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Effect of 3-Phenylindole on Lipophilic Ion and Carrier-Mediated Ion Transport Across Bilayer Lipid Membranes

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Summary. The physical effects of 3-phenylindole, an antimicrobial compound which interacts with phospholipids, on ion transport across phosphatidylcholine-cholesterol bilayers have been investigated using three lipophilic ions and one ion-carrier complex. It was found that 3-phenylindole increased membrane electrical conductance of positively charged membrane probes and decreased electrical conductance of negatively charged probes. The enhancement of conductance detected by nonactin-K^+ complex and tetraphenylarsonium$^+$ was several orders of magnitude, whereas the suppression of conductance due to tetraphenylborate$^-$ and dipicrylamine$^-$ was less than a factor of ten. Presence of 3-phenylindole in aqueous phase slightly decreased adsorption of tetraphenylborate$^-$ and dipicrylamine$^-$ at the membrane surface. From the voltage dependence of the steady-state conductance it was shown that 3-phenylindole induced kinetic limitation of membrane transport of potassium mediated by nonactin. No such limitation was found in the case of tetraphenylarsonium$^+$ transport. These results are shown to be consistent with the present concept of ion diffusion in membranes and the assumption that 3-phenylindole decreases the electric potential in the membrane interior. The asymmetry of the effect of 3-phenylindole on the magnitude of conductance changes for positively and negatively charged membrane permeable ions is also discussed as a reflection of the discreteness of both the adsorbed 3-phenylindole and lipid dipoles.

Key Words 3-phenylindole · ion transport · bilayer lipid membranes · membrane permeability · dipole potential · fungicide

Introduction

The process of ion transport across membranes is a vital part of the function of a cell or organism. Some insight into the mechanism of ion transport in membranes has been gained from studies of electrical conductance of bilayer lipid membranes induced by lipophilic ions, antibiotics acting as ion-carriers, and weak acid uncouplers of oxidative phosphorylation (McLaughlin & Eisenberg, 1975; Hladky, 1979; McLaughlin & Dilger, 1980). The ways in which the membrane electrical conductance induced by carriers and lipophilic ions can be modified by electrically neutral compounds is a logical extension of the investigation of membrane transport processes. McLaughlin (1973) found that salicylamide increased cationic membrane conductance and depressed anionic conductance. Szabo (1976) determined that cholesterol modifies the electrical conductance of bilayer lipid membranes primarily because the dipole moment of cholesterol increases the electric potential in the interior of the membrane.

Andersen, Finkelstein, Katz and Cass (1976) studied the effect of phloretin and several related compounds on electrical conductance of membranes. These authors concluded that the neutral form of phloretin adsorbs at the surface and introduces additional electrical potential difference across the membrane/water interfacial region, which changes the concentration of ions in the interior of the membrane. In addition, they also considered the possibility of phloretin-induced changes of membrane fluidity. This was an important finding in that phloretin inhibits several energy independent transport processes, among them the transport of chloride ions across red blood cell membranes (Wieth, Dalmark, Gunn & Tosteson, 1973), and increases potassium conductance in *Aplysia* neurons (Owen, 1974). Cousin and Motais (1978) have shown that the ability of phloretin and acetophenone derivatives to reduce chloride transport in red blood cells is determined by two primary factors: lipid solubility and dipole moment of membrane modifier.

The presence of a layer of adsorbed oriented dipolar molecules has been also proposed by Smejtek and Paulis-Illangasekare (1979*a*; *b*) to explain the effect of the widely used herbicide 2,4-D (2,4-Dichlorophenoxyacetic acid) on electrical conductance of lipid bilayers induced by membrane permeable ions. They noted that the degree of enhancement of membrane cationic conduc-

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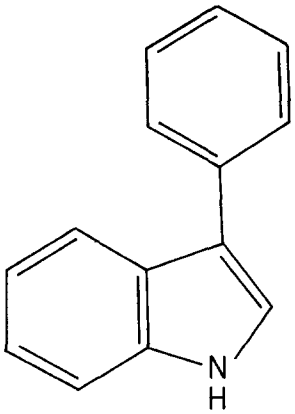

