Biophysical Studies of Ion Channels

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Summary. Ionic channels, the integral membrane proteins responsible for the brain's electrical activity, have long been studied with standard electrophysiological and biochemical methods. Recently, however, newly developed electrical and molecular biological methods have been brought to bear on long-standing questions in neurobiology. Goals of current channel research include elucidating the primary amino acid sequence and three-dimensional structure of channel species; the mechanisms of synthesis, sorting, membrane insertion, and degradation; and aspects of function such as gating, ion permeation and selectivity, and regulation. The latest research combines the new biochemical and electrophysiological techniques to reveal relations between molecular structure and function.

The electrical activity of the nervous system is an expression of ionic fluxes across neuronal membranes. A large class of integral membrane proteins, known as channels, has evolved to mediate these ionic fluxes. The properties of channels, then, determine the nature of the brain's electrical activity and play a central role in neuronal function. This article summarizes the properties of channels and describes recent research on this class of proteins.

Nature of Channels

Channels regulate the passive transmembrane flow of ions through water-filled pores. Tens or hundreds of different channel varieties are present in the brain, although an individual neuron expresses only about a dozen types. All channels have selectivity and gating properties, and some are sensitive to various natural toxins and pharmacological agents. Selectivity refers to the fact that a particular channel species selects the types of ions that will pass through its pore. For example, potassium may be excluded and sodium might be permitted to traverse the channel and enter the cell. Gating refers to the fact that channels have a door that opens and closes to permit or prevent the transmembrane flux of favored ions.

What causes a channel to open and close its gate? Two general classes of mechanisms are recognized, voltage gating and ligand gating. In voltage gating, the physical quantity that determines whether a gate is open or closed—or, more precisely, the probability of a gate being open or closed—is the difference in membrane potential between the inside and outside of the cell. For example, depolarization generally causes closed gates to open. In ligand gating, a channel is caused to open by binding a particular ligand, usually a neurotransmitter released by another neuron.

Although ligand and voltage gating are not logically mutually exclusive, in practice most channels use only one of these gating modes. Thus, all the channels may be divided into two almost nonoverlapping subclasses: voltage-gated channels and ligand-gated channels.

Each of these subclasses may be further subdivided according to specific gating properties. For example, voltage-gated channels differ according to their sensitivity to membrane voltage, the length of time a channel remains in the open state, and the rapidity with which the open state is entered after an appropriate change in voltage. Ligand-gated channels are like enzymes in that they exhibit great specificity in the ligands that they bind. Binding drives the channel into its open state. One channel may be opened by acetylcholine (ACh) and ignore dopamine, whereas another may be opened by dopamine and be totally insensitive to ACh. As a rule, a particular channel type recognizes just one natural ligand.

Channels also differ in their selectivity. Some, for example, permit chloride to pass through their pores and exclude all cations; others exclude all anions and accept only sodium. The selectivity of a channel is usually denoted by the name of the ion that passes through most readily; the five main types of selectivity are sodium, potassium, calcium, chloride, and all small cations.

Channels are named in two different ways. Voltage-gated channels are identified by the ion that best passes through them (one speaks of a sodium or a potassium channel, for example), whereas ligand-gated channels are designated by the ligand type most effective in causing the channel to open (ACh channel, for example). Note that sodium does not cause a sodium channel to open but rather is the main permeant ion; ACh does not pass through the ACh channel's pore but rather causes it to open. In an attempt to minimize this ambiguity, many investigators prefer, for instance, "ACh receptor (AChR) channels" to "ACh channels."

A number of natural toxins and pharmacological agents bind specifically to one or another channel type and serve as a basis for making distinctions between the various channel proteins. For example, puffer fish tetrodotoxin binds to certain sodium channels with nanomolar dissociation constants and blocks ion transport through the channel's pore. A snake toxin, α-bungarotoxin, binds even more tightly to a class of AChR channels and also blocks function. Both tetrodotoxin and α-bungarotoxin are sufficiently specific and bind tightly enough to be used for identification of the two corresponding channel types.

