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Structure of the Axolemma of Frog Myelinated Nerve: Relaxation Experiments with a Lipophilic Probe Ion

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Summary. Asymmetrical displacement currents are measured in the absence and in the presence of the lipophilic anion dipicrylamine (DPA) in the extracellular solution of nerve fibres of the frog *Rana esculenta*. DPA (30 nM -3 μM) enhances the current by a component that has the properties expected for a translocation current of DPA ion across the lipid membrane. Analysis in terms of a single-barrier model yields the translocation rate constant (k), the total surface density of DPA absorbed to the membrane (N_t), and the equidistribution voltage (Ψ). The value of k of about 10^4 s^{-1} is similar to that for a solvent-free artificial bilayer formed by the Montal-Mueller method. The surface density N_t varies with the DPA concentration as it does in the artificial bilayer, but is about tenfold smaller at all concentrations. The DPA ions sense an intrinsic electric field that is offset by a transmembrane voltage between 0 and 30 mV (inside positive). The part of the axolemma probed by the DPA ion appears as a thin (<2.5 nm), fluid bilayer of lipids. DPA ions seem, however, to be excluded from the major part of the axolemma as if this area is occupied by integral proteins or negative charges.

Transport properties of lipophilic ions have been extensively studied in lipid bilayers and have been found to reflect the structure and dynamics of such artificial membranes [1, 2, 6–9, 30, 33]. Thus transport kinetics are strongly sensitive to the dipolar potential and the thickness of the membrane [7, 8, 30, 33]. The adsorption of negatively charged lipophilic ions to the lipid bilayer is probably governed by the electrostatic potential of the lipid phase, which results from the superposition of surface charge potentials and dipolar potentials.

The fluid mosaic model for biological membranes postulates that such membranes contain areas of pure lipid bilayers [32]. Some properties of biological membranes, like electrical capacity, seem to be determined by these bilayer areas, whereas functions such as the transport of substrates and the respiratory chain are achieved by the activities of integral and peripheral proteins [32]. Although the fluid mosaic model has been questioned occasionally [15], it has been widely used. Nevertheless, little is known about the lipid areas of particular biological membranes such as the electrically excitable membrane of nerve, and it is not clear to what extent artificial bilayers are a model for the lipid part of the biological membranes [16].

In this paper we explore the lipid part of a nerve membrane using the lipophilic anion dipicrylamine (DPA) as a probe. Translocation of this ion across the membrane phase is studied with a technique already developed for measuring displacement currents associated with the opening and closing of the Na channels in nerve membranes (gating currents) [3, 21, 24, 29]. We find that adding DPA to the extracellular solution generates an additional displacement current. Analysis of this current reveals kinetics of DPA movements similar to those in a 'solvent-free' artificial membrane made from monolayers [6, 7]. The nodal axolemma, however, adsorbs about ten times less translocatable ion than a bilayer of neutral lipids [34], indicating that integral proteins and negative surface charges dominate most of the area in this nerve membrane.

Materials and Methods

Single myelinated nerve fibers of *Rana esculenta* with diameters between 15 and 20 μm were studied under voltage-clamp con-

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ditions [27]. The nerve fiber was cut in isosmotic CsCl solution to block current in K channels; this block was enforced by including tetraethylammonium (TEA) ion to external solutions. Sodium currents were measured with an external solution containing 110.5 mM NaCl, 2 mM N-morpholino-3-propane-sulfonic acid (MOPS)-NaOH buffer at pH 7.4, 2 mM $CaCl_2$ and 10 mM TEACl. For measurements of displacement currents, tetramethylammonium ion was substituted for Na ion and 300 nM tetrodotoxin (TTX) was added for blocking current in Na channels. Dipicrylamine (Fluka, Buchs, Switzerland; puriss.) was dissolved in water or in ethanol. Small aliquots of the stock solution were added to the external solutions for final concentrations of DPA between 3×10^{-8} and 3×10^{-6} M. The ethanol content of these solutions was less than 0.1 % (v/v) and did not affect the properties of the nerve membrane. The temperature was 13 °C if not otherwise indicated.

