteolysis, as was the case with the peripheral nicotinic AChR. Meanwhile, however, the insect neuronal AChR and the peripheral *Torpedo* AChR, purified using affinity chromatography with snake venom toxin, after reconstitution in planar lipid bilayers exhibited nicotinic pharmacology (*see* next section).

B) The voltage-dependent sodium channel from mammalian brain and skeletal muscle and from the electric eel *Electrophorus electricus*. The voltage-dependent sodium channel mediates the inward sodium current during the depolarizing phase of a nerve action potential (Hodgkin & Huxley, 1952). Voltage-dependent sodium channels have been isolated and purified predominantly from three tissue sources: Mammalian brain and muscle as well as eel electroplax (Agnew et al., 1986; Catterall, 1986; Hartshorne et al., 1986*b*; Tanaka et al., 1986). All three preparations share in common a glycopeptide with an apparent M_r between 260,000–295,000. Brain sodium channels contain, in addition, two smaller glycopeptides with apparent $M_r \sim 36{,}000$ and ~33,000, also known as β_1 and β_2. β_2 is linked to the larger glycopeptide (α) by a disulfide linkage. In muscle, there is at least one small glycopeptide with an apparent M_r of ~38,000 (Roberts & Barchi, 1987). In contrast, no small peptides were detected in the electric eel preparations (*cf.* Agnew et al., 1986; Levinson et al., 1986).

Numa's group (Numa & Noda, 1986) cloned and sequenced the complementary DNA's (cDNA), encoding the large peptide of electric eel (Noda et al., 1984) and of the rat brain sodium channels (Noda et al., 1986*a*). In brain, three sodium channel genes were discovered with closely related homologies to one another as well as to the electric eel protein. Furthermore, mRNA generated by transcription of the cloned cDNA encoding the rat brain sodium channel large polypeptide, especially rat brain II, when microinjected into *Xenopus* oocytes led to the expression of voltage-dependent sodium currents. These currents were tetrodotoxin (TTX) sensitive, sodium selective and displayed the voltage-dependent activation and inactivation characteristic of genuine voltage gated sodium channels (Noda et al., 1986*b*). A similar result was obtained by Goldin et al. (1986). Thus, it appears that α, the large polypeptide subunit of the sodium channel, is sufficient to form a functional sodium channel. The selective removal of β_2 subunits has no effect on the functional properties of sodium channels, while removal of β_1 subunits leads to the loss of saxitoxin binding activity (Messner & Catterall, 1986; Messner et al., 1986).

Given the primary structure of the electric eel protein and the brain sodium channel proteins, several proposals were suggested concerning the folding of the α polypeptide chain across the bilayer membrane (Noda et al., 1984; Greenblatt, Blatt & Montal, 1985; Kosower, 1985; Guy & Seethara-

lamu, 1986). These models are currently being tested by probing the protein accessibility to binding of antibodies generated to specific synthetic peptides corresponding to given sequences in the protein (Gordon et al., 1987).

C) The voltage-dependent calcium channel from skeletal muscle transverse tubules. Voltage-dependent Ca^{2+} channels are essential in neurotransmission, secretion, excitation-contraction coupling in muscle and modulation of neuronal function (*cf.* Miller, 1987). The transverse or T-tubule membranes of skeletal muscle appear to be the richest source of voltage-sensitive calcium channels currently available. The identification and availability of calcium channel antagonists of the 1,4-dihydropyridine class (e.g., nifedipine, nitrendipine, PN 200-110) has led to the purification of the receptor protein as a putative voltage-sensitive calcium channel. The calcium antagonist receptor was solubilized with digitonin or CHAPS (3-[(3-cholamidopropyl)dimethylammonio]-1-propane-sulfonate) and purified by affinity chromatography using wheat germ aglutinin, followed by ion exchange chromatography and sedimentation through sucrose density gradient. These procedures lead to ≥300-fold purification and a preparation that appears to be better than 40% homogeneous. The isolated calcium antagonist receptor is a large glycoprotein with a subunit composition $\alpha\beta\gamma$ with apparent M_r of 130,000, 50,000, and 33,000, respectively (Borsotto et al., 1984, 1985; Curtis & Caterall, 1984, 1986). Immunoblot analysis suggest that the dihydropyridine receptor in cardiac and smooth muscles has a polypeptide composition similar to that of the T-tubule skeletal muscle protein (Schmid et al., 1986; Glossmann et al., 1987).

What has been Learned from Reconstituted Channel Proteins in Lipid Bilayers?

A) The acetylcholine receptor from *Torpedo californica* and *Torpedo marmorata* and the acetylcholine receptor from *Locust migratoria*. One of the initial motivations to reconstitute the purified AChR in planar lipid bilayers was to assess if the purified complex composed of $\alpha_2\beta\gamma\delta$ subunits was sufficient to express the ligand-regulated cation-selective channel characteristic of the postsynaptic receptor. Indeed, the reconstituted *Torpedo* receptor exhibited the macroscopic phenomena associated with the AChR in native membranes, namely, activation and desensitization in the presence of cholinergic agonists and blockade by cholinergic antagonists. Furthermore, the planar lipid bilayer allowed the resolution of the opening and closing of single AChR channels activated by a variety of cholinergic ligands, namely, ACh, carbamylcholine, and suberyldicholine (Nelson et al., 1980; Schindler & Quast, 1980; Boheim et al., 1981; Suarez-Isla et al., 1983; Tank et al., 1983; Labarca, Lindstrom & Montal, 1984*a*,*b*; Montal et al., 1984; Schindler, Spilleke & Neumann, 1984). The result that the pentameric complex of $\alpha_2\beta\gamma\delta$ is the minimum entity necessary for function was confirmed by experiments where mRNA generated by transcription of the cloned cDNA encoding each one of the *Torpedo* receptor subunits were injected into *Xenopus* oocytes leading to the expression of functional cholinergic receptors (Mishina et al., 1984, 1985; Sakmann et al., 1985). Studies on the ion conduction properties through the reconstituted AChR using the single-channel assay demonstrated that the reconstituted receptor had a single-channel conductance, γ, in symmetric 0.5 M NaCl of 45 pS and showed the same apparent ionic selectivity sequence determined for the receptor in muscle cells, namely: $NH_4^+ > Cs^+ > Rb^+ > Na^+ \gg Cl^-, F^-, SO_4^{2-}$ (Adams, Dwyer & Hille, 1980; Horn & Patlak, 1980; Dani & Eisenman, 1984; Labarca et al., 1984*b*). In addition, the reconstituted receptor exhibited the saturation characteristics known in muscle cells (Horn & Patlak, 1980; Tank et al., 1983; Labarca et al., 1984*b*; Montal et al., 1984). Thus, ion conduction through the reconstituted receptor channel is fully retained in the planar lipid bilayer.

The analysis of single-channel gating kinetics of the reconstituted receptor led to the realization that the distribution of channel open times was well fitted by the sum of two exponentials reflecting the existence of, at least, two channel open states (Labarca et al., 1982, 1984*b*, 1985*a*,*b*; Tank et al., 1983). For ACh, the typical values of the time constants for the short lived (S) and long lived (L) open states are 0.5 msec and about 4 msec (Labarca et al., 1985*a*,*b*). The time constants for these two states exhibited cholinergic pharmacology, being longest for suberyldicholine and shortest for carbamylcholine, in agreement with single-channel data obtained in native muscle AChR (Colquhoun & Sakmann, 1985). In contrast, the time constants were not affected systematically by changing agonist concentration in the range 0.01 μM ≤ ACh ≤ 0.1 mM. Similar results were obtained with carbamylcholine (Labarca et al., 1984*a*, 1985*a*). Since the channel lifetimes are not affected by agonist concentration some other parameters of the channel open state must increase to account for the established dependence of the cholinergic response or conductance on ACh concentration (*cf.* Dionne,

Steinbach & Stevens, 1978). Indeed, the frequency of occurrence of the long openings increases with agonist concentration (Labarca et al., 1985*b*). These results led Labarca et al. (1985*a,b*) to suggest the following kinetic scheme to account for the results:

$$\begin{array}{ccccc} C + 2L & \rightleftharpoons & CL + L & \rightleftharpoons & CL_2 \\ & & \downarrow\uparrow & & \downarrow\uparrow \\ & & OL & & OL_2 \end{array}$$

where C represents closed channel states and O open states and L denotes the ligand.

The independence of the time constants for both the short- and long-lived open state on agonist concentration are readily accounted for by the scheme, implying that the liganding step occurs into the closed states. Furthermore, such a model predicts a linear dependence with a unitary slope of the area (A) ratio of the long-lived and short-lived open states (A_L/A_S ratio) on agonist concentration when plotted as a log (A_L/A_S) *versus* log of ACh concentration. The function, determined experimentally, indeed shows a unitary slope within the range of ACh concentration between 0.01 and 0.1 μM. At higher concentration, the A_L/A_S ratio approaches a constant value (Labarca et al., 1985*a*). Similar results were obtained by Colquhoun and Sakmann in adult muscle cells (1985). Considering that the AChR contains two distinguishable ACh binding sites on each one of the α subunits, the simplest mechanism proposed to account for the described results suggested that both singly and doubly liganded states of the AChR molecule could undergo transitions to the open conformation and that the short-lived open state corresponded to the singly liganded receptor, while the long-lived open state was associated with the biliganded form of the receptor (Labarca et al., 1985*a,b*).