Fig. 1. Molecular structure of 3-phenylindole (3PI)

tance by 2,4-D was greater than the degree of suppression of anionic conductance. The problem of charge asymmetry in membrane conductance response to various factors affecting the structure of bilayer membranes was addressed by Pickar and Benz (1978). These authors found that the inclusion of various sterols in monoolein and phosphatidylcholine membranes influenced the transmembrane transport of the positively charged lipophilic ion tetraphenylarsonium much more than it influenced transport of the negatively charged lipophilic ion tetraphenylborate, and attributed this asymmetric behavior to a combination of dipolar and fluidity effects.

In this paper we report on the alteration of membrane ionic permeability characteristics by 3-phenylindole (3PI) whose molecular structure is shown in Fig. 1. 3-phenylindole is a lipophilic compound which is electrically neutral at physiological pH and inhibits the growth of many species of fungi and Gram-positive bacteria (Dekker, Selling & Overeem, 1975; Hoppe, Kirkenaar & Sijpesteijn, 1976*a*). Hoppe et al. (1976*a*; *b*) report that 3PI interacts selectively with the phospholipid fraction of the fungus *Aspergillus niger* and affects the uptake and incorporation of several biosynthetically important molecules. They also detected a leakage of ^{32}P-labeled compounds from *A. niger* in growth conditions. These biological experiments suggested that 3PI could influence processes occurring in or on the membrane, including, for example, ion transport. It is not yet clear, however, to what extent physical processes, as distinct from metabolic processes, are responsible for the observed biological phenomena.

The goal of research described in this paper was to clarify the physical effects of 3PI on ion transport in lipid bilayer membranes regarded as the matrix of biological membranes. The possibility that 3PI changes the ion potential energy in the membrane was tested by studying the voltage dependence of membrane steady-state conductance induced by nonactin-K^+ and tetraphenylarsonium$^+$, as well as the transient membrane conductance due to tetraphenylborate$^-$ and dipicrylamine$^-$ in "voltage clamp" experiments.

Transport Models

Nonactin Carrier Model

The nonactin-mediated transport of potassium ions is well described by a kinetic model whose merits and deficiencies have been discussed in detail by other investigators (Stark & Benz, 1971; Hladky, 1972; 1973; 1979). We repeat here only the basic features of the carrier model as it applies to our experiments. The carrier mechanism of transport consists of the following steps: combination of a potassium ion (K^+) with uncomplexed nonactin molecule near the interface between aqueous and membrane phases, diffusion of the nonactin-K^+ complex across the membrane, dissociation of the complex into K^+ and uncomplexed nonactin near the opposite interface, and diffusion of uncomplexed nonactin (free carrier) to the original interface. Each step of the process can be characterized by a rate constant which may or may not depend on applied voltage. We use the standard version of the model according to which the rate of back diffusion of the neutral carrier as well as the rates of recombination and dissociation of K^+ and nonactin are independent of transmembrane electric field. Only the rate constants of nonactin-K^+ complex translocation across membrane core are assumed to be dependent on the applied voltage. Their explicit forms, based on the model of ion electrodiffusion in image force field due to ion polarization of aqueous phase, are given below. The rate constant for translocation of the ion/carrier complex from left to right, k', is

$$k' = k_{is} \exp(-\omega u^2) \exp(zu/2) \tag{1}$$

and the rate constant for translocation from right to left, k'', is

$$k'' = k_{is} \exp(-\omega u^2) \exp(-zu/2) \tag{2}$$

where u is the reduced voltage ($u = FV/RT$), F the Faraday, V the externally applied voltage, R the gas constant, T the absolute temperature, z the valency of the ion, ω a parameter dependent on thickness of the membrane, and k_{is} the zero voltage value of both k' and k'' (Smejtek & Paulis-Illangasekare, 1979*a*; Andersen & Fuchs, 1975).

