PARAMAGNETIC HYDROPHOBIC IONS AS PROBES FOR ELECTRICALLY ACTIVE CONFORMATIONAL TRANSITIONS IN ION CHANNELS

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One important and fascinating aspect of many gated ion channels is their capacity to be modulated by membrane electric fields. These voltage-sensitive processes are necessarily result of conformational transitions termed "electrically active," referring not to electrical changes accompanying the bulk movement of ions across a membrane but rather to electrial changes that are the result of minute changes in the net charge, dipole moment, or orientation of charges in the channel-protein structure. Electrophysiologically, such events are believed to result in "gating" currents. Where channels are not voltage-dependent, the existence of electrically active events associated with gating is not precluded.

Examples of several plausible electrically active conformational events are shown in Fig. 1. Notice that in many cases the electrical changes are unique to a particular conformational transition. For example, changes in dipole orientation within a membrane would not be expected to change surface potential, whereas charge movements across the membrane-solution interface will alter the membrane surface potential. Therefore, methods that permit an estimation of the electrical changes resulting from gating events should help determine the nature of these events.

A novel set of techniques utilizing paramagnetic derivatives of hydrophobic ions and other amphiphiles has been particularly useful in characterizing membrane electrical properties (1). Hydrophobic ions, in addition to being membrane permeable, are believed to bind to membranes at two potential-energy minima, termed "boundary" regions, located below the membrane-solution interface. The phase partitioning of these probes, as determined by electron paramagnetic resonance spectroscopy (EPR), is dependent both on the transmembrane potential, $\Delta\psi$, and on potentials (termed boundary potentials) at the two free energy minima where the probe binds. By using a number of different paramagnetic amphiphiles sensitive to membrane surface potentials, an estimation of several distinct membrane potentials can be made. To characterize gating events in the nicotinic acetylcholine receptor (AcChR), we have studied the behavior of several nitroxide-labeled phosphonium ions in receptor-rich vesicles isolated from the electric organ of *Torpedo californica*.

RESULTS AND DISCUSSIONS

Phosphonium labels, such as the amid-linked nitroxide shown in Fig. 2 *A*, in AcChR-rich vesicles yield composite EPR spectra, arising from both "aqueous" and "membrane-associated" probes. As shown previously for a number of model and native membrane systems (1), this phase partitioning also varies with ionophore-induced transmembrane potentials in AcChR vesicles. A significant fraction of the membrane-associated phosphonium arises from a population undergoing motions relatively slow on the EPR time scale, an observation generally made for membrane-associated nitroxides in this system. In previous studies using radioactive tracers (2, 3) and ion-selective electrodes (4), agonists have been shown to induce the partition of the phosphonium ion into AcChR vesicles. Spin-labeled phosphonium ions as shown in Fig. 2 *A* also change their phase partitioning upon the addition of agonists on a relatively slow time scale (seconds). This partitioning change is unaffected by the addition of agents that rapidly dissipate $\Delta\psi$ in this system (for example the ionophores nonactin or gramacidin). The addition of α-bungarotoxin also induces an identical uptake of the phosphonium nitroxide and quantitatively blocks the agonist-induced partitioning change. The changes measured are not affected by a decrease in the spin population resulting from spin reduction or the activity of the acetylcholine esterase.

There are several possible causes of the rather dramatic partitioning change seen in Fig. 2 *A*. Electrical changes in either the surface potential or potential changes in a

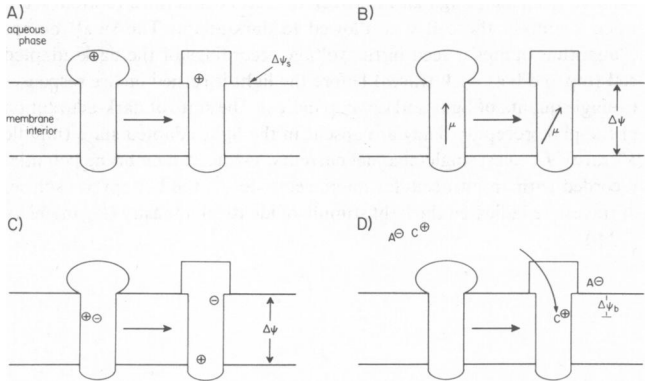

FIGURE 1 Examples of electrically active conformational transitions in membrane proteins: *A*, displacement of a bound protein charge through the surface double-layer; *B*, reorientation of a molecular dipole; *C*, intramembrane change displacement; *D*, interfacial charge transfer. These transitions generate small currents and potential changes, and are associated with free energy differences that vary with local membrane potentials.