It was with the reconstituted AChR channel that a new approach for the analysis of single channels, which takes advantage of autocorrelation and autocovariance functions, was developed (Fredkin, Montal & Rice, 1985). This approach allows the extraction of information about the pathways that connect the open and the closed states. Application of autocovariance analysis to the reconstituted AChR indicated that there are at least two entry/exit states through which the open and closed state aggregates communicate (Labarca et al., 1985*b*), consistent with the kinetic scheme shown above. Recently, we have calculated the two-dimensional distribution of single-channel open and closed intervals which provide the relative occurrence of open $\rightarrow$ closed and closed $\rightarrow$ open transitions as function of both the open and closed time intervals. The results of such computations on the AChR as a function of agonist concentration confirm the occurrence of two open states and suggest a kinetic scheme where the short-lived open state ($\tau_{os} \simeq 0.5$ msec) is primarily accessed through a long-lived closed state ($\tau_{cl} \simeq 2.5$ msec) while the long-lived open state ($\tau_{ol} \simeq 5$ msec) communicates with the short-lived closed state ($\tau_{cs} \leq 0.5$ mmsec). These two predominant gating patterns are suggested to arise from transitions proceeding in the monoliganded and biliganded forms of the AChR, respectively (Fredkin et al., 1985; Labarca et al., 1985*b*; B.U. Keller & M. Montal, *unpublished results*). The cumulative evidence renders support to the notion that the AChR channel can open from the singly liganded form as well as from the doubly liganded form and that agonist concentration increases effectively the probability of channel opening by promoting the frequency of occurrence of the long-lived open state (biliganded form). The results of this extensive kinetic analysis of the reconstituted receptor have contributed to our current understanding of the kinetics of the ligand regulated ion channel.

The reconstituted receptor was used to evaluate the role of protein phosphorylation on the functional activity of the channel. For this purpose, the *Torpedo* receptor was phosphorylated by purified catalytic subunit of the cyclic AMP-dependent kinase. This kinase specifically phosphorylates the AChR γ and δ subunits (Huganir & Greengard, 1983; Huganir, Miles & Greengard, 1984). This site was located to be within the sequence bounded by residues 350–354 of the γ subunit (Huganir et al., 1984, 1986) and 354–367 of the δ subunit (Huganir et al., 1984, 1986; Safran, Neumann & Fuchs, 1986). Inspection of single-channel records show that receptor phosphorylation increases the channel opening probability without affecting the single-channel conductance (Montal & Montal, 1987). Detailed analysis of the single-channel currents indicates that the increment in the opening frequency arises primarily from a shortening of the characteristic long silent periods (channel closed times) that separate the bursts of channel openings. Agonist-activated cation flux measurement in reconstituted vesicles show that AChR phosphorylation accelerates the rate to enter into the desensitized state (Huganir et al., 1986). These results are consistent with the view that AChR phosphorylation modifies the kinetics of its ability to enter into and exit from the desensitized state (Huganir et al., 1986; Montal & Montal, 1987). They also point to a critical role of the γ and δ subunits in AChR gating. This notion is further supported by the effects of monoclonal antibodies specific to the β and γ subunits of the AChR on single-channel activity. Three monoclonal anti-

bodies directed against determinants on the β and γ subunits inhibit channel activity (Lindstrom et al., 1983; Blatt et al., 1984, 1986). One of them, 168, directed against the cytoplasmic domain of γ inhibited single AChR channels only when added to the compartment opposite to that containing ACh, in agreement with its specificity for determinants on the cytoplasmic surface of AChR (Lindstrom, 1986). Furthermore, substitution of mRNA encoding for bovine δ subunit for mRNA coding *Torpedo* δ subunit in a mixture of mRNAs encoding a complete set of AChR subunits injected into *Xenopus* oocytes expresses AChR with considerable longer open channel lifetimes (Sakmann et al., 1985). Taken together, the results point to a critical role of γ and δ subunits in the control of AChR gating.

The neuronal AChR from locust was first reconstituted into lipid vesicles and then fused with preformed lipid bilayers (Hanke, 1985, 1986). Single channels were activated by the cholinergic ligands carbamylcholine and suberyldicholine and were inhibited by curare, thus exhibiting nicotinic pharmacology (Hanke & Breer, 1986). The single-channel conductance in symmetric 0.1 M NaCl was 75 pS while in KCl it was 70 pS, indicating poor selectivity between these two monovalent ions. Analysis of the channel open times indicated the existence of a single-channel open state with a lifetime for suberyldicholine of ~14 msec while for carbamylcholine of ~3–5 msec. Analysis of the channel closed times indicated the occurrence of two channel closed states with apparent lifetimes of 25 msec for the mean closed times within the bursts and 300 msec for the mean closed times between bursts. It should be interesting to extend the kinetic analysis to resolve faster openings that could express the existence of two open states and three closed states as is the case for the *Torpedo* AChR. Nevertheless, having demonstrated that the purified insect neuronal AChR exhibits the single-channel activity expected from a chemically activated channel comparable to that of the peripheral AChR, the question now arises as to whether the insect neuronal receptor is the predicted ancestral AChR, that is a homooligomer of α-like subunits that constitutes the archetype of the contemporary vertebrate receptors. The primary structure of the locust neuronal receptor is eagerly awaited for to establish homologies between insect, fish and vertebrate receptors. In this context, it is noteworthy that the primary structure of a nicotinic AChR from *Drosophila melanogaster* was recently elucidated (Hermans-Borgmeyer et al., 1986). This AChR shows extensive homology to known AChR subunits from vertebrate and fish AChRs.

B) The voltage-dependent sodium channel from mammalian brain and skeletal muscle transverse tubule and from electric eel of *Electrophorus electricus* were reconstituted in planar lipid bilayers essentially by the same strategy. The technique basically consists of making membrane vesicles containing purified proteins interact with preformed planar bilayers (*cf.* Cohen, 1986; Hanke, 1986). The technical details of the experimental protocols were described extensively in the methodological papers by Agnew et al., (1986), Hartshorne et al. (1986*b*), Levinson et al. (1986), and Tanaka et al. (1986). The properties of the reconstituted sodium channels were studied in the presence of batrachotoxin (BTX). BTX removes the inactivation of the sodium channel and shifts the voltage dependence for activation towards more negative voltage values (towards more hyperpolarizing potentials, in the electrophysiological convention) (*see* Khodorov, 1975; Khodorov & Revenko, 1979; for review *see* Khodorov, 1985).

A remarkable property of the voltage-sensitive channel is its exquisite sensitivity to the applied voltage: negative (hyperpolarizing) voltages favor channel closing, whereas positive (depolarizing) voltages favor channel opening (Hartshorne et al., 1984, 1985, 1986*a,b*; Keller et al., 1985, 1986; Furman et al., 1986). The fraction of time that the channel spends in the open state is a function of applied voltage. The probability of channel opening can be calculated as a Boltzmann function of the applied voltage. From the calculated function, two parameters are derived; q, the apparent gating charge, and V_{50}, the voltage at which the probability of channel opening is 0.5. For the reconstituted mammalian brain and skeletal muscle T-tubule channels, q is 3.8 (Hartshorne et al., 1985) and 3.7 (Furman et al., 1986; Tanaka et al., 1986). In contrast, for the electric eel reconstituted channel $q = 1.7$ and, under some conditions, can be as high as 3.4 (Levinson et al., 1986). Thus, eel sodium channels appear to be less sensitive to the applied field than either brain or skeletal muscle channels. For all three systems, V_{50} is, as expected, at far more negative values than that present in the unmodified native sodium channels. For the mammalian channels, the V_{50} is in the range of -90 to -95 mV (Hartshorne et al., 1985, 1986*a,b*; Furman et al., 1986; Tanaka et al., 1986): for the eel channel, $V_{50} = -77$ mV (Levinson et al., 1986). The V_{50} of purified sodium channels varies from channel to channel. This variation can be as large as 30 mV to either side of the average V_{50} (-90 mV). Channel opening, however, remains steeply voltage dependent over a limited voltage range for each channel (Hartshorne et al., 1985, 1986*a,b*). The variation in V_{50} from channel to channel can be accounted for by heterogeneity in the sodium chan-

nel population, perhaps arising from variations in the extent of protein glycosylation. The V_{50} and q parameters calculated for reconstituted sodium channels are in good agreement with those determined in BTX modified sodium channels derived from native membranes and measured after insertion of the channels into preformed lipid bilayers by the fusion technique. These preparations include synaptosomes (French et al., 1984, 1986) and rat sarcolemma (Moczydlowski, Garber & Miller, 1984). Similar results were obtained from electrophysiological recordings of BTX modified sodium channels in frog node of Ranvier (Khodorov & Revenko, 1979) and in NG108-15 neuroblastoma cells (Huang, Moran & Ehrenstein, 1982, 1984). These measurements indicate that the voltage-sensing mechanism as well as the BTX sensitivity remain intact in the purified and reconstituted sodium channels.