In the experiment, the conductance, $G(V)$, is measured as a function of voltage applied across the bilayer. The normalized membrane conductance is equal to (Smejtek & Paulis-Illangasekare, 1979*a*)

$$\frac{G(V)}{G(O)}=\frac{2}{u}(1+A)\exp(-\omega u^2)\cdot\frac{\sinh(u/2)}{1+A\exp(\omega u^2)\cosh(u/2)}. \quad (3)$$

The kinetic limitation parameter A is related to the rate constants by

$$A=(2k_{is}/k_d)+(k_r k_{is} a_i/k_s k_d) \quad (4)$$

where k_s is the rate constant for back diffusion of the free carrier, k_r the rate constant for recombination, k_d the rate constant for dissociation, and a_i the concentration of ion in the bulk aqueous phase. The existence of kinetic limitation of carrier-mediated ion transport in lipid bilayers and the usefulness of parameter A as the measure of kinetic limitation have been demonstrated (Stark & Benz, 1971; Hladky, 1975; Smejtek & Paulis-Illangasekare, 1979*a*).

Lipophilic Cation Transport Model

The transport of lipophilic ions across a lipid bilayer membrane can be described by a kinetic model in which the lipophilic ions adsorb to the membrane, cross the membrane upon application of a voltage, and desorb from the opposite interface (Ketterer, Neumcke & Läuger 1971). Due to similarities in the transport schemes, the expression for the normalized conductance induced by lipophilic cations is identical to that for nonactin-K^+, i.e., the normalized conductance for lipophilic cations is given by Eq. (3) with the modification that the kinetic limitation parameter A for lipophilic cations is defined as

$$A=2k_i/k_{ma} \quad (5)$$

where k_i is the rate constant for translocation and k_{ma} the rate constant of ion desorption from membrane to the aqueous phase (Pickar & Benz, 1978). In a comparative study of oppositely charged lipophilic ions, Pickar and Benz (1978) found that for the lipophilic cation tetraphenylarsonium, the normalized conductance fit the case of $A\approx 0$, indicating that $k_{ma}>>k_i$. With this condition, it can be shown that the conductance in the limit of zero applied voltage, $G(0)$, is related to the rate constants by

$$G(0)=F^2\beta c k_i/RT \quad (6)$$

where β is the partition coefficient for the lipophilic cation and c is the concentration of the cation in the bulk aqueous phase (Ketterer et al., 1971). As Pickar and Benz (1978) point out, the absence of any transient component in the current due to tetraphenylarsonium precludes the possibility of obtaining the individual values of β and k_i.

Lipophilic Anion Transport

Lipophilic anions, such as tetraphenylborate and dipicrylamine, adsorb to lipid bilayers to a much greater degree than positively charged structurally similar ions (Liberman & Topaly, 1969). An important feature of lipophilic anion transmembrane transport is that it is limited by a relatively slow rate of interfacial transfer as compared to that of transmembrane transfer, i.e., $k_{ma}<<k_i$. This gives rise to a transient current that provides information on membrane transport characteristics (Ketterer et al., 1971; Anderson & Fuchs, 1975). We reiterate the main features of the lipophilic anion transport models which are relevant for the interpretation of the experimental results on the effect of 3PI on bilayer membranes.

In the absence of an applied potential, lipophilic anions are located in deep potential energy minima close to the aqueous/membrane interface on each side of the membrane (Ketterer et al., 1971); consider that N_{ads} is the number of anions adsorbed per unit area to one boundary region of the membrane. When voltage is applied, a net flux of ions occurs as the anions redistribute themselves between the two membrane surfaces. On a short time scale the total number of ions on the membrane is conserved so that the number of ions which have crossed the membrane is $\Delta N=N_{ads}\tanh(bu/2)$, where b is presumed to represent the fraction of applied voltage effective in moving ions through the membrane. As a consequence of the fact that the probe molecules used in this study each carry one electronic charge, it will also be true that

$$\Delta Q=Q_{ads}\tanh(bu/2) \quad (7)$$

where ΔQ is the transferred charge density and Q_{ads} is the adsorbed charge density (Andersen & Fuchs, 1975).