Methods for Studying Channels

Because channels are proteins, all techniques by which proteins are studied can be used for channels. In addition, the function of channels can be investigated electrophysiologically by measuring the ionic current they regulate. Although protein biochemical and electrophysiological methods are commonly used, two new methods have been developed that are unprecedented in their contributions to our understanding of channel structure and function: genetic engineering and single-channel recording.

The techniques of molecular biology are permitting channel proteins to be sequenced, the structure of their genes to be analyzed, and the channels themselves to be expressed in cells where they normally would not be present. Some of the results of these approaches

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are described later in this article and in other articles in this issue (*1*).

When a channel opens, a small current—on the order of 1 pA—flows. Largely because of the work of Erwin Neher, a method has been developed to record the currents regulated by a single channel (*2–4*). The primary problem is one of detecting single-channel currents in the presence of background electrical noise. The problem is solved by pressing a glass microelectrode, with an opening of about 1 μm^2, onto the cell's surface and forming a very tight seal between the phospholipids of the membrane and the electrode glass. This tight seal, together with the small membrane area involved, results in very low extraneous noise levels and permits the measurement of picoampere currents that flow for milliseconds.

What determines whether a single channel's currents can be detected? If a channel remains open for a relatively long period (>100 msec), one may filter the recorded currents to remove high-frequency components of the noise without removing the single-channel current and consequently detect very small single-channel currents. Conversely, if a channel remains open for only a brief time (<1 msec), then high frequencies in the record must be retained in order not to lose the channel currents themselves, and the high-frequency components in the background noise tend to obscure the currents being studied. As a result of these factors, a rule of thumb for the limits of resolution for this method is $i\sqrt{t} > 0.5$, where i is the single-channel current in picoamperes and t is the mean open time in milliseconds (*3*). For example, if the mean open time is 1 msec, currents of 0.5 pA or larger can be studied.

Areas of Research

Like all research on proteins, channel research focuses on structure, metabolism, and function. The primary amino acid sequence, subunit organization, and three-dimensional structure must be discovered for each channel species. Channel biosynthesis and degradation are, of course, regulated to suit the needs of the cell, and channels, like other membrane proteins, must be processed (sugar chains added, for example), sorted, and inserted into appropriate areas of the neuronal surface membrane. All these topics are areas of active research. The mechanisms of channel function must be discovered for each channel type: gating and selectivity must be understood, and the role a particular channel plays in the overall function of the cell must be elucidated. Functional characteristics (gating, for example) of many types of channels are modulated by second messenger systems, and it is important to understand this process.

Examples of research in these three general areas are presented below. Because we know more about the AChR channel (the ligand-gated channel involved in neuromuscular transmission) and the sodium channel (the voltage-gated channel responsible for nerve impulses), most of the examples will deal with these proteins. Structure and metabolism will be treated more briefly than questions about function.

AChR structure. The AChR is a pentameric integral membrane protein composed of four distinct subunits with the stoichiometry α_2, β, γ, δ and a molecular weight of about 250,000 (*5*). All the subunits span the membrane, and the major portion of the protein mass is extracellular. Electron micrographs of negatively stained preparations show the subunits arranged like the staves of a barrel around a central pit that is presumed to be the vestibule of the transmembrane pore.

The complementary DNA coding for all four subunits has been cloned and sequenced and reveals that each subunit shares the same general structure (*6–11*). Starting from the amino terminus, one finds a long stretch of hydrophilic peptides, then four hydrophobic regions long enough to span the membrane, and finally a series of hydrophilic amino acids near the carboxyl terminus. Between the third and fourth hydrophobic (membrane-spanning) portions is another region that, when coiled as an alpha helix, would present a charged face with a hydrophobic body and be long enough to traverse the membrane. Five such helices, one contributed by each subunit, are thought to form the charge-lined pore through which ions pass (*12, 13*).