The exquisite sensitivity of voltage gated sodium channels to TTX was used to ascertain the fidelity of the reconstituted system. The reconstituted sodium channel indeed shows blocking by TTX (Hartshorne et al., 1984, 1985; Furman et al., 1986; Levinson et al., 1986). TTX blocks the reconstituted channels with K_i in the nM range: for the rat brain channel the K_i at -50 mV = 8.3 nM (Harsthorne et al., 1985) and for the eel channel at -60 mV K_i = 10 nM (Levinson et al., 1986), both measurements in 0.5 M NaCl. As with native sodium channels, the K_i for TTX block is voltage dependent (Krueger, Worley & French, 1983; French et al., 1984, 1986; Hartshorne et al., 1985; Levinson et al., 1986). The voltage dependence of this shift in K_i for the brain channel corresponds to an e-fold increase in K_i for each 43 mV of the depolarization (Hartshorne et al., 1985). For the skeletal muscle sodium channels, these parameters were not determined. In addition, the TTX block of the reconstituted brain channel displayed the reversibility characteristic of this blockade in native membranes (Hartshorne et al., 1985). The reconstituted mammalian channels exhibited blockade by TTX only from the normally extracellular face of the channel (Hartshorne et al., 1985; Furman et al., 1986), in agreement with the established topography of the TTX binding site.

The single-channel conductance of the reconstituted sodium channel was characterized in detail. For the BTX-modified sodium channel is symmetric 0.5 M NaCl the single-channel conductance (γ) is 25 pS, 23.7 and 20 pS for rat brain, electric eel, and rabbit T-tubule membrane reconstituted sodium channels, respectively (Hartshorne et al., 1985; Furman et al., 1986; Levinson et al., 1986). For veratridine-modified sodium channels, γ = 13 and 13.5 pS in rat brain and electric eel reconstituted sodium channels (R.P. Hartshorne, B.U. Keller and M. Montal, *unpublished observations*; Levinson et al., 1986). γ in symmetric solutions of the chloride salts of Na^+, K^+ and Rb^+ is ohmic (Hartshorne et al., 1985; Furman et al., 1986; Levinson et al., 1986). For rat brain, γ in symmetric K^+ = 3.2 pS and in Rb^+ = 1.1 pS, yielding apparent conductance ratios of Na^+/K^+ of ~8 and Na^+/Rb^+ of ~23 (Hartshorne et al., 1985). For the eel reconstituted sodium channel, the apparent selectivity of Na^+/K^+ is 4.5 (Levinson et al., 1986). For the reconstituted brain sodium channel γ increases with sodium activity and displays saturation behavior. The two parameters extracted from a fit to this function are γ_{max}, the maximum conductance observed at high sodium activity, and $K_{1/2}$, the sodium activity for $\frac{1}{2}$ maximum conductance. For the reconstituted brain sodium channel, $K_{1/2}$ = 52 mM and γ_{max} = 28 pS (B.U. Keller, R.P. Hartshorne and M. Montal, *unpublished results*). For the native rat brain and muscle sodium channels incorporated into lipid bilayers, the equivalent parameters are 37 mM and 32 pS, respectively (Andersen, Green & Urban, 1986). All the properties of ion conduction through purified sodium channels studied are in remarkable agreement with those measured in native sodium channels incorporated into bilayers by similar techniques.

It is worthy of note that, in a study of rat brain sodium channels in the absence of BTX and in symmetric solutions of 140 mM NaCl, γ = 25 pS (Hanke et al., 1984). Likewise, reconstituted eel channels in proteoliposomes that were subjected to freezing and thawing and subsequently sealed to the tip of a patch electrode (Suarez-Isla et al., 1983; Tank et al., 1983) provided measurements of γ in the absence of BTX of 11 pS in solutions containing, in one compartment 90 mM NaCl and in the other 10 mM NaCl+ 80 mM KCl; in the presence of BTX and a similar aqueous solution, γ = 24 pS (Rosenberg, Tomiko & Agnew, 1984; Agnew et al., 1986). These different values of γ from those reported in other preparations have not yet been accounted for.

The study on the reconstituted eel sodium channel by the patch-clamp recording technique provided single-channel recordings of sodium channels activated by voltage in the absence of BTX (Rosenberg et al., 1984). A fraction of depolarization steps showed distinct opening events. Summation of several of these transient recordings showed that, following the steps of depolarization, there was a transient inward current which declined exponentially to 0 current level. The decay was fitted by a single exponential with a time constant of 6–7 msec, corresponding to similar numbers for the time course

of inactivation determined from sodium channels in native membranes (Sigworth & Neher, 1980; Aldrich et al., 1983; Rosenberg et al., 1984; Vandenberg & Horn, 1984; Agnew et al., 1986).

A detailed study of the channel-gating kinetics of purified sodium channels modified by BTX was conducted on the brain channel. Probability density analysis of dwell times in the closed and open states of the channel indicates the occurrence of a single open state but of multiple distinct closed states in the voltage (V) range -120 mV $\leq V \leq +120$ mV. The transition rates between states are exponentially dependent on the applied voltage, as described also in mouse neuroblastoma cells (Huang et al., 1984). There are two opening rates: the fast opening rate is exponentially voltage dependent for negative applied voltages with a slope of 13.5 ± 0.9 mV/e-fold change, while the slow opening rate decreases exponentially with a slope of -20.2 ± 2.3 mV/e-fold change. At negative applied voltages, the closed rate is exponentially voltage dependent with a slope of -13.6 ± 0.6 mV/e-fold change. In contrast, for positive applied voltages, all the transition rates for opening and closing are virtually voltage independent. Autocorrelation analysis shows that there is no correlation in the durations of successive open or closing events (Keller et al., 1985; 1986; Labarca et al., 1985*b*). Several kinetic schemes that are consistent with experimental data were considered. For the entire studied range of apparent voltages, BTX-modified sodium channel gating can be explained by the 5-state model which considers the sequence: *C-C-O-C-C*, where *C* denotes closed state and *O* an open state of the channel. This model considers exponentially voltage-dependent transition rates. A *C-C-O* model with exponentially voltage-dependent and saturating transition rates is equally adequate. At present, the latter model is favored because of its inherent simplicity (Keller et al., 1986). This characterization of the gating kinetics of purified sodium channels should serve as a fingerprint for further comparisons with reconstituted sodium channels where chemical or genetic modifications are introduced. This approach may provide some insights into the mechanism underlying the voltage dependence of channel activation. Definitely, an immediate goal should be the detailed characterization of the channel gating kinetics in the absence of BTX.

It is clear, therefore, that all the properties tested for the purified reconstituted sodium channels in lipid bilayers match in remarkable agreement those of the native sodium channel: the voltage dependent for channel opening, the V_{50} for channel opening, the apparent gating charge, the sensitivity to neurotoxins such as BTX, veratridine, and TTX, the single-channel conductance, the apparent selectivity between the monovalent ions. Furthermore, the kinetic analysis of purified sodium channels modified by BTX suggested a scheme with one open state and several closed states. The transition rates calculated for a *C-C-O* model of native (Huang et al., 1984) and reconstituted (Keller et al., 1986) sodium channels provided values for the transition rates in both systems of comparable magnitude. This indicates that native and purified sodium channels can be described in terms of the same kinetic scheme.

C) The voltage-dependent calcium channel from skeletal muscle transverse tubule. Calcium channels from cardiac sarcolemma membranes (Rosenberg et al., 1986), skeletal muscle T tubule membranes (Affolter & Coronado, 1985, 1986), brain synaptosomes (Nelson, French & Krueger, 1984) and *Paramecium* cilia (Ehrlich et al., 1984) were incorporated into planar lipid bilayers by fusion of native membrane vesicles to the preformed bilayer (*cf.* Nelson, 1986). Black lipid membranes containing organic solvent or solvent-free bilayers formed at the tip of patch pipette or across holes in hydrophobic septi were used. The detection of calcium channels from native membranes in planar lipid bilayers was instrumental in the identification as well as the characterization of the single-channel properties of voltage-dependent calcium channels. The availability of the dihydropyridine agonist which prolong the open channel time and the antagonists which competitively block the action of the agonist provided the pharmacological reliability to ascertain the origin of the single-channel currents detected in the bilayer. In addition, the selectivity for divalent cation (but not to Mg^{2+}) over monovalent cation, the voltage-dependent kinetics as well as the loss of calcium selectivity when membranes are studied in the absence of divalent cations provided an assay with operational criteria to identify the putative voltage-dependent calcium channel in the bilayer (*cf.* Nelson, 1986; Miller, 1987).