Membrane currents were sampled and averaged on line with a digital computer, which also generated the voltage pulses to be applied to the membrane [28]. For measuring the nonlinear part of the capacity current (which represented gating and DPA current) each positive test pulse ($Ep+$) was followed at 0.5-sec intervals by two negative control pulses ($Ep-$) that had half the amplitude of the test pulse. As a rule 64 such pulse sequences were applied for each voltage, and all records were added algebraically yielding the asymmetrical displacement current. Voltage pulses were superimposed on a holding potential of $E_H=-98$ mV (inside negative). The asymmetrical displacement current was first measured without, and again with, DPA in the external solution. DPA currents were calculated as differences of such records. Least-squares fits of DPA currents were done on the computer using expressions for a one-barrier model.

Description of the Transport Model

Lipophilic ions have been postulated to move across a lipid bilayer membrane in three steps [20]: (*i*) adsorption from the aqueous phase to the lipid-water interface, (*ii*) translocation over the central barrier in the middle of the membrane, and (*iii*) desorption into the aqueous phase. The transport is strongly limited by the rate of diffusion in the water [8, 19], so voltage-jump experiments yield a large transient current, carried by adsorbed DPA ions that are translocated to the other side of the lipid phase, and a much smaller sustained current. For an analysis of the transient current, it is correct to consider the number of adsorbed DPA ions as a constant quantity. Then, the sum of both surface concentrations N' and N'' equals a constant total concentration of DPA in the membrane

$$N'+N''=N_t \tag{1}$$

and the variations of the surface concentrations by jumps across the membrane (rate constants k' and k'')

$$\frac{dN'}{dt}=-k'N'+k''N'', \tag{2}$$

$$\frac{dN''}{dt}=k'N'-k''N'', \tag{3}$$

cancel each other:

$$\frac{dN'}{dt}+\frac{dN''}{dt}=0. \tag{4}$$

The DPA ion senses the sum of several voltages on its way across the membrane. One is due to the external field imposed by the voltage clamp E. The others are the surface potentials and dipole potentials at both sides of the membrane, resulting in a bias potential ψ [8, 20]. Finally, the bias potential ψ can be thought to include also a possible 'chemical' asymmetry between the two layers of the lipid bilayer. The rate constants k' and k'' are then functions of the total voltage $E-\psi$. For a symmetrical central barrier and a monovalent anion that senses the fraction α of the total voltage, the rate constants are given by the Eyring expressions

$$k'=k\exp\left[\frac{\alpha F(\psi-E)}{2RT}\right] \tag{5a}$$

$$k''=k\exp\left[\frac{\alpha F(E-\psi)}{2RT}\right]. \tag{5b}$$

(F is the Faraday constant, R the gas constant, and T the absolute temperature.)

According to Eqs. (4) and (5), the surface concentrations N' and N'' are functions only of $E-\psi$, of the parameter α and of the total concentration N_t of lipophilic ion absorbed to the membrane. Under the conditions of our experiments voltage steps to levels E_{p^+} (test) and E_{p^+} (control) were applied to the membrane beginning from the holding voltage E_H. The time course of the current relaxations and the charge movements may be calculated from the differential Eq. (2) and the boundary conditions for the surface concentrations N' and N'' at the voltages $\psi-E_H$ and $\psi-E_{p^+}$ or $\psi-E_{p^-}$.