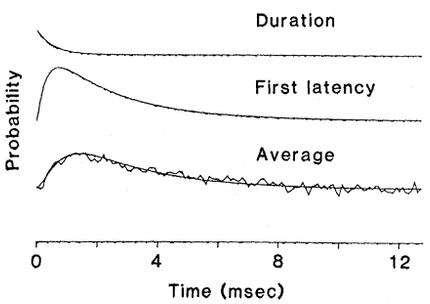

Fig. 1. Occupancy of the open state in sodium channels. The upper curve is an exponential with its decay constant set by the mean open time of channels determined by single-channel records. The middle curve presents a theoretical function that was fitted to the measured probability density for first latencies in the same record. Although not described here, the theory for first latencies was developed by methods similar to those described. The bottom curve presents theoretical and empirical occupancy probabilities as a function of time after a depolarization. The theoretical (smooth) curve was calculated, according to the equation in the text, by convolving the top two functions.

Although each channel species will have unique structural features, several features of the AChR channel may be shared by other species. For example, one expects all channels (i) to be composed of multiple subunits or multiple domains of a single peptide to form a stable structure by spanning the membrane a number of times and (ii) to provide a charged, water-filled pore through which the ions may pass. The various AChR subunits have similar general structures and show regions with strong homologies (*8*); these features also may be shared by other types of channels. Generalizations from a single example are, of course, suspect, and only after the structure of other channels has been ascertained will the common patterns be determinate. Whatever the generality of the conclusions about channel structure based on the AChR, this protein has been important for indicating which techniques are useful for approaching structural questions.

Channel metabolism. Each of the four types of AChR subunits is coded for by a separate gene, and each has a hydrophobic leader ("signal") sequence at the amino terminus—characteristic of membrane proteins (*6, 7, 14*). The subunits are synthesized in rough endoplasmic reticulum, processed and assembled into the mature pentameric structure, and transported to the surface membrane (*14–17*). The general features of this biosynthetic scheme are typical of membrane proteins and so can be expected to hold true for all types of channels. Because different kinds of channels will have different subunit structures, their biosynthesis will necessarily require somewhat modified assembly processes. The most important—and least understood—mechanism, however, involves sorting and insertion. Each channel species has a special distribution over the cell surface, so that the neuron's biosynthetic apparatus must have some method for labeling the various types of channels. Solving the problem of how channels in particular and membrane proteins in general are directed to their proper locations is an important goal of neurobiology.

Channel numbers are regulated. This

regulation occurs both in the rate of synthesis and in degradation. AChR's are degraded in lysosomes after internalization by an endocytotic process. The mechanisms of endocytosis, as well as the controls on rates of destruction and synthesis, are key unsolved issues in channel metabolism. Again, the overall processes will probably be the same for all channels, but details will differ because each channel species is treated separately.

Function: selectivity and permeation. Most channel species are highly selective, preferring one ion type for permeation over alternatives by an order of magnitude. The AChR channel is highly selective for cations over anions, but discriminates among various cations only weakly as long as their radius is less than about 0.6 nm (*18–21*). Although much is known about the selectivity of many types of channels, the AChR channel will be discussed here because its weak selectivity simplifies the theoretical treatment described later.

Most small, positively charged species, including organic ions like ammonium and its substituted derivatives, can traverse the AChR pore, but we will limit our discussion here to the alkali metals (lithium through cesium) and the alkaline earths (magnesium through barium). These two groups of ions are especially simple because their noble gas outer electron structure makes them chemically inert and limits interactions with surroundings to electrostatic ones.

Of the monovalent alkali metals, cesium passes through the AChR channel most easily, lithium is least permeant, and the others fall between with permeabilities that decrease in order of decreasing ionic crystal radius. The selectivity sequence for the monovalent ions, then, is "larger go better." For the divalent alkaline earth ions, the sequence is reversed ("smaller go better") with magnesium most permeant and barium least. Because the size range for the two groups of ions overlap, these observations seem paradoxical as long as the selectivity mechanism is viewed as simple sieving. However, selectivity can occur on the basis of electrostatic interactions rather than stearic factors (*22*). This point is made especially clear by the AChR channel data.

For an ion to pass through the pore in a channel protein, it must surmount an energy barrier. Ions that pass through readily have a relatively low energy barrier and species that are excluded have a high barrier. Of course, stearic factors contribute to the barrier height—an ion that is larger than the pore will, because of van der Waals forces, have a very high barrier—but permeant ion barriers are determined mostly by electrostatic interactions of the ion with the water in the pore and with the structure of the pore's walls. Data from electrophysiological experiments can give estimates of the barrier heights for the various ions (*23*), so the problem is to develop a physical theory that can account for barrier heights.