The properties of calcium channels from bovine cardiac sarcolemma membrane vesicles incorporated into planar lipid bilayers by the fusion technique were compared with those of cardiac channels studied in intact cells by the patch-clamp technique (Rosenberg et al., 1986). As in most studies concerned with voltage-dependent calcium channels, a dihydropyridine calcium channel agonist BAY K8644 was included in all experiments to increase the frequency of channel opening and to prolong the lifetime of the opening events (Hess, Lansman & Tsien, 1984). Depolarization increased the probability of channel opening: at -50 mV the channel opening probability was virtually null while

at 100 mV it was 0.9. In symmetric solutions of 0.1 M $BaCl_2$, 50 mM NaCl, at pH 7.4, $\gamma = 22.7 \pm 1.1$ pS (Rosenberg et al., 1986). The distribution of channel open times was well fitted by a single exponential with a time constant at 0 mV of 20 msec (Rosenberg et al., 1986). Presumably, brief opening events were undetected due to bandwidth limitations. The voltage dependence of channel activation, the single-channel conductance and open-time distributions were similar to those recorded for cardiac L-type channels in intact cells (Brown et al., 1982; Reuter et al., 1982). By contrast, skeletal muscle T-tubule calcium channels studied under similar conditions and with similar techniques, exhibit a $\gamma = 10.6 \pm 0.8$ pS. Likewise, the skeletal channels activate more slowly and have a longer mean open time (Rosenberg et al., 1986; Affolter & Coronado, 1985, 1986; Ehrlich et al., 1986; Coronado & Affolter, 1986).

Two independent groups described the reconstitution of the purified voltage-sensitive calcium channel from skeletal T-tubule in planar lipid bilayers. Flockerzi et al. (1986) purified calcium channels according to Curtis and Catterall (1984) with the exception that TSK DEAE-5PW was used instead of DEAE Sephadex. The protein was recombined with phosphatidylethanolamine (PE)/phosphatidylserine (PS)/cholesterol at the weight ratio of 2.8 : 1.2 : 1 and the lipid receptor mixture in detergent was dialyzed. The dialyzed proteoliposomes were added to the aqueous bath. Lipid bilayers were formed at the tip of patch pipettes from monolayers composed of bovine heart PE, bovine brain PS and cholesterol at the ratio of 70 : 15 : 15 in hexane. The solutions on both sides of the bilayer contained 90 mM $BaCl_2$ at pH 7.3. Under this condition, spontaneous single-channel openings were detected in the range of applied voltage of ±165 mV. The current voltage relationship was ohmic with a slope conductance of 20 pS, in agreement with results obtained from cell-attached L-type calcium channels as well as from planar lipid bilayers containing native skeletal muscle T-tubule and cardiac sarcolemma membranes (Rosenberg et al., 1986). In the presence of dihydropyridine calcium agonist BAY K8644, the probability of channel opening increased from 0.2 ± 0.01 to 0.81 ± 0.08 with a significant increase in the frequency of long opening events. Furthermore, the phenylakylamine calcium channel blocker gallopamil (25 μM) reduced the probability of channel opening from 0.22 ± 0.5 to 0.02 ± 0.01 with a considerable abbreviation of the open durations. As in native L-type calcium channels (Bean, Nowycky & Tsien, 1984; Cachelin et al., 1984), the purified and reconstituted tubule calcium channel was modulated by protein phosphorylation. The α ($M_r \sim 142{,}000$) and β ($M_r \sim 56{,}000$) peptides of the purified calcium channel were phosphorylated by cyclic AMP-dependent protein kinase (Flockerzi et al., 1986; Hosey, Borsotto & Lazdunski, 1986; Curtis & Catterall, 1984). Bath application of catalytic subunit of the cyclic AMP-dependent kinase in the presence of Mg^{2+} and ATPγS prolonged the open channel lifetimes and abbreviated the closed intervals between channel openings. The probability of channel opening increased from 0.05 ± 0.02 to 0.57 ± 0.11 after phosphorylation. Control experiments using denatured receptor, or in the absence of cyclic AMP-dependent protein kinase, Mg^{2+} or ATP, established the specificity of the phosphorylation effect. In a similar study McKenna et al. (1987) reconstituted the purified voltage-dependent calcium channel from rabbit skeletal muscle T-tubule into planar lipid bilayers. This purified channel was composed primarily of the α polypeptide with an apparent M_r of 150,000 with other minor polypeptide components. This preparation exhibited spontaneous channel activity. The most prominent channel showed properties indistinguishable from those recorded from native T-tubule membranes: $\gamma = 10$ pS in 100 mM Ba^{2+}; voltage-dependent kinetics; selectivity for divalent cation (not Mg^{2+}); minimum permeability ratio for monovalents $P_{Ba^{2+}}/P_{Na^+} = 20$; sensitivity to the dihydropyridine agonist BAY K8644 with the consequent prolongation of the channel open times and with an increase in the frequency of channel opening. In addition, a second type of calcium channel with $\gamma = 22$ pS was detected. The major difference between these two reports is the apparent lack of voltage-dependent activation in the preparation purified by Flockerzi et al. (1986) and its occurrence in the preliminary report by McKenna et al. (1987).

The purified and reconstituted calcium channels seem to match remarkably well the electrophysiological properties characterized for L-type calcium channels (Nowycky, Fox & Tsien, 1985; *cf.* Miller, 1987): they exhibit voltage-dependent kinetics with an activation range for applied voltages from −10 mV to positive, and inactivation range from −60 to 10 mV; single-channel conductances of 22–25 pS; modulation by dihydropyridine agonists and antagonists; as well as blockade by external Cd^{2+} or Co^{2+}. It is worth noting that injection of RNA isolated from rat brain, heart and skeletal muscle, into *Xenopus* oocytes leads to the expression of functional voltage-gated calcium channels in the oocyte membrane. A detailed characterization of the electrophysiological properties of these channels demonstrates that the calcium channels from different sources exhibit distinct properties in terms of time course, sensitivity to calcium channel agonists and

antagonists as well as modulation by hormones and neurotransmitters (Dascal et al., 1986). This approach may lead to the identification of the minimum components necessary to express functional calcium channels and may clarify the role of the smaller β and γ subunits in the activity of the calcium channel.

Which Channels Are Likely to be Reconstituted in the Near Future?

The three channel proteins discussed thus far, which have demonstrated functional activity in the reconstituted planar lipid bilayer, were isolated and purified due to the availability of a tissue source naturally enriched in the protein as well as the availability of a high affinity specific inhibitor which was used in binding assays to follow the purification of the protein, or as affinity ligand to develop purification strategies using affinity chromatography techniques. Such strategy can be applied to other channel proteins.

Calcium-Activated K^+ Channels and Calcium Channels

Recently, a component of the scorpion venom *Leiurus quinquestriaus* was identified as a very specific inhibitor of the calcium-activated potassium channel from skeletal muscle (Miller et al., 1985). This scorpion toxin, was named charibdotoxin. Its activity was originally identified during studies of the properties of the calcium-activated potassium channel of skeletal muscle detected in planar lipid bilayers (Latorre, 1986). That observation was further exploited as an assay for the activity of charibdotoxin during the purification procedure. Recently, charibdotoxin was purified; it is a highly basic protein with a M_r of about 10,000. It corresponds to approximately 0.1% of the total venom protein. The protein is remarkably stable to denaturing reagents including heat, organic solvents and extreme pH. However, its channel-specific inhibitory activity is sensitive to chymotrypsin treatment and to acylation of lysine groups. Charibdotoxin was iodinated without loss of activity. The purified toxin inhibits the calcium-activated potassium channel with a K_d of about 3.5 nM (Smith, Phillips & Miller, 1986). It is anticipated that the availability of ^{125}I-labeled charibdotoxin will facilitate direct binding measurements of the toxin to membrane vesicles and its remarkable stability will be of extreme value in designing strategies for the isolation and purification of the channel protein. It is likely that the combination of these factors will allow purification of the channel protein in the not too distant future.

Apamin is a polypeptide component from the bee venom which also acts on calcium-dependent potassium channels in many excitable cells. However, it is inactive on the calcium-activated potassium channels from skeletal muscle T-tubule. The polypeptide can be iodinated without the loss of activity. Radiolabeled monoiodoapamin binds specifically to calcium-dependent potassium channels from synaptosomes, neuroblastoma cells and primary neuronal cell culture, smooth muscle and skeletal muscle cells (*cf.* Lazdunski, 1983). Radiolabeled apamin binds specifically also to rat brain sections with high affinity (K_d = 25 pM) to a single class of sites (Mourre, Hugues & Lazdunski, 1986). In addition, an endogenous equivalent of apamin was detected in rat brain (Fosset et al., 1984). The apamin receptor protein from rat brain synaptosomes was solubilized in cholate (Seagar, Marquez & Couraud, 1987). The solubilized receptor retained the high affinity for ^{125}I-apamin (K_d = 40 pM) characteristic of the native form (K_d = 30 pM). Binding was inhibited by K^+ channel blockers quinidine and tetraethylammonium (Seagar et al., 1987). Thus, apamin is also a potential candidate for the purification of calcium-activated potassium channels, especially from neuronal sources.