Results

Dipicrylamine Does Not Interact with Na Channels

In the experiment of Fig. 1, Na current was first measured with the Na^+-containing external solution (solid curve). Then 10^{-7} M DPA^- was added to the same solution, and Na current was measured again (dashed curve). Both records are closely superimposed indicating that the presence of DPA ions does not modify the gating process of Na channels. If DPA ions are taken up by the nerve membrane, they

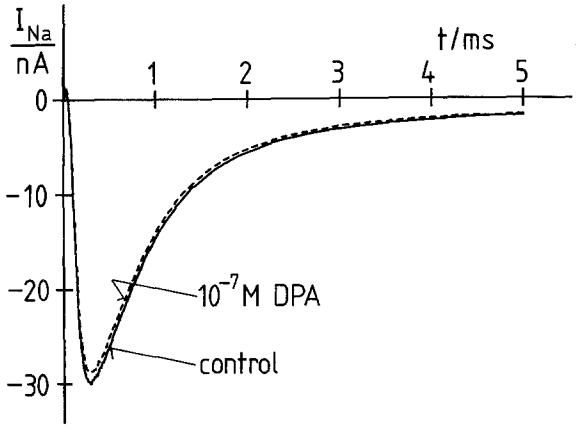

Fig. 1. Effect of dipicrylamine on Na current. Solid line: control; dashed line: 5 min after adding 10^{-7} M DPA to the extracellular solution. Test pulse to −10 mV, applied after 50 msec hyperpolarizing prepulse to −110 mV. Temperature 13 °C, fiber 4A/77. Holding potential was −70 mV in this experiment

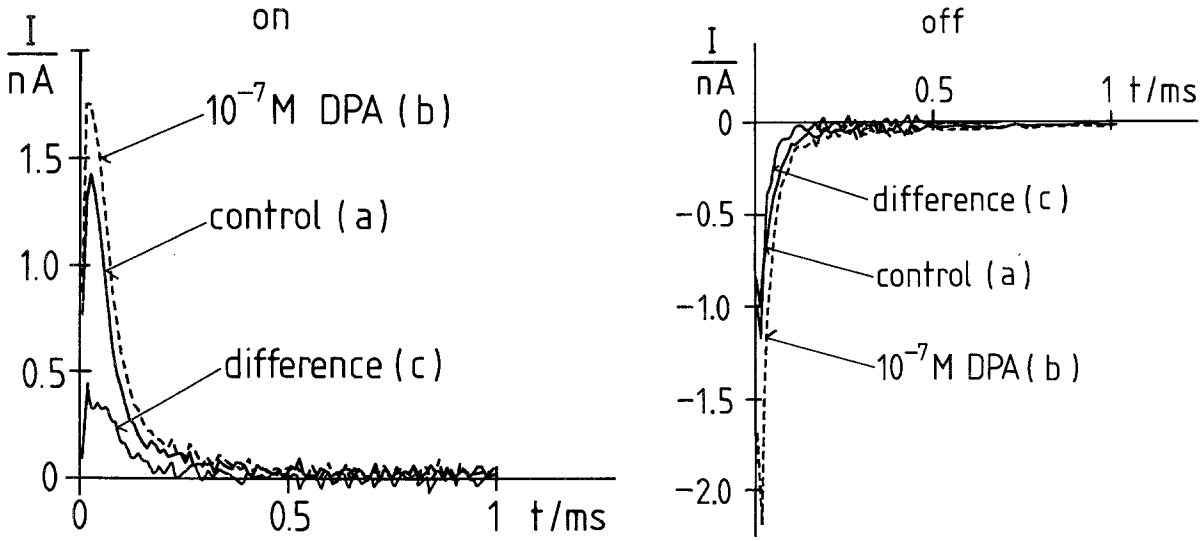

Fig. 2. Asymmetrical displacement currents. Solid line: control (*a*); dashed line: 10^{-7} M DPA (*b*). DPA translocation current (*c*) (difference of records (*b*) and (*a*)). Depolarization to 30 mV from holding potential of −98 mV. Fiber 3B/77. The areas under the on- and off-transients (*c*) represent charges of 40.8 and 44 fC

obviously are confined to membrane areas where they do not appreciably interact with the Na channels.

A large fraction of the normal asymmetry current arises from the voltage-gated Na channels (at least 90% in the axolemma of the squid giant axon) [10] and reflects transitions among different configurations of the Na channel protein. Since DPA ions do not interfere with this process, translocation currents of DPA ions in the nerve membrane should also be practically independent of the gating currents or vice versa, and may be separated by a simple subtraction method as described now.