The AChR channel has a pore that is only weakly selective for small cations. What does this weak selectivity imply about barrier heights? If barrier height B is expressed as a power series in reciprocal ion radius X—reciprocal radius is a natural variable to use because length enters in the denominator of Coulomb's law and other electrostatic relations—then "weak selectivity" is equivalent to saying that B is slowly varying with X so that only early terms in the series expansion need be retained. The AChR channel is sufficiently nonselective, in fact, that no terms past the linear one appear to be necessary (*23*). Thus

$$B(z,X) = B_0(z) + A(z)X$$

where $B(z,X)$ is barrier height for an ion with valence z and reciprocal crystal radius X and B_0 and A are coefficients in the series expansion. ($A(z)$ specifies the direction of the selectivity in that it is positive for the monovalent ($z = 1$) "bigger goes better" sequence and negative for the divalent ($z = 2$) "smaller goes better" sequence.

Why does A reverse sign when z increases from 1 to 2? Analysis of the ion's electrostatic interactions with its environment in the selectivity region of the pore (*23*) reveals that A has two components, $A(z) = zM + z^2N$, where M is related to the average fixed dipole moments of the ion's surroundings and N to the average induced dipole moment. Both M and N specify differences between the selectivity region electrical environment and free water. When z increases from 1 to 2, then, the first term doubles while the second term quadruples. For example, if $M = 3$ and $N = -2$, then $A(1) = 1$ and $A(2) = -2$, and the slope of the relation would reverse.

By having a very small fixed dipole component to the selectivity region environment and by decreasing slightly the ability of water molecules to be oriented in this same region, the observed slope reversal can be accounted for (*23*). Estimates of the actual values involved indicate that AChR channel selectivity can easily arise from an environment in the pore whose electrical characteristics differ from free water by less than 0.1 percent. Because protein-ion interactions could not be sufficiently water-like, these findings suggest that ions in the selectivity regions of the AChR pore are completely surrounded by water.

Although the selectivity of the AChR channel is weak, these results indicate some of the likely general features of ion permeation: the pore environment is basically an aqueous one in which ion-water and water-protein interactions are crucial for determining the selectivity barrier heights. Further, to achieve selection between chemically similar ions whose radii differ by only a few hundredths of a nanometer, channels do not primarily use mechanical sieving mechanisms but rather create appropriate energy barrier heights by relatively small differences in the electrostatic interactions of ions with their environment.

Function: gating. Although the simplest possible channel protein must have access to at least two distinct states (open and closed) to achieve gating, almost all real channels are much more complicated in that they also exhibit other states (*23*). These other states represent distinct major conformations of the channel protein, so that gating involves driving a channel from resting conformations to open ones. Gating has been studied in different channel types for many years with a variety of methods; the discussion here, however, will focus on the new recording technique of studying gating in sodium channels.

Understanding the gating function of the sodium channel (or of another type of channel) involves four steps (*24*). First, one must find some way of defining the various states that the channel can enter. For example, three states, or groups of states, of the sodium channel are closed, open, and inactivated. Open states are detected by the current flow associated with them. Sodium channels are normally driven open by a depolarization, but continued depolarization causes them to enter an inactivated state that is absorbing; that is, once this state is entered, the channel must remain in it until the membrane voltage is returned for a period to a more negative value. Channels that are not open but able to open (that is, not inactivated) are designated closed.

In the second step toward understanding gating, one must discover a way of estimating the occupancy of each state as a function of time and voltage. Occupancy of open states is directly measured and that of inactivated states can be estimated by inducing depolarization at various times and observing the number

of channels that open; decreases in this number reflect inactivation. Traditionally, occupancy has mostly been estimated by fitting kinetic schemes to the occupancy of the open state in an effort to calculate the occupancies of unseen states. More recently, techniques have started to be developed for estimating occupancy from a statistical analysis of data from single-channel recording experiments.