It is interesting to note the recent isolation of *Coral goniopora* toxin (Qar et al., 1986). This polypeptide toxin has an apparent M_r of 90,000. The toxin behaves as a calcium channel activator in a variety of functional assays: it contracts guinea pig ileum; it stimulates the uptake of $^{45}Ca^{2+}$ into cardiac cells in culture. Both of these effects are prevented or abolished by calcium channel blockers. Furthermore, the polypeptide toxin prevents the binding of (+) − [^{3}H] PN 200-110 to the calcium channel protein of skeletal muscle T-tubule membranes. All these effects are observed at μM toxin concentrations. Thus, the coral toxin may prove valuable in the study of calcium channels, the identification of voltage-dependent calcium channels in other tissues as well as the purification of calcium channel from other sources. Included in this category are the ω-conotoxins purified from the piscivorous marine gastropods *Conus geographs* and *Conus magus* which recognize distinct voltage-dependent calcium channel types in neuronal membranes (Cruz, Johnson & Olivera, 1987).

The Gap Junctional Channel

Communication between neighboring cells proceeds through specialized transcellular channels, known as gap junctions. These channels provide a

pathway between cells to exchange, by passive diffusion, ions and small molecules through a large nonselective pore. They are ubiquitous in the animal kingdom but have been characterized biochemically, physiologically, immunologically and molecularly especially in liver, heart, and lens tissues. Gap junctions can be isolated without detergent treatment. The isolated gap junction can then be solubilized with detergents. The purified junctional protein appears to be a single polypeptide with an apparent M_r of 28 kDa. The gap junction protein from lens and liver was cloned and sequenced (Revel, Nicholson & Yancey, 1985; Kumar & Gilula, 1986; Paul, 1986). The derived sequence corresponds to a polypeptide with relative mass of 32 kDa devoid of an amino-terminal signal sequence. The complete sequence of the cardiac junction proteins derived from the cDNA clones may be available soon.

From a biophysical point of view, the gap junctional conductance exhibits a cut-off diameter for molecules up to 8,000–12,000 daltons. The junctional conductance can be modulated by a variety of physiological effectors: protons and calcium decrease the junctional conductance; phosphorylation by the cyclic AMP-dependent protein kinase increases the junctional conductance (*cf.* Spray & Bennett, 1985). Isolated liver gap junctions were incorporated into planar lipid bilayers formed at the tip of patch pipettes (Spray et al., 1986). The single-channel conductance was 150 pS in 0.15 M electrolyte solution. The single-channel currents were blocked by a polyclonal antibody generated against rat liver junctional membranes and were blocked by similar concentrations of protons and octanol as exhibited by the macroscopic junctional currents recorded from pairs of hepatocytes. Isolated lens junctions were solubilized with octylglucoside. The solubilized material was reconstituted into liposomes. The proteoliposomes were added both to aqueous compartments separated by a preformed lipid bilayer (Zampighi, Hall & Kreman, 1985). Voltage-dependent channels were detected 5–10 min after this operation. The channels exhibited a conductance of 1,500–2,000 pS in 1 M salt and 200 pS in 0.1 M salt solutions, resembling values measured in native junctions (Neyton & Trautman, 1985). The channel showed no selectivity between Na^+ and K^+ and no modulation by calcium up to mM concentrations. Microinjection of mRNA from estrogen-stimulated rat myometrium into *Xenopus* oocytes results in the functional expression of the junctional channel (Werner et al., 1985). The availability of the genes for these channel-forming proteins allows one to anticipate that the mRNAs generated by transcription of the cloned cDNAs encoding the junctional channel, when injected into *Xenopus* oocytes, should translate and express functional junctional channels. This experiment should establish the minimum protein component necessary for functional activity. Progress in the high resolution structural analysis of the gap junction (Unwin & Zampighi, 1980; Makowski et al., 1984) will provide a unique opportunity to integrate the molecular structure of the junctional channel, its biophysics, with its three dimensional arrangement in the lipid bilayer.

The Light-Sensitive and Cyclic GMP-Dependent Channel of Retinal Rod Outer Segments

Visual transduction in retinal rod photoreceptors is initiated by the light-induced isomerization of the 11 *cis*-retinal chromophore in the visual pigment rhodopsin. Photoexcited rhodopsin activates the cylic GMP phosphodiesterase via a GTP-binding protein or transducin. This flow of information from rhodopsin to transducin to the phosphodiesterase results in a very fast and highly amplified cascade of information transfer (Stryer, 1986). The subsequent hydrolysis of cyclic GMP leads to the hyperpolarization of the rod photoreceptor due to a decrease in the plasma membrane sodium conductance. The light modulated sodium conductance has now been identified with a 3′,5′-cyclic GMP-dependent conductance located in the rod outer segment plasma membrane. These results were obtained from recordings of cyclic GMP-activated single-channel currents from excised rod outer segment patches. The most prominent single-channel current exhibited a conductance of about 25 pS. The membrane current increased with the third power of cyclic GMP concentration and with a half saturation cyclic GMP concentration of about 15 μM. Sub mM concentrations of divalent cations (magnesium or calcium) led to a fast flickering block of the open channel (Cobbs & Pugh, 1985; Fesenko, Kalenskov & Lyubarsky, 1985; Haynes & Yau, 1985; Yau & Nakatani, 1985; Haynes, Kay & Yau, 1986; Zimmerman & Baylor, 1986).

Sonicated outer segment preparations from dark-adapted frog or from bovine retinal rod outer segments were fused to preformed planar lipid bilayers (Tanaka et al., 1987). Cyclic GMP activated the membrane conductance exhibiting saturation behavior with a $K_{\frac{1}{2}}$ between 12 and 27 μM. Cyclic GMP-activated currents were detected in the presence of sodium, lithium, cesium or potassium but not in the presence of choline. In agreement with the results obtained from native rod outer seg-

ments, the presence of divalent cations, calcium or magnesium, led to the blockade of the conductance. Thus, the incorporated retinal rod membrane material in the planar lipid bilayers exhibits the characteristics expected for a cyclic GMP-activated channel.

Bovine rod outer segment membranes were solubilized in the presence of the zwitterionic detergent CHAPS in the presence of calcium and phospholipids. The rod membrane material was incorporated into liposomes following the removal of detergent by dialysis, leading to the formation of calcium-containing liposomes. The assay for channel activity was the cyclic GMP-induced release of calcium from liposomes as monitored with the calcium indicator dye arsenazo III. Under these conditions and with this assay the cyclic GMP-activated permeability pathway was promoted by cyclic GMP concentration with a $K_{\frac{1}{2}}$ of 19 μM and exhibited a Hill coefficient, $n = 2.7$ (Cook et al., 1986).

These two reports suggest that the stage is now set for the purification of the cyclic GMP-activated channel protein from retinal rod outer segments and its reconstitution into planar lipid bilayer. A potential strategy to attempt the purification of the channel protein is based on the known sensitivity of the cyclic GMP conductance in retinal rods to the calcium channel antagonist and blocker diltiazem, the 1-*cis* isomers (Stern, Kaupp & MacLeish, 1986). It may be interesting to explore different analogs of the diltiazem channel blocker type in their ability to permit the identification and potential purification of the cyclic GMP-dependent channel using them as affinity ligands.

Cook et al. (1987) recently purified a 63-kDa protein that when incorporated into liposomes exhibited a cyclic GMP-activated flux response for mono- and divalent cations. Furthermore, vesicle fusion with planar bilayers led to the transfer of channels, which were also activated by cyclic GMP with an apparent Michaelis constant of 11 μM and a Hill coefficient $n = 3.1$. The single-channel conductance, in the absence of divalent cations and in symmetric 0.15 M NaCl solutions was 26 pS. It is worth noting that 1-*cis* diltiazem did not block the reconstituted channel. Matesic and Liebman (1987) solubilized retinal rod outer segment membranes in cholate-PC solutions and fractionated the extract by molecular sieve chromatography. A fraction enriched in a 39-kDA protein, which was photoaffinity labeled with 8-azido GMP, when incorporated into lipid vesicles mediated cyclic GMP-dependent cation fluxes which were blocked by 1-*cis* diltiazem. The reconstitution of this protein in planar bilayers and the characterization of the putative cyclic GMP-dependent cation channel is eagerly awaited.

Rapid advances are occurring in the purification of other neurotransmitter receptors, and their reconstitution in bilayers can be anticipated in the not too distant future. Among them, it is worth noting the brain glutamate receptor (e.g., Tashmukhamedov et al., 1984) and the brain GABA-benzodiazepine receptor complex (e.g. Sigel & Barnard, 1984). From immune cells, the IgE-Fc epsilon receptor was purified and reconstituted in lipid bilayers in conjunction with the cromolyn-binding protein (Mazurek et al., 1984). Cation channels were activated by antigen specific for either of the two proteins. The channels showed a single-channel conductance of 1–2 pS in the presence of 1.8 mM cation. It was suggested that the cromolyn-binding protein participates in the formation of the channel (Corcia et al., 1986). Perforins, or pore forming proteins (PFP) have been purified from cytotoxic T lymphocytes and shown to induce channel-like activity in lipid bilayers (Young et al., 1984; Young, Unkeless & Cohn, 1985; Young, Podack & Cohn, 1986). The channels may be an expression of the lesions proceeding during the assembly of the protein in the target cell which, in due turn, leads to cell killing by the immune cell.