Dipicrylamine Ions are Translocated Across the Nerve Membrane

Fig. 2 shows asymmetrical displacement currents measured with the node bathed in the Na-free, TTX-containing external solution. The current was first measured without (*a*) and again with 10^{-7} M DPA added to this solution (*b*). It is clear that the displacement current is larger with DPA (dashed trace) than without (solid trace (*a*)). The difference of the two records (*c*) follows a time course basically similar ot that of the gating current (*a*) and reveals properties expected for a DPA translocation current. Thus, the integrals of the forward current during the pulse and of the reverse current after the pulse are almost equal (*see* legend of Fig. 2), and, as is shown later, this equality holds over a large range of potentials (Fig. 4*B*). The time constants of decay are about 90 μsec for the forward, and 20 μsec for the reverse, current and are thus comparable to DPA translocation time constants of Montal-Mueller membranes [7].

Fig. 3 shows time courses of the integrated currents obtained for various test voltages between −70 and +90 mV. The traces (*A*) give the charge movements in the absence of DPA (gating charge movement), traces (*B*) those in the presence of 10^{-7} M DPA, and traces (*C*) the algebraic differences (DPA charge movement). Obviously, DPA enhances the charge movement over a large range of voltages.

Fig. 4 plots the charges moved within the first 0.5 msec after the make (circles) or break (squares) of the pulse. The DPA-induced charge movement in Fig. 4*B* is somewhat less steeply dependent on voltage than the gating charge movement in Fig. 4*A* and follows, as described in the subsequent analysis, the voltage dependence expected for a monovalent charge that moves between the membrane surfaces. These observations support the view that the additional displacement current is due to a translocation of DPA ions adsorbed to the axolemma. It will, therefore, in the following be referred to as translocation current.

The translocation current appeared, and reached its full size, within 3–4 min after DPA was applied in the bath. The measurements analyzed in the subsequent sections were begun no earlier than 5 min after application of DPA. The effect of DPA develops here faster than in lipid bilayer experiments [7, 8] probably because of the continuous perfusion of the nerve chamber (bilayers can adsorb as many DPA

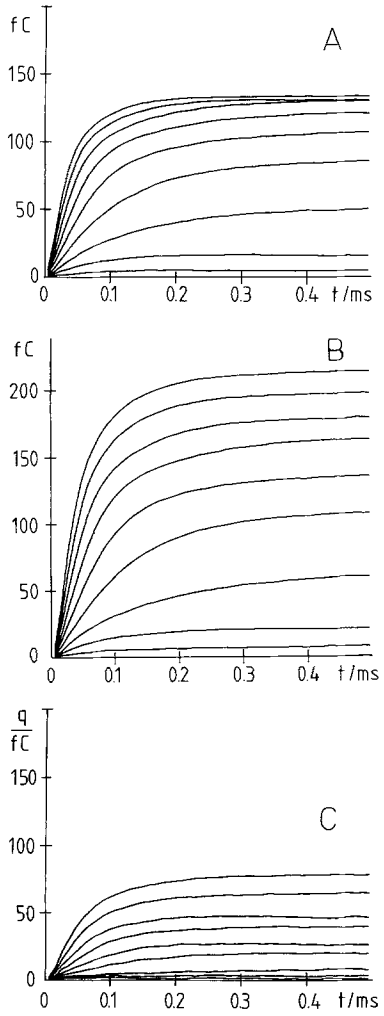

Fig. 3. Families of integrated asymmetrical displacement currents. (*A*) control, (*B*) 10^{-7} M DPA, (*C*) difference of (*B*) and (*A*). Test pulse varied in 20-mV steps between −70 and +90 mV. (*A*) gives the movement of gating charge, (*C*) the translocation of DPA. From same fiber as Fig. 2