Third, rates at which transitions occur between states must be determined. If the occupancy of all states is known as a function of time, the transition rates can be determined directly from these data. (Conversely, knowledge of all transition rates specifies the evolution of the occupancies of all states, given an initial occupancy pattern.) Frequently, however, full occupancy information is not available, so other methods for finding transition rates must be used (one such method is described below). Note that the transition rates and the occupancies as a function of time vary with membrane potential and so must be determined over an appropriate operating voltage range for the neuron.

Finally, once states are defined, their occupancy estimated, and the rates of transition between them found, then all of these data must be combined in a theory that accounts for the channel gating behavior. The general formulation of such a theory is implicit in the concept of states and transitions between them, but the full theory must also account for the voltage dependence of all the transition rates (*24*).

A relatively simple example of this mode of analysis follows. In this example there are three sodium channel states: open, closed, and inactivated. Occupancy of the open state is measured directly (Fig. 1). Single sodium channels were studied in neuroblastoma N1E115 cells, and the membrane patch containing one to four sodium channels was presented with a 15-msec depolarization epoch once per second (*25*). Although the analysis is somewhat complicated by the presence of more than one channel in the patch, the description here circumvents some of these complications by considering only patches with just one active channel. For each depolarization epoch, the following were measured: first latency (time from onset of depolarization to first opening of the channel), number of openings per epoch, and time in the open state.

A channel remains in the open state for random lengths of time that are exponentially distributed (*25, 26*). This suggests that the open state is a unique one. An open channel can return to one of the closed states (from which reopening is possible) or can enter the absorbing inactivated state; the following argument shows that open channels typically pass directly into the inactivated state instead of returning to a closed state.

Suppose that an open channel enters the inactivated state with rate a and the closed states with rate b. Then the mean dwell time m in the open state is $m = 1/(a + b)$. From measurements of m the sum of the two transition rates $(a + b)$ is about two per millisecond (*25*). We must now find a second relation to determine the individual rates.

Consider an open channel with transition rates a and b as above. When a channel finally does make a transition, the probability that it will enter the inactivated state is $a/(a + b)$. Note that this quantity does not depend on time (the units of a and b are transitions per millisecond, and they cancel in the fraction) and approaches 1 as a becomes large compared to b, and 0 as a becomes small compared to b. In other words, if an open channel has a large rate of transition into the inactivated state, it virtually never will achieve the closed state and so will rarely if ever reopen.

In sodium channel recordings a single channel frequently opens once in a depolarization epoch, but it hardly ever reopens (*25*). How can this happen? If an open channel sometimes returned to the closed state, it would sometimes have to open a second time and, in fact, should have as good a chance of reopening as of opening the first time. The only way for a channel never to reopen is to always enter the inactivated state, where it is trapped and cannot escape for the duration of the depolarization epoch. If open channels always enter the inactivated state, a must be large compared to b, so a must be close to $1/m$, or two per millisecond, and b must be much smaller.

In summary, then, the time in the open state is exponentially distributed; the rate of leaving this state and going into the inactivated state is two per millisecond, and the rate of going into the closed state is negligible.

Although the preceding conveys the principle of the analytical technique, the actual method (*25*) is more complicated. Nevertheless, the main conclusions are essentially correct.

This approach can now be extended to provide a partial theory for the occupancy of the open state and to indicate the first steps in developing a more complete theory. We seek now an expression for $P(t)$, the probability that a channel occupies the open state at time t after the onset of the depolarization epoch. A channel can only be open at t by first having opened at a previous time and then not closing (because a channel never reopens after closing). The probability density for opening the first time is $w(\tau)$, and this can be obtained directly by recording channel behavior or through a theory (not described here); the probability of remaining open is exponentially distributed with the rate constant a. Thus an enumeration of the mutually exclusive ways a channel can be found open at t gives

$$P(t) = \int_0^t w(\tau)\, e^{-a(t-\tau)} d\tau$$

Figure 1 shows the results of a test of this equation in which the predicted occupancy probability after a depolarization is superimposed on the observed one obtained directly form channel openings during a number of depolarization epochs.