What Can Be Learned from Reconstituted Systems?

The reconstituted channel proteins provide a highly sensitive assay for the study of the mechanism of action of drugs and toxins that modify channel proteins. This was exemplified by the use of monoclonal antibodies in the case of cholinergic AChR (Blatt et al., 1986), by the extension of the studies on the mechanism of TTX action on the reconstituted sodium channel (Hartshorne et al., 1985; French et al., 1986), and by the information obtained concerning the sidedness of the dihydropyridine blocking side on calcium channels (Coronado, 1986). For charibdotoxin, the planar bilayer with the incorporated calcium-dependent potassium channel provided a sensitive assay to identify the toxin and to follow its activity during the purification procedures (Miller et al., 1985; Smith et al., 1986).

The effects of chemical modification of the channel proteins can also be detected with very high sensitivity in the reconstituted bilayer as was demonstrated by the effect of protein phosphorylation by the catalytic subunit of the cyclic AMP-dependent protein kinase on the purified cholinergic AChR (Montal & Montal, 1987) and on the purified calcium channel protein (Flockerzi et al., 1986). The results on these two purified channel proteins

clearly demonstrated that protein phosphorylation brought about modulation of channel activity without the requirement of additional protein components (*see also* Levitan, 1986).

One of the salient advantages of the reconstituted system emerges from the ready possibility of modifying the lipid composition of the membrane as well as the facility to assemble asymmetric membranes where the chemical composition in the two component monolayers can be radically different (Montal, 1973; Sherwood & Montal, 1975). The effect of lipid surface charge on membrane conductance was explored for the sarcoplasmic reticulum K^+ channel (Bell & Miller, 1984; Bell, 1986); and for the calcium-activated K^+ channel (Moczydlowski et al., 1985). The results demonstrate significant effects introduced by the presence of negatively charged lipids in the membranes. Questions related to the specific presence of charged phospholipids on one or another monolayer of the bilayer with respect to the functional binding sites of the purified channel proteins are amenable to experimental evaluation.

The role of the membrane lipid environment in modulating the final folding of the channel protein and in determining the dynamics of the channel protein function can be uniquely investigated in reconstituted systems. In this respect, expression systems, such as the valuable *Xenopus* oocytes, cannot be readily used to establish the lipid dependence of channel protein function. In contrast, reconstituted membranes are ideally suited to explore the effects of specific lipid composition, surface charge and membrane thickness and fluidity on channel gating kinetics, ionic conduction and selectivity, ligand(s) and toxin(s) binding affinities or other tools aimed at probing precise biophysical parameters of channel function.

Perspective

This is a time of excitement in channel protein research. The convergence of knowledge extracted from classical physiology and biochemistry of excitable membranes with the contemporary approaches to molecular structure and function, should yield strategic insights into the fundamental structural principles which govern channel gating. It can be anticipated that reconstituted membranes will continue to contribute key information to this fascinating and challenging field.

I acknowledge with pleasure the collaboration of Robert Anholt, Robert Hartshorne, Bernhard Keller, Pedro Labarca and Myrta Montal. I am indebted to Mrs. Rita Della Fave for her invaluable assistance.

References

Adams, D.J., Dwyer, T.M., Hille, B. 1980. *J. Gen. Physiol.* **75:**493–510

Affolter, H., Coronado, R. 1985. *Biophys. J.* **48:**341–347

Affolter, H., Coronado, R. 1986. *Biophys. J.* **49:**767–771

Agnew, W.S., Rosenberg, R.L., Tomiko, S.A. 1986. *In:* Ion Channel Reconstitution. C. Miller, editor. pp. 307–335. Plenum, New York—London

Aldrich, R.W., Corey, D.P., Stevens, C.F. 1983. *Nature (London)* **306:**436–441

Andersen, O.S., Green, W.N., Urban, B.W. 1986. *In:* Ion Channel Reconstitution. C. Miller, editor. pp. 385–404. Plenum, New York—London

Anholt, R., Fredkin, D.R., Deerinck, T., Ellisman, M., Montal, M., Lindstrom, J. 1982. *J. Biol. Chem.* **257:**7122–7134

Anholt, R., Lindstrom, J., Montal, M. 1981. *J. Biol. Chem.* **256:**4377–4387

Bean, W.P., Nowycky, M.C., Tsien, R.W. 1984. *Nature (London)* **307:**371–375

Bell, J. 1986. *In:* Ion Channel Reconstitution. C. Miller, editor. pp. 469–482. Plenum, New York—London

Bell, J.E., Miller, C. 1984. *Biophys. J.* **45:**279–287

Benz, R. 1986. *In:* Ion Channel Reconstitution. C. Miller, editor. pp. 553–573. Plenum, New York—London

Blatt, Y., Montal, M.S., Lindstrom, J., Montal, M. 1984. *Biophys. J.* **45:**311*a*

Blatt, Y., Montal, M.S., Lindstrom, J., Montal, M. 1986. *J. Neurosci.* **6:**481–486

Boheim, G., Hanke, W., Barrantes, F.J., Eibl., H., Sakmann, B., Fels, G., Maelicke, A. 1981. *Proc. Natl. Acad. Sci. USA* **78:**3586–3590

Borsotto, M., Barhanin, J., Fosset, M., Lazdunski, M. 1985. *J. Biol. Chem.* **260:**14255–14263

Borsotto, M., Barhanin, J., Norman, R.A., Lazdunski, M. 1984. *Biochem. Biophys. Res. Commun.* **122:**1357–1366

Breer, H., Kleene, R., Hinz, G. 1985. *J. Neurosci.* **5:**3386–3392

Brown, A.M., Camerer, H., Kunze, D.L., Lux, H.D. 1982. *Nature (London)* **299:**156–158

Cachelin, A.B., Peyer, J.E. de, Kokubun, S., Reuter, H. 1984. *Nature (London)* **304:**462–464

Catterall, W.A. 1986. *Annu. Rev. Biochem.* **55:**953–985

Changeux, J.-P., Heidmann, T., Popot, J.-L., Sobel, A. 1979. *FEBS Lett.* **105:**181–187

Claudio, T., Ballivet, M., Patrick, J., Heinemann, S. 1983. *Proc. Natl. Acad. Sci. USA* **80:**111–115

Cobbs, W.H., Pugh, E.N. 1985. *Nature (London)* **313:**585–587

Cohen, F.S. 1986. *In:* Ion Channel Reconstitution. C. Miller, editor. pp. 131–139. Plenum, New York—London

Colombini, M. 1986. *In:* Ion Channel Reconstitution. C. Miller, editor. pp. 533–552. Plenum, New York—London

Colquhoun, D., Sakmann, B., 1985. *J. Physiol. (London)* **369:**501–557

Cook, N.J., Zeilinger, C., Koch, K.-W., Kaupp, B. 1986. *J. Biol. Chem.* **261:**17033–17039

Corcia, A., Schweitzer-Stenner, R., Pecht, I., Rivnay, B. 1986. *EMBO J.* **5:**849–854

Coronado, R. 1986. *Annu. Rev. Biophys. Biophys. Chem.* **15:**259–277

Coronado, R., Affolter, H. 1986. *In:* Ion Channel Reconstitution. C. Miler, editor. pp. 483–505. Plenum, New York—London