Lipophilic anion transmembrane transport results in a transient current, $I(t)$, which decays exponentially with time: $I(t)=I_o\cdot\exp(-t/\tau)$, where I_o is the initial current and τ the time constant. The specific membrane conductance $G_o(V)=I_o/V\cdot S$, where S is the area of the aperture across which the lipid is applied. The experimental results are

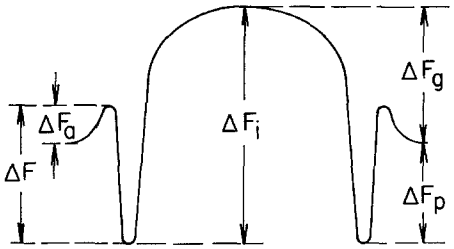

Fig. 2. Ion potential energy profile of membrane. Adapted from Ketterer, Neumcke, and Läuger (1971), with additional details in text

given in terms of the ohmic limit: the relaxation current time constant, $\tau(0)=[\tau(V)]V_{V\to 0}$, and the initial conductance, $G_o(0)=[G_o(V)]_{V\to 0}$. These zero voltage values are related to the partition coefficient β and the kinetic constant for transmembrane translocation k_i according to

$$G_o(0)=z^2F^2\beta c k_i/RT \tag{8}$$

and

$$\tau(0)=(2k_i)^{-1}. \tag{9}$$

In contrast to the case of tetraphenylarsonium$^+$, it is possible to obtain the individual values of β and k_i for lipophilic anion transport from Eqs. (8) and (9). The partition coefficient can also be obtained more directly due to the fact that N_{ads} can be experimentally determined from Q_{ads} [Eq. (7)]. For the low concentrations of anion used in these studies $N_{ads}=\beta c$. These relationships become important when one considers that anion partition coefficient β reflects changes occurring near the aqueous/membrane interface whereas changes in k_i are an indication of changes occurring in the interior region of the membrane.

It is instructive at this point to examine the potential energy barrier encountered by a lipophilic anion as it crosses the membrane. The shape of this barrier is determined by several factors (Ketterer et al., 1971; Andersen & Fuchs, 1975). We will be particularly interested in the changes of electrostatic potential difference associated with changes of dipole moment per unit of membrane surface area caused by the introduction of a neutral lipophilic compound into membrane. These changes were found to alter membrane conductance (Szabo, Eisenman, McLaughlin & Krasne, 1972; Haydon & Myers, 1973). The following model of an ion free energy profile across the membrane has been found useful for the purpose of discussion of ion transport alteration by membrane modifiers (Fig. 2 with free energies in units of RT). The main features of the intrinsic barrier are deep potential energy minima near the aqueous/membrane interface, associated with ion adsorption phase, and a large potential energy rise in the middle of the membrane, ΔF_i. The transmembrane translocation rate constant, k_i, is sensitive to changes of ΔF_i since it is proportional to the exponential of $(-\Delta F_i)$. The partitioning of the ion is determined by the rates of adsorption and desorption, so β is proportional to the exponential of ΔF_p, where $\Delta F_p=\Delta F-\Delta F_a$. The zero voltage conductance is proportional to the product βk_i; consequently, it is evident that $G_o(0)$ is proportional to the exponential of $(-\Delta F_g)$, where $\Delta F_g=\Delta F_i-\Delta F_p$.

Separation of Electrostatic and Nonelectrostatic Effects on Ion Transport

The adsorption of a neutral lipophilic compound such as 3PI onto the lipid bilayer can be expected to change the dipole potential near the aqueous/membrane interface in two ways. First, if 3PI adsorbs in preferred orientation, then 3PI dipole moment will contribute to the interfacial dipole potential difference. Second, if 3PI adsorption changes the density or orientation of the lipid molecules in the bilayer, the dipolar potential difference due to the lipid molecules will also be changed. Let ΔV_D be the change in electric potential of the middle plane of the bilayer with respect to the aqueous phase due to both effects. Note that depending on the location of the adsorption plane of 3PI, ΔV_D could modify either ΔF_i or both ΔF_i and ΔF_p (refer to Fig. 2).