We have thus measured some of the transition rates and occupancy probabilities and have formulated an expression that clearly defines what must next be explained, namely, the first latency density, $w(\tau)$. A version of these same methods is being used to analyze $w(\tau)$ and therefore to extend the theory so that it explicitly includes other states.

Function: neuromodulation. Part of the adaptability of the nervous system resides in its capacity to modify, through its own neural activity, the computational capacity of neurons by changing the characteristics of channels. This is generally accomplished through second messenger systems (*27*). For example, a neurotransmitter activates an adenylate cyclase, which in turn activates a protein kinase that phosphorylates a substrate. In some instances the substrate is a channel (or some molecule that regulates a channel), and the result of phosphorylation is appropriately modified channel function. A few instances of this phenomenon have been investigated at the molecular level with single-channel recording; in the case of a calcium channel in the heart, the probability of the open state is increased so that the influx of calcium is larger (*28–30*). In another example, a potassium channel is turned off so that the electrical properties of an axon terminal favor enhanced synaptic transmission (*31*). Neuromodulatory phenomena promise to be especially important areas of channel research in coming years.

Future Directions

A combination of molecular biological and electrophysiological methodology offers new opportunities for investigating the molecular basis of the brain's electrical activity. Electrophysiological analysis can provide detailed functional descriptions (like that in Fig. 1), but the exact nature of the underlying molecular mechanisms cannot be revealed. Structural techniques, including molecular biological methods, can elucidate the structure of channels, but are not appropriate for investigating the extremely rapid events characteristic of channel function. A combined approach should, however, offer the key for linking structure and function. The basic idea behind the combined approach is to make specific changes in structure and then evaluate the functional sequelae. This approach is now possible through the use of site-directed mutagenesis to substitute specific amino acids, expression systems to manufacture the modified proteins, and single-channel recording and other modern electrophysiological methods for analyzing altered function (*32*).

References and Notes

1. M. G. Rosenfeld, S. G. Amara, R. M. Evans, *Science* **225**, 1315 (1984); J. F. Gusella *et al.*, *ibid.*, p. 1320; R. H. Scheller *et al.*, *ibid.*, p. 1300; J. G. Sutcliffe *et al.*, p. 1308.
2. B. Sakmann and E. Neher, Eds. *Single-Channel Recording* (Plenum, New York, 1983).
3. C. F. Stevens, *Trends Pharmacol. Sci.* **56**, 131 (1984).
4. D. P. Corey, *Neurosci. Comment.* **1**, 99 (1983).
5. J.-P. Changeux, A. Devillers-Thiery, P. Chemouilli, *Science* **225**, 1335 (1984).
6. M. Noda *et al.*, *Nature (London)* **299**, 793 (1982).
7. M. Noda *et al.*, *ibid.* **301**, 251 (1983).
8. M. Noda *et al.*, *ibid.* **302**, 528 (1983).
9. T. Claudio, M. Ballivet, J. Patrick, S. Heinemann, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 1111 (1983).
10. K. Sumikawa *et al.*, *Nucleic Acids Res.* **10**, 5809 (1982).
11. A. Devillers-Thiery, J. Giraudat, M. Bentaboulet, J.-P. Changeux, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 2067 (1983).
12. H. R. Guy, *Biophys. J.* **45**, 249 (1984).
13. J. Finer-Moore and R. M. Stroud, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 155 (1984).
14. D. J. Anderson, P. Walter, G. Blobel, *J. Cell Biol.* **93**, 501 (1982).
15. D. Anderson and G. Blobel, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 5598 (1981).
16. J. P. Merlie, R. Sebbane, S. Tzartos, J. Lindstrom, *J. Biol. Chem.* **257**, 2694 (1982).
17. J. P. Merlie and R. Sebbane, *ibid.* **256**, 3605 (1981).
18. L.-Y. Huang, W. A. Catterall, G. Ehrenstein, *J. Gen. Physiol.* **71**, 397 (1978).
19. P. W. Gage and D. Van Helden, *J. Physiol. (London)* **288**, 509 (1979).
20. D. J. Adams, T. M. Dwyer, B. Hille, *J. Gen. Physiol.* **75**, 493 (1980).
21. C. A. Lewis and C. F. Stevens, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 6110 (1983).
22. G. Eisenman, *Biophys. J.* **2**, 259 (1963); S. Krasne and G. Eisenman, in *Membranes: A Series of Advances*, G. Eisenman, Ed. (Dekker, New York, 1973), vol. 2, p. 179.
23. A. L. Hodgkin and A. F. Huxley, *J. Physiol. (London)* **117**, 500 (1952).
24. C. F. Stevens, *Biophys. J.* **22**, 295 (1978).
25. R. W. Aldrich, D. P. Corey, C. F. Stevens, *Nature (London)* **306**, 436 (1983).
26. R. W. Aldrich and C. F. Stevens, *Cold Spring Harbor Symp. Quant. Biol.* **158**, 147 (1983).
27. J. Nestler, I. Walaas, P. Greengard, *Science* **225**, 1357 (1984).
28. H. Reuter, C. F. Stevens, R. W. Tsien, G. Yellen, *Nature (London)* **297**, 501 (1982).
29. H. Reuter, *ibid.* **301**, 569 (1983).
30. A. B. Cachelin, J. E. de Peyer, S. Kokubun, H. Reuter, *ibid.* **304**, 462 (1983).
31. S. A. Siegelbaum, J. S. Camardo, E. R. Kandel. *ibid.* **299**, 413 (1982).
32. C. F. Stevens, *Trends Neurosci.*, in press.