Cruz, L.J., Johnson, D.S., Olivera, B.M. 1987. *Biochemistry* **26:**820–824

Curtis, B.M., Catterall, W.A. 1984. *Biochemistry* **23:**2113–2118

Curtis, B.M., Catterall, W.A. 1986. *Biochemistry* **25:**3077–3083

Dani, J.A., Eisenman, G. 1984. *Biophys. J.* **45**:10–12
Darszon, A. 1986. *Methods Enzymol.* **127**:486–502
Dascal, N., Snutch, T.P., Lubert, H., Davidson, N., Lester, H.A. 1986. *Science* **231**:1147–1150
Devilliers-Thiery, A., Giraudat, J., Bentaboulet, M., Changeux, J.-P. 1983. *Proc. Natl. Acad. Sci. USA* **80**:2067–71
Dionne, V.E., Steinbach, J.H., Stevens, C.J. 1978. *J. Physiol. (London)* **281**:421–444
Ehrlich, B.E., Finkelstein, A., Forte, M., King, C. 1984. *Science* **225**:427–428
Ehrlich, B.E., Schen, C.R., Garcia, M.L., Kaczorowski, G.J. 1986. *Proc. Natl. Acad. Sci. USA* **83**:193–197
Epstein, M., Racker, E. 1978. *J. Biol. Chem.* **253**:6660–6662
Etemadi, A.-H. 1985. *Adv. Lipid Res.* **21**:281–428
Fesenko, E.E., Kalenskov, S.S., Lyubarsky, A.L. 1985. *Nature (London)* **313**:310–313
Flockerzi, V., Oeken, H.-J., Hofmann, F., Pelzer, D., Cavalier, A., Trautwein, W. 1986. *Nature (London)* **323**:66–68
Forte, M., Guy, H.R., Mannella, C.A. 1987. *J. Bioenerg. Biomembr. (in press)*
Fosset, M., Schmid-Antomarchi, H., Hugues, M., Romey, G., Lazdunski, M. 1984. *Proc. Natl. Acad. Sci. USA* **81**:7228–7232
Fredkin, D.R., Montal, M., Rice, J.A. 1985. L.M. Le Cam and R.A. Olshen, editors. pp. 269–289. Wadsworth, Belmont, California
French, R.J., Worley, J.F., III, Blaustein, M.B., Romine, W.O., Jr., Tam, K.K., Krueger, B.K. 1986. *In:* Ion Channel Reconstitution. C. Miller, editor. pp. 363–383. Plenum, New York—London
French, R.J., Worley, J.F., Krueger, B.K. 1984. *Biophys. J.* **45**:301–310
Furman, R.E., Tanaka, J.C., Mueller, P., Barchi, R.L. 1986. *Proc. Natl. Acad. Sci. USA* **83**:488–492
Goldin, A.L., Snutch, T., Lubert, H., Dowsett, A., Marshall, J., Auld, V., Downey, W., Fritz, L.C., Lester, H.A., Dunn, R., Catterall, W., Davidson, N. 1986. *Proc. Natl. Acad. Sci. USA* **83**:7503–7507
Goldin, S.M., Moczydlowski, E.G., Papazian, D.M. 1983. *Annu. Rev. Neurosci.* **6**:419–446
Gordon, R.D., Fieles, W.E., Schotland, D.L., Hogue-Angeletti, R., Barchi, R.L. 1987. *Proc. Natl. Acad. Sci. USA* **84**:308–312
Greenblatt, R.E., Blatt, Y., Montal, M. 1985. *FEBS Lett.* **193**:125–134
Guy, H.R., Seetharalamu, P. 1986. *Proc. Natl. Acad. Sci. USA* **83**:508–512
Hanke, W. 1985. *CRC Crit. Rev. Biochem.* **19**:1–44
Hanke, W. 1986. *In:* Ion Channel Reconstitution. C. Miller, editor. pp. 131–139. Plenum, New York—London
Hanke, W., Boheim, G., Barhanin, J., Pauron, D., Lazdunski, M. 1984. *EMBO J.* **3**:509–515
Hanke, W., Breer, H. 1986. *Nature (London)* **321**:171–174
Hartshorne, R.P., Keller, B.U., Talvenheimo, J.A., Catterall, W.A., Montal, M. 1984. *Soc. Neurosci.* **10**:864
Hartshorne, R.P., Keller, B.U., Talvenheimo, J.A., Catterall, W.A., Montal, M. 1985. *Proc. Natl. Acad. Sci. USA* **82**:240–244
Hartshorne, R.P., Keller, B.U., Talvenheimo, J.A., Catterall, W.A., Montal, M. 1986*a*. *Ann. NY Acad. Sci* **479**:293–305
Hartshorne, R.P., Tamkun, M., Montal, M. 1986*b*. *In:* Ion Channel Reconstitution. C. Miller, editor. pp. 337–362. Plenum, New York—London
Haynes, L.W., Kay, A.R., Yau, K.-W. 1986. *Nature (London)* **321**:66–70
Haynes, L.W., Yau, K.-W. 1985. *Nature (London)* **317**:61–64
Hermans-Borgmeyer, I., Zopf, D., Ryseck, R.-P., Hovemann, B., Betz, H. Gundelfinger, E.D. 1986. *EMBO J.* **5**:1503–1508
Hess, P., Lansman, J.B., Tsien, R.W. 1984. *Nature (London)* **311**:538–544
Hodgkin, A.L., Huxley, A.F. 1952. *J. Physiol. (London)* **116**:449–506; **117**:500–544
Horn, R., Patlak, J. 1980. *Proc. Natl. Acad. Sci. USA* **77**:6930–6934
Hosey, M.M., Borsotto, M., Lazdunski, M. 1986. *Proc. Natl. Acad. Sci. USA* **83**:3733–3737
Huang, L.Y., Moran, N., Ehrenstein, G. 1982. *Proc. Natl. Acad. Sci. USA* **79**:2082–2085
Huang, L.Y., Moran, N., Ehrenstein, G. 1984. *Biophys. J.* **45**:313–322
Huganir, R.L., Delcour, A.H., Greengard, P., Hess, G.P. 1986. *Nature (London)* **321**:774–777
Huganir, R.L., Greengard, P. 1983. *Proc. Natl. Acad. Sci. USA* **80**:1130–1134
Huganir, R.L., Miles, K., Greengard, P. 1984. *Proc. Natl. Acad. Sci. USA* **81**:6968–6972
Huganir, R.L., Schell, M.A., Racker, E. 1979. *FEBS Lett.* **108**:155–160
Kao, P.N., Dwork, A.J., Kaldany, R.-R.J., Silver, M.L., Wideman, J., Stein, S., Karlin, A. 1984. *J. Biol. Chem.* **259**:11662–11665
Kao, P.N., Karlin, A. 1986. *J. Biol. Chem.* **261**:8085–8088
Karlin, A., Kao, P.N., DiPaola, M. 1986. *Trends Pharmacol. Sci.* **8**:304–308
Keller, B.U., Hartshorne, R.P., Talvenheimo, J.A., Catterall, W.A., Montal, M. 1985. *Biophys. J.* **47**:439*a*
Keller, B.U., Hartshorne, R.P., Talvenheimo, J.A., Catterall, W.A., Montal, M. 1986. *J. Gen. Physiol.* **88**:1–23
Khodorov, B.I. 1975. *Brain Res.* **84**:541–546
Khodorov, B.I. 1985. *Prog. Biophys. Mol. Biol.* **45**:57–148
Khodorov, B.I., Revenko, S.V. 1979. *Neuroscience* **4**:1315–1330
Kosower, E.M. 1985. *FEBS Lett.* **182**:234–242
Krueger, B.K., Worley, J.F., French, R.J. 1983. *Nature (London)* **303**:172–175
Kumar, N.M., Gilula, B.N. 1986. *J. Cell Biol.* **103**:767–776
Labarca, P., Lindstrom, J., Montal, M. 1982. *Biophys. J.* **37**:170*a*
Labarca, P., Lindstrom, J., Montal, M. 1984*a*. *J. Neurosci.* **4**:502–507
Labarca, P., Lindstrom, J., Montal, M. 1984*b*. *J. Gen. Physiol.* **83**:473–496
Labarca, P., Montal, M.S., Lindstrom, J., Montal, M. 1985*a*. *J. Neurosci.* **5**:3409–3413
Labarca, P., Rice, J.A., Fredkin, D.R., Montal, M. 1985*b*. *Biophys. J.* **47**:469–478
Latorre, R. 1986. *In:* Ion Channel Reconstitution. C. Miller, editor. pp. 431–467. Plenum, New York—London
Latorre, R., Alvarez, O., Cecchi, X., Vergara, C. 1985. *Annu. Rev. Biophys. Biophys. Chem.* **14**:79–111
Lazdunski, M. 1983. *Cell Calcium* **4**:421–428
Levinson, S.R., Duch, D.S., Urban, B.W., Recio-Pinto, E. 1986. *Ann. NY Acad. Sci.* **479**:162–178
Levitan, I.B. 1986. *In:* Ion Channel Reconstitution. C. Miller, editor. pp. 523–532. Plenum, New York—London
Lindstrom, J. 1986. *In:* Ion Channel Reconstitution. C. Miller, editor. pp. 205–229. Plenum, New York—London
Lindstrom, J., Anholt, R., Einarson, B., Engel, A., Osame, M., Montal, M. 1980. *J. Biol. Chem.* **255**:8340–8350