In addition, we should expect that adsorption of 3PI onto the lipid bilayer would change nonelectrostatic properties of the bilayer. For instance, the viscosity of the bilayer, and hence the diffusion constant of the ions in the membrane interior, may be altered by 3PI.

The relative conductance, $\bar{G}[\bar{G}=G_o(0)/G_o^*(0)$, where $G_o(0)$ and $G_o^*(0)$ are the conductances of a membrane formed in the presence and absence of 3PI, respectively], depends on both electrostatic interactions and nonelectrostatic interactions. The relative conductance can be expressed as the product of two factors:

$$\bar{G}=f(x)\exp(-zF\Delta V_D/RT) \tag{10}$$

where $f(x)$ reflects the nonelectrostatic changes which are a function of the concentration of 3PI in the aqueos phase, x (Szabo, 1974). If fluidity effects at a given 3PI concentration are approximately the same for the lipophilic anion and lipophilic cation, then by comparing the relative conductances of oppositely charged lipophilic ions

(Szabo, 1974; Pickar & Benz, 1978), we can determine both the value of the dipole potential difference ΔV_D and the nonelectrostatic factor f. ΔV_D can be obtained according to

$$\exp(-F\Delta V_D/RT)=[\bar{G}^+/\bar{G}^-]^{1/2} \quad (11)$$

where the superscript $^+$ denotes the positively charged lipophilic ion ($z=+1$), and $^-$ denotes the negatively charged lipophilic ion ($z=-1$). The nonelectrostatic factor is given by

$$f(x)=[\bar{G}^+\cdot\bar{G}^-]^{1/2}. \quad (12)$$

Materials and Methods

Bilayer lipid membranes were formed by the brush technique of Mueller, Rudin, Tien and Wescott (1963) across the aperture (1.7 mm diameter) in a Teflon cup set in an acrylic container; the membrane was bathed by symmetrical aqueous solutions. The membrane forming solution contained a mixture of egg phosphatidylcholine and cholesterol in decane. Cholesterol mole fraction was 0.22 and the total lipid content was typically 12 mg/ml. The width of membrane torus was kept at minimum by using a small amount of membrane forming solution on the paint brush. Under these circumstances it was justifiable to use area of hole in computations of specific membrane conductance instead of membrane area. Egg phosphatidylcholine from Sigma Chemical Co., St. Louis, Mo., showed a single spot by thin layer chromatography and was used without further purification. In experiments involving nonactin-mediated K^+ transport, the membrane forming solution contained 3×10^{-5} M nonactin (gift of Dr. B. Stearns, Squibb Institute).

A modification of the method of Fischer and Schmidt (1888) was used to synthesize the 3-phenylindole (3PI). Phenylhydrazine (Matheson, Coleman & Bell) was added in equimolar quantity to 100 grams (0.83 mole) phenylacetaldehyde (Aldrich, Milwaukee, Wisc., technical grade), heated in a steam bath for an hour and then diluted with about 300 ml absolute alcohol. To the mixture was added 200 ml of absolute alcohol which had been saturated with hydrogen chloride gas. The solution was then refluxed in nitrogen environment for 45 min, again using the steam bath. When the solution had cooled to room temperature, enough water ($\approx$1500 ml) was added to dissolve the ammonium chloride and the excess hydrazine salts. The solution was filtered several times, discarding the liquid at each step. The precipitate was dried in a dessicator in the presence of KOH pellets for 90 min. The product was then recrystallized from toluene/hexane, being less soluble in hexane. After sublimation the product was granular, ivory color, and had a distinctive, not unpleasant odor. The melting point was 86.5–87.5 °C, in agreement with published values (Fischer & Schmidt, 1888; Dekker et al., 1975).