Message Transmission: Receptor Controlled Adenylate Cyclase System

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The adenylate cyclase system is located in the cell membrane and is apparently present in every cell type of higher organisms; it is controlled by specific receptors for neurotransmitters and hormones which turn the enzyme on, while other receptors cause its inhibition. Cyclic adenosine monophosphate (cyclic AMP), which the enzyme produces from adenosine triphosphate (ATP), has been implicated in many processes, from activation of glycogenolysis and lipolysis (*1*) to actions more specific for the nervous system, such as the function of opiates (*2, 3*), the control of electrical activity (*3, 4*) and the initiation of simple behavior patterns (*5*). Thus, it is accepted by now that the receptor activated adenylate cyclase serves as a major transmembrane signaling system: A neurotransmitter released presynaptically binds to the postsynaptic receptor facing outward in the cell membrane, and the receptor communicates this information inward, through the membrane, by activating the adenylate cyclase (*6, 7*). The cyclic AMP formed in turn activates the specific protein kinase originally discovered by Krebs and his collaborators (*8*). The kinase then phosphorylates certain specific proteins that cause the final biological response. This protein kinase is the only molecule known at present to interact specifically with cyclic AMP in eukaryotes.

The early studies of Sutherland and co-workers already revealed that adenylate cyclase activity is extremely high in brain (*1*). Later, it was shown by Bloom *et al.* (*4*) that noradrenaline acting on beta-adrenergic receptors stimulates cyclic AMP synthesis in the Purkinje cells of the cerebellum and that the cyclic AMP formed inhibits specific electrical activities of these cells.

The functions of two other neurotransmitters, adenosine and dopamine, have been studied in the nervous system in connection with the adenylate cyclase. Adenosine raised special interest when it was shown to cause a dramatic increase in cyclic AMP in brain slices (*1*). Various electrophysiological effects of adenosine have been studied (*3*), and the location and characteristics of the receptors in brain have been elucidated by Snyder and his collaborators (*9*). The role of cyclic AMP in an adenosine effect must, however, be ascertained in each case since it has been shown that there are two classes of adenosine receptors at the cell membrane, facing out: Ra, stimulating adenylate cyclase, and Ri, inhibiting the enzyme (*10*). Because of the great variety of biological responses elicited by adenosine and because of the medical implications, a considerable number of adenosine analogs with receptor selectivity are being studied (*11*).

When the dopamine-stimulated adenylate cyclase was discovered (*12, 13*), it was hoped that it might explain all the major actions of dopamine. However, like epinephrine, adenosine, and several other neurotransmitters, the action of dopamine is not limited to one type of receptor. Kebabian and Calne proposed a D_1 receptor which activates the adenyl-

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