Lindstrom, J., Merlie, J., Yogeeswaran, G. 1979. *Biochemistry* **18**:4465–4470
Lindstrom, J., Tzartos, S., Gullick, W., Hoechschwender, S., Swanson, L., Jacob, M., Sargent, P., Montal, M. 1983. *Cold Spring Harbor Symp. Quant. Biol.* **48**:89–99
Makowski, L., Caspar, D.L.D., Phillips, W.L., Baker, T.S., Goodenough, D.A. 1984. *Biophys. J.* **45**:208–218
Matesic, D., Liebman, P.A. 1987. *Nature (London)* **326**:600–603
Mazurek, N., Schindler, H., Schurholz, T., Pecht, I. 1984. *Proc. Natl. Acad. Sci. USA* **81**:6841–6845
McKenna, E.J., Smith, J.S., Ma, J., Vilven, J., Vaghy, P., Schwartz, A., Coronado, R. 1987. *Biophys. J.* **51**:29*a*
McNamee, M.G., Jones, O.T., Fong, T.M. 1986. *In:* Ion Channel Reconstitution. C. Miller, editor. pp. 231–273. Plenum, New York—London
Messner, D.J., Catterall, W.A. 1986. *J. Biol. Chem.* **261**:211–215
Messner, D.J., Feller, D.J., Scheuer, T., Catterall, W.A. 1986. *J. Biol. Chem.* **261**:14882–14890
Miller, C. 1984. *Physiol. Rev.* **63**:1209–1242
Miller, C. 1986. Ion Channel Reconstitution. Plenum, New York—London
Miller, C., Moczydlowski, E., Latorre, R., Phillips, M. 1985. *Nature (London)* **313**:316–318
Miller, R.J. 1987. *Science* **235**:46–52
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
Mishina, M., Tobimatsu, T., Imoto, K., Tanaka, K., Fujita, Y., Fukuda, K., Kurasaki, M., Takahashi, H., Morimoto, Y., Hirose, T., Inayama, S., Takahashi, T., Kuno, M., Numa, S. 1985. *Nature (London)* **313**:364–369
Moczydlowski, E., Alvarez, O., Vergara, C., Latorre, R. 1985. *J. Membrane Biol.* **83**:273–282
Moczydlowski, E., Garber, S.S., Miller, C. 1984. *J. Gen. Physiol.* **84**:687–704
Montal, M. 1973. *Biochim. Biophys. Acta* **298**:750–754
Montal, M. 1976. *Annu. Rev. Biophys. Bioeng.* **5**:119–175
Montal, M. 1986. *In:* Techniques for Analysis of Membrane Proteins. C.I. Ragan, and R.J. Cherry, editors. pp. 97–128. Chapman and Hall, London–New York
Montal, M., Anholt, R., Labarca, P. 1986. *In:* Ion Channel Reconstitution. C. Miller, editor. pp. 157–204. Plenum, New York—London
Montal, M., Darszon, A., Schindler, H. 1981. *Q. Rev. Biophys.* **14**:1–79
Montal, M., Labarca, P., Fredkin, D.R., Suarez-Isla, B.A. 1984. *Biophys. J.* **45**:165–174
Montal, M.S., Montal, M. 1987. *Soc. Neurosci. Abstr. (in press)*
Mourre, C., Hugues, M., Lazdunski, M. 1986. *Brain Res.* **382**:239–249
Nelson, M.T. 1986. *In:* Ion Channel Reconstitution. C. Miller, editor. pp. 507–522. Plenum, New York—London
Nelson, M.T., French, R.J., Krueger, B.K. 1984. *Nature (London)* **308**:77–80
Nelson, N., Anholt, R., Lindstrom, J., Montal, M. 1980. *Proc. Natl. Acad. Sci. USA* **77**:3057–3061
Neyton, J., Trautman, A. 1985. *Nature (London)* **317**:331–335
Noda, M., Ikeda, T., Kayano, T., Suzuki, H., Takeshima, H., Kurasaki, M., Takahashi, H., Numa, S. 1986*a*. *Nature (London)* **320**:188–192
Noda, M., Ikeda, T., Suzuki, H., Takeshima, H., Takahashi, T., Kuno, M., Numa, S. 1986*b*. *Nature (London)* **322**:826–828
Noda, M., Shimizu, S., Tanabe, T., Takai, T., Kayano, T., Ikeda, T., Takahashi, H., Nakayama, H., Kanaoka, Y., Minamino, N., Kangawa, K., Matsuo, H., Raftery, M.A., Hirose, T., Inayama, S., Hayashidall, H., Miyata, T., Numa, S. 1984. *Nature (London)* **312**:121–127
Noda, M., Takahashi, H., Tanabe, T., Toyosato, M., Fusutani, Y., Hirose, T., Asai, M., Inayama, S., Miyata, T., Numa, S. 1982. *Nature (London)* **299**:793–797
Noda, M., Takahashi, H., Tanabe, T., Toyosato, M., Kikyotani, S., Hirose, T., Asai, M., Takashima, H., Inayama, S., Miyata, T., Numa, S. 1983. *Nature (London)* **301**:251–255
Nowycky, M.C., Fox, A.P., Tsien, R.W. 1985. *Nature (London)* **316**:440–443
Numa, S., Noda, M. 1986. *Ann. NY Acad. Sci.* **479**:338–355
Paul, D. 1986. *J. Cell Biol.* **103**:123–134
Qar, J., Schweitz, H., Schmid, A., Lazdunski, M. 1986. *FEBS Lett.* **202**:331–336
Raftery, M.A., Hunkapiller, M.W., Strader, C.D., Hood, L.E. 1980. *Science* **208**:1454–1457
Ragan, C.I., Cherry, R.J. 1986. Techniques for Analysis of Membrane Proteins. Chapman and Hall, London–New York
Revel, J.P., Nicholson, B.J., Yancey, S.B. 1985. *Annu. Rev. Physiol.* **47**:263–279
Reuter, H., Stevens, C.F., Tsien, R.W., Yellen, G. 1982. *Nature (London)* **297**:501–504
Reynolds, J.A., Karlin, A. 1978. *Biochemistry* **17**:2035–2038
Roberts, R.H., Barchi, R.L. 1987. *J. Biol. Chem.* **262**:2298–2303
Rosenberg, R.L., Hess, P., Reeves, J.P., Smilowitz, H., Tsien, R.W. 1986. *Science* **231**:1564–1566
Rosenberg, R.L., Tomiko, S.A., Agnew, W.S. 1984. *Proc. Natl. Acad. Sci. USA* **81**:5594–5598
Safran, A., Neumann, D., Fuchs, S. 1986. *EMBO J.* **5**:3175–3178
Sakmann, B., Methfessel, C., Mishina, M., Takahashi, T., Takai, T., Kurasaki, M., Fukuda, K., Numa, S. 1985. *Nature (London)* **318**:538–543
Schindler, H., Quast, U. 1980. *Proc. Natl. Acad. Sci. USA* **77**:3052–3056
Schindler, H., Spillecke, F., Neumann, E. 1984. *Proc. Natl. Acad. Sci. USA* **81**:6222–6226
Schmid, A., Barhanin, J., Coppola, T., Borsotto, M., Lazdunski, M. 1986. *Biochemistry* **25**:3492
Seagar, M.J., Marqueze, B., Couraud, F. 1987. *J. Neurosci.* **7**:565–570
Sherwood, D., Montal, M. 1975. *Biophys. J.* **15**:417–434
Sigel, E., Barnard, E.A. 1984. *J. Biol. Chem.* **259**:7219–7223
Sigworth, F.J., Neher, E. 1980. *Nature (London)* **287**:447–449
Smith, C., Phillips, M., Miller, C. 1986. *J. Biol. Chem.* **261**:14607–14613
Spray, D.C., Bennett, M.V.L. 1985. *Annu. Rev. Physiol.* **47**:281–303
Spray, D.C., Saez, J.C., Brosins, D., Bennett, M.V.L., Hertzberg, E.L. 1986. *Proc. Natl. Acad. Sci. USA* **83**:5494–5497
Stern, J.H., Kaupp, B.U., MacLeish, P.R. 1986. *Proc. Natl. Acad. Sci. USA* **83**:1163–1167
Stryer, L. 1986. *Annu. Rev. Neurosci.* **9**:87–119
Suarez-Isla, B.A., Wan, K., Lindstrom, J., Montal, M. 1983. *Biochemistry* **22**:2319–2323
Tanaka, J.C., Furman, R.E., Barchi, R.L. 1986. *In:* Ion Channel Reconstitution. C. Miller, editor. pp. 277–305. Plenum, New York—London
Tanaka, J.C., Furman, R.E., Cobbs, W.H., Mueller, P. 1987. *Proc. Natl. Acad. Sci. USA* **84**:724–728

Tank, D.W., Huganir, R.L., Greengard, P., Webb, W.W. 1983. *Proc. Natl. Acad. Sci. USA* **80:**5129–5133

Tashmukhadmedov, B.A., Makhumudova, E.M., Usmanov, P.B., Kazakov, I., Atakuziev, B.U. 1984. *Dokl. Akad. Nauk SSSR* **276:**977–979

Unwin, P.N., Zampighi, G. 1980. *Nature (London)* **283:**545–549

Vandenberg, C.A., Horn, R. 1984. *J. Gen. Physiol.* **84:**535–564

Werner, R., Miller, T., Azarnia, R., Dahl, G. 1985. *J. Membrane Biol.* **87:**253–268

Yau, K.-W., Nakatani, K. 1985. *Nature (London)* **317:**252–255

Young, G.P., Young, J.D., Deshpande, A.K., Goldstein, M., Koide, S.S., Cohn, Z.A. 1984. *Proc. Natl. Acad. Sci. USA* **81:**5155–5159

Young, J.D., Podack, E.R., Cohn, Z.A. 1986. *J. Exp. Med.* **164:**144–155

Young, J.D., Unkeless, J.C., Cohn, Z.A. 1985. *J. Cell Biochem.* **29:**289–297

Zampighi, G.A., Hall, J.E., Kreman, M. 1985. *Proc. Natl. Acad. Sci. USA* **82:**8468–8472

Zimmerman, A.L., Baylor, D.A. 1986. *Nature (London)* **321:** 70–72

Received 24 March 1987