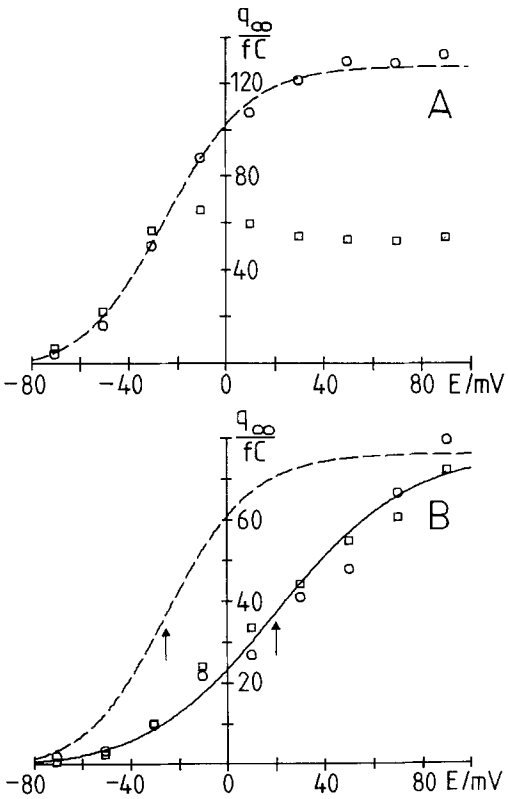

Fig. 4. Steady-state gating charge (*A*) and DPA translocation (*B*) *vs.* voltage. Symbols: charges measured at 0.5 msec after the make (circles) and the break (squares) of the test pulse. Note that gating charge movement is not fully reversible within 0.5 msec because of 'immobilization' [28], but DPA translocation is. The dashed curve in (*A*) represents Eqs. (6) and (10) with $q_{max}=135$ fC, effective valency 1.4, $\psi=-25$ mV and fits the gating charges. The solid curve in (*B*) describes the DPA translocation with $q_{max}=80$ fC, $\alpha=0.9$, $\psi=20$ mV; the dashed curve in (*B*) is the renormalized curve from (*A*) and shows the difference between the gating charge and DPA translocation characteristics. Arrows mark the midpoint potentials of the charge distributions. Fiber 3B/77

ions as are contained in a 1-mm thick layer of 10^{-7} M DPA solution [7, 34]).

Analysis of DPA Translocation Currents

Our method of measuring the translocation current exploits the observation on bilayers that the distribution of DPA ions saturates at extreme voltages. Then a voltage step between a strongly negative voltage towards a more negative one should produce very little translocation (nearly all DPA ions being already on the respective surface), whereas a positive step should translocate a fraction, or almost all, DPA ions to the other surface. Our procedure measures the net charge movement

$$\Delta q(t)=[q_+(t)-q_0]+2[q_-(t)-q_0]. \tag{6}$$

The time course of the charge distribution function is

$$q(t)=q_\infty+[q_0-q_\infty]\,e^{-t/\tau}. \tag{7}$$

Since the distribution curve of a monovalent charge is rather flat, a self-consistent analysis of the charge translocation has to consider the charge translocated at the holding potential (q_0) and the charge moving during the control pulses toward a more extreme

distribution $(q_-(t)-q_0)$. Indeed, our translocation currents revealed a marked rising phase during pulses [Fig. 2, (c)] as expected if a rapid inward transient current was subtracted from the outward transient.

The one-barrier model described before predicts at a given voltage E the time constant

$$\tau=\frac{\tau_{max}}{\cosh\left[\frac{\alpha F}{2RT}(\psi-E)\right]}. \tag{8}$$

$$\tau_{max}=\frac{1}{2k} \tag{9}$$

and the steady-state distribution of charge

$$q_\infty=\frac{q_{max}}{1+\exp\left[\frac{\alpha F}{RT}(\psi-E)\right]}. \tag{10}$$

The total concentration of lipophilic ions in the nodal membrane can be calculated from

$$N_t=\frac{q_{max}}{F\alpha A} \tag{11}$$

where A is the surface area of a frog node. In the following, this area will be assumed to be 50 μm^2 in agreement with a recent morphological study on the same preparation, yielding nodal areas of 30–60 μm^2 (B. Uhrik and R. Stämpfli, *personal communication*).