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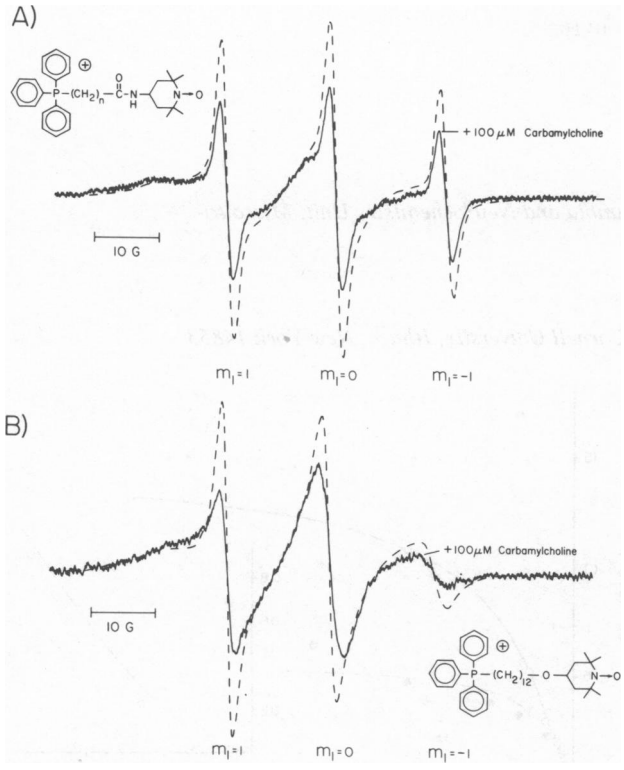

FIGURE 2 EPR spectra of spin-labeled phosphonium ions in receptor-rich vesicles isolated from fresh electric organ tissue of *T. californica*. Vesicles were isolated and characterized as previously described (6), and contain ~3 nm of toxin-binding sites per mg of protein. The vesicles are suspended in a Ringer's solution at a concentration of ~20 μm in a α-bungarotoxin binding sites. *A*, spectra of the indicated label, ($n = 10$) at 20 μm with (—) and without (---) added agonist. The ratio of bound/free label (N_b/N_f) is ~2.9 in the absence of agonist; the change in free signal corresponds to decrease in the aqueous concentration of label of 1.1 μm. *B*, spectra of the ether-linked phosphonium label (20 μM), which is entirely membrane associated, are shown: with (—) and without (---) added agonist.

membrane low-dielectric region will alter the partitioning of phosphonium spin labels (5). Changes in the affinity of the label to a specific site or area on the receptor could account for this effect. Because the measured partitioning change is largely independent of ionic strength, a change in surface potential is not likely to be a major component of this effect. A change in the affinity of the phosphonium to a site on the receptor protein might be expected to show changes in the membrane-associated spectra of the phosphonium nitroxide, and we have examined this possibility by utilizing a phosphonium that does not partition but is completely membrane-associated. The EPR spectra of this probe in AcChR vesicles are shown in Fig. 2 *B*. The spectra are apparently the result of at least two populations of membrane-associated probe, one having relatively slow motions and another that is more freely diffusing (and similar to that seen for the label in pure lipid vesicles). The addition of agonist results in a dramatic change in the membrane-associated spectra, which clearly reflect an increase in the population of slowly diffusing spins.

These results indicate that the changes in the partitioning of phosphonium seen in Fig. 2 *A* may be largely due to a shift in the distribution of membrane-bound probe. Thus, a modulation of the affinity of the receptor for the phosphonium at a specific site, and not necessarily a less localized charge in membrane electrostatics, could account for these results. This possibility is also strongly suggested by earlier work utilizing radiolabeled phosphonium ions (2, 3). The likelihood that part of this partitioning difference is due to electrostatic effects can not be ruled out, nor do these interesting complications preclude the possibility that significant membrane electrostatic changes occur during gating. We are currently investigating these phenomena utilizing a number of other negatively and positively charged amphiphilic probes.

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