For analyzing a family of records as in Fig. 3*C*, the individual curves were fit according to Eq. (6). The parameters α and ψ were given tentative values and were maintained for all curves of a family. The fit of each individual curve yielded an estimate for q_{max} and τ_{max}. The procedure was repeated for different choices of α and ψ until the variation of q_{max} and τ_{max} among all individual curves was minimal. The translocation rate constant, k, and the total concentration in the membrane of DPA, N_t, were then calculated from the average τ_{max} and q_{max} by Eqs. (9) and (11).

The fraction of the membrane voltage that is effective between the adsorption planes of DPA ions, α, had to be close to one for a minimal variation of q_{max} estimates, but had to be about 0.8 for a minimal variation of τ_{max} estimates. The phenomenon has been observed in experiments on artificial lipid bilayers [8] and indicates that the empirical rate constant of DPA translocation varies less steeply with voltage than is consistent with the single-barrier model. As a compromise we finally assigned to α the value 0.9, and calculated the other parameters on this basis. As already mentioned, this result shows that the translocation current reflects movements of monovalent charges between two surfaces that are close to the lipid-water interfaces of the nerve membrane.

The optimal values of the midpoint potential, ψ, were found between 0 and 30 mV ($\alpha=0.9$). Fig. 4*B* plots the translocation charges from one experiment versus the test voltage (symbols). The solid line represents the net charges $\Delta q(\infty)$ calculated according to Eqs. (6) and (10); the parameters are $q_{max}=80$ fC, $\alpha=0.9$, $\psi=20$ mV. In this and in other experiments the translocated charge did not saturate within the range of voltages studied (saturation was, however, observed in one experiment with more positive test voltages of up to 126 mV). Therefore, the estimates of the midpoint potential were mainly determined through the voltage dependence of the translocation kinetics. They indicate that the DPA ions sense a bias potential different from that of the gating charges of Na channels. Evaluation of the gating charges in Fig. 4*A* in terms of the same model (dashed curve) yields a midpoint potential of −25 mV, close to that observed previously [29].

We also studied the variation of the DPA ion concentration in the membrane (column N_t in Table 1) with the DPA concentration of the bath (column C_{DPA}). The membrane concentration increased proportionally between 30 and 100 nM, but saturated and decreased again for larger concentrations (up to 3 μM). The partition coefficient

$$\beta=\frac{N_t}{2C_{DPA}} \tag{12}$$

therefore became smaller at large bath concentrations of DPA. The saturation of the membrane concentration of DPA was accompanied by a decrease of the translocation rate constant k (Table 1), which has also been observed for the transport of lipophilic ions through artificial lipid bilayer membranes [8, 9, 33].

The concentration of DPA adsorbed in the nerve membrane, as will be discussed later, is much smaller than that adsorbed in artificial bilayers of lipid. For detecting artifacts due to complexation between DPA anions and some large cations used in our experiments on nerve, control experiments were done on bilayers with 0.1 M solutions of NaCl, KCl, CsCl, NH_4Cl, TMACl, and TEACl for two DPA concentrations in the bath. Bilayers of egg phosphatidylcholine (3×10^{-8} and 10^{-7} M DPA, 25 °C) gave rate constants k of 420, 470, 460, 410, 480, 450 per second, and adsorption coefficients, in 10^{-2} cm, of 2.9, 3.1, 2.8, 3.0, 2.9, and 2.7, respectively. Thus there is no indication that the concentration of free DPA ion in the solutions used for nerve was lower than that present in solutions commonly used in bilayer experiments.

Table 1. Analysis of translocation current in the axolemma

C_{DPA}/M	r	$q_{max}/10^{-15}$ A · sec	τ_{max}/μsec	$k/10^3\,s^{-1}$	$N_t/10^{-12}$ mol cm^{-2}	$\beta/10^{-3}$ cm
3×10^{-8}	2	12±4	41±13	12 ±2.9	0.28±0.09	4.7
10^{-7}	6	39±5	54± 5	9.3±0.8	0.90±0.12	4.6
3×10^{-7}	4	29±4	78±15	6.4±1.0	0.67±0.09	1.1
10^{-6}	2	26±9	110±41	4.5±1.2	0.60±0.20	0.30
3×10^{-6}	1	18±1	77± 7	6.5±0.5	0.41±0.02	0.07

The analysis was done for a fixed value $\alpha=0.9$ and the optimal values of ψ, ranging between 0 and +30 mV. r is the number of translocation current families analyzed for each DPA concentration.

In experiments with one nerve fiber (C_{DPA} = 100 nM) the temperature was varied between 13 and 23 °C. The total concentration of DPA in the membrane decreased by the factor of two between 13 and 23 °C (from 0.98 pmol/cm^2 to 0.53 pmol/cm^2), whereas k increased simultaneously by 30%. This finding is consistent so far with the idea that in lipid bilayer membranes the adsorption of lipophilic ions is partly driven by entropy [8, 9]. More measurements are required, however, for an accurate estimate of the activation energies.

Discussion

The experiments show that the lipophilic anion dipicrylamine is adsorbed to the axolemma of myelinated nerve and can be translocated across the membrane by brief voltage pulses. On the assumption that the total number of adsorbed DPA ions is constant during such relaxation measurements [8, 19], our experiments directly reveal the rate constant of translocation across the membrane lipid, k, and the density of DPA ions adsorbed to the axolemma, N_t (Table 1). These quantities are known for a variety of artificial lipid bilayers [6–8, 34]. Hence, properties of the nerve membrane may be deduced by comparison.

Let us first consider the rate of translocation. In artificial systems, it has been shown to be strongly sensitive to the thickness of the lipid phase and to the dipolar potential of the membrane lipid. Electrostatic image forces create a potential energy barrier for ions in the dielectric of the membrane; the height of this barrier increases with thickness [26]. The dipolar potential presumably originates at the level of the ester linkages of the membrane lipid. These linkages form dipoles, with the negative poles (oxygens) oriented towards the water. The interior of the membrane becomes therefore electrically positive with respect to the aqueous phases. The difference can be several hundred millivolts [23]. Reducing the dipolar potential (e.g. by forming the film from lipids with ether linkages) reduces the rate of translocation for lipophilic anions [7].

We find for the rate constant, k, values near 10^4/sec at 13 °C (Table 1). Such a rate constant is clearly at the upper end of the range measured for artificial membranes. Solvent-containing, 4–5 nm thick bilayers yield values between 4×10^2/sec and 10^3/sec at 25 °C. Montal-Mueller membranes [5, 25], which are almost free of solvent, are thinner and give larger rate constants, e.g., 8.5×10^3/sec (25 °C) for a 2.5 nm thick bilayer of dioleoyl phosphatidylcholine [7]. Thus the part of the axolemma probed by the DPA ion is likely to be even somewhat thinner than 2.5 nm, and its dipolar potential is not likely to be smaller than that of the dioleoyl phosphatidylcholine membrane. This thickness is consistent with capacitance and X-ray diffraction measurements on other natural membranes (*see* Ref. [31] for a summary). It is considerably smaller than the twofold length of the fully extended C_{18}-chain (4 nm) and would indicate that the hydrocarbon chains of the axolemma are in the disordered state implied by a fluid membrane. A large dipolar potential of the axolemma might seem incompatible with the large cation currents through the Na and K channels. Gramicidin pores, however, are shielded from the lipid by only a single layer of polypeptide and conduct monovalent cations at an even higher rate; their conductance varies little among lipid bilayers with different dipolar potentials [4].

While the axolemma appears as quite similar to a thin lipid bilayer so far, the amount of dipicrylamine adsorbed to the axolemma is considerably smaller. The apparent partition coefficient β (Table 1) is no larger than 5×10^{-3} cm (13 °C). In Mueller-Rudin membranes of egg phosphatidylcholine, the partition coefficient has a maximal value of 6×10^{-2} cm (15 °C) or, in membranes of egg phosphatidylethanolamine, of 2×10^{-2} cm (15 °C) (R. Benz, *unpublished*). On the other hand, the saturation characteristics of the axolemma are very similar to those of an artificial bilayer [34]. The surface density of DPA, N_t, is maximal at a bath concentration near 10^{-7} M, and it falls to *ca.* one-half maximum at 3×10^{-6} M (Table 1). Thus the axolemma behaves as if about one-tenth of

its total area translocates DPA like an ordinary bilayer, and nine-tenths of its area either does not adsorb DPA or does not allow the translocation of DPA. We also find no obvious deviation from the exponential time course in the translocation current, like a superposition of several exponentials. If different lipid domains [31] exist, their translocation characteristics for DPA must be either similar or much slower than those of the rest.

Part of the nodal membrane area must be formed by integral proteins like the electrically excitable cation channels and cation pumps. For example, if each Na channel protein fills an area of 50 nm^2, then 10^5 Na channels [12] would contribute 5 μm^2, or 10%, to the nodal membrane area. All of the integral proteins together may form a considerably larger area, but are probably not the only factor for excluding DPA from the axolemma. Thus such proteins may be surrounded by an ordered shell of lipid molecules ('boundary' lipid). The Ca^{2+} pump protein of the sarcoplasmic reticulum, for instance, has been suggested to be intimately associated with 27 molecules of phospholipid [22]; this would be a lipid area of about $27 \times 0.6\ nm^2 \simeq 16\ nm^2$. Such boundary lipids could be in an immobilized state preventing translocations of a lipophilic ion. Another important factor for exclusion of DPA anions may be negative charges of the lipids or integral proteins. In bilayers of negatively charged phosphatidyl serine, the partition coefficient for DPA follows the ionic strength in the way predicted by the Gouy-Chapman theory [7]. An increase of the negative surface potential by -58 mV reduces the partition coefficient for 10^{-8} M DPA *ca.* tenfold. Indeed, surface potentials of the nodal membrane have been predicted from the effects of external protons or divalent cations on the gating properties of Na and K channels [13, 14, 17, 18]; estimates range from *ca.* -50 to -90 mV at pH 7.4. A surface potential of this size would explain our observation, but it should be noted that these values may apply only to the vicinity of the ionic channels and that the surface potential may be smaller elsewhere. Both a shell of boundary lipids or negative charges of the Na channel protein could exclude DPA from the vicinity of the channels and prevent effects of DPA anions on the voltage-sensitive gating process (Fig. 1). Finally, the midpoint potential of the DPA distribution curve (Fig. 4*B*) indicates that there is only a small electrical asymmetry in the axolemma when the external voltage is zero. Hence, the DPA ion probes a membrane in which the electrical surface and dipolar potentials and the chemical asymmetries are approximately balanced between both sides of the membrane.

Thus, partitioning and transport of DPA in the axolemma seem either to be very similar to those in a lipid bilayer membrane or at least the differences between both kinds of membranes can easily be explained. In any case it is not necessary to postulate a fundamental difference between the lipid part of the nerve membrane and lipid bilayers in order to explain the somewhat different behavior of DPA. Conrad and Singer [11], who studied the partitioning of chlorpromazine, 2,4-dinitrophenol, and 1-decanol, found the partition coefficient of these amphipatic compounds to be at least by 1000 times smaller in cell membranes than in lipid vesicle membranes. They explained the discrepancy by postulating that cell membranes, but not vesicle membranes, have a large internal pressure and therefore exclude amphipatic molecules [11]. Our experiments with the amphipatic compound DPA reveal a much smaller ratio of about 10 between the partition coefficients of a nerve membrane and lipid bilayer membranes and do not support the hypothesis of a large internal pressure in biological membranes.

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