Nonactin and sodium tetraphenylborate ($TPhB^-$, reagent grade from Mallinckrodt, St. Louis, M.) were stored as ethanol solutions, while 2,2′,4,4′,6,6′-hexanitrodiphenylamine (Dipicrylamine, abbreviated DPA^-, from Aldrich Chemical Co., Milwaukee, Wisc.) was prepared as a stock solution in 10^{-2} M sodium hydroxide. Tetraphenylarsonium chloride hydrate from Aldrich was used to make an aqueous stock solution which also contained electrolyte and buffer. In the experiments to determine the effect of 3PI on tetraphenylarsonium ($TPhA^+$) or nonactin transport, the aqueous solution consisted of 0.1 M KCl and a buffer (B^{-3}) of potassium phosphate/potassium citrate/boric acid 0.002:0.002:0.0005 M plus either 10^{-7} M nonactin or 5×10^{-3} M $TPhA^+$. For the experiments involving $TPhB^-$ and DPA^-, sodium salts were substituted for potassium salts, and the aqueous phase contained either 10^{-7} M $TPhB^-$ or 10^{-8} M DPA^-. The lipophilic anion concentrations were low enough that conductivity saturation effects were avoided (Ketterer et al., 1971; Wang & Bruner, 1978a). In all cases the desired amount of 3PI was added from a stock solution in acetone by injecting it below the surface of the aqueous solution during continuous stirring. To mimic physiological conditions the membrane transport studies were done at pH 6.9.

To determine the effect of 3PI on nonactin and $TPhA^+$ transport, the steady-state technique was used to obtain the zero voltage conductance, $G(0)$. A constant voltage was applied across the membrane and the current measured with a picoammeter (Keithley, Model 480). Current was measured at voltages ranging from 25 to 200 mV, or until the membrane broke; the conductance at low voltages ($\leq$125 mV) extrapolated by parabolic curve fit to zero voltage was the value used as $G(0)$.

The voltage jump technique was used to determine the effect of 3PI on transport of $TPhB^-$ and DPA^- (Ketterer et al., 1971; Andersen & Fuchs, 1975). The electrodes, which were similar in both techniques, were silver/silver chloride and of surface area $\approx$1.1 cm^2. A voltage pulse was applied across the membrane by a fast-settling digital-to-analog convertor (DAC80, Burr-Brown, Tucson, Ariz.) with the voltage output taken across a resistive load. Membrane current was converted to a voltage by a differential amplifier (LH0062, National Semiconductor, Santa Clara, Calif.) in a virtual ground configuration. A transient recorder (Biomation Model 802, Palo Alto, Calif.) digitized the information and stored it as 8-bit words in a 1,000-word memory. A multiplexer permitted automatic reading of the settings of the transient recorder and transfer of the data by burst mode from the transient recorder through a DRV11-B direct memory access interface to a minicomputer (PDP 11/03, Digital Equipment Corp., Maynard, Mass.). The voltage pulse amplitude, pulse duration, and number of pulses were controlled by computer software (using Laboratory Support Library from DEC) via DRV11 (DEC) interface. The average of a number of pulses was displayed graphically on the computer terminal as current versus time and as logarithm of current versus time, and then curve fit to an exponential decay. In this way the time constant and the intitial current were obtained, and the transferred charge density, $\Delta Q[\Delta Q=I_o\cdot\tau/S$, where S is the geometrical area of the aperture], was computed. The zero voltage conductance, $G_o(0)$, was determined from a parabolic curve fit of the low voltage ($\leq$125 mV) initial conductances. The zero voltage time constant, $\tau(0)$, was also determined from a parabolic curve fit by extrapolating low voltage data ($\leq$125 mV) to zero voltage.

To investigate the possibility of chemical interaction between components of membrane transport system we have done (i) standard thin layer chromatography on mixture of 3PI, DPA and cholesterol, and (ii) spectrophotometric studies (using Beckman DU 7 spectrophotometer) on aqueous solutions of 3PI and DPA.

Results

Cation Conductance and Kinetic Limitation Parameter

The effect of 3PI on transport of the lipophilic cation $TPhA^+$ and on the nonactin-mediated transport of K^+ is illustrated by the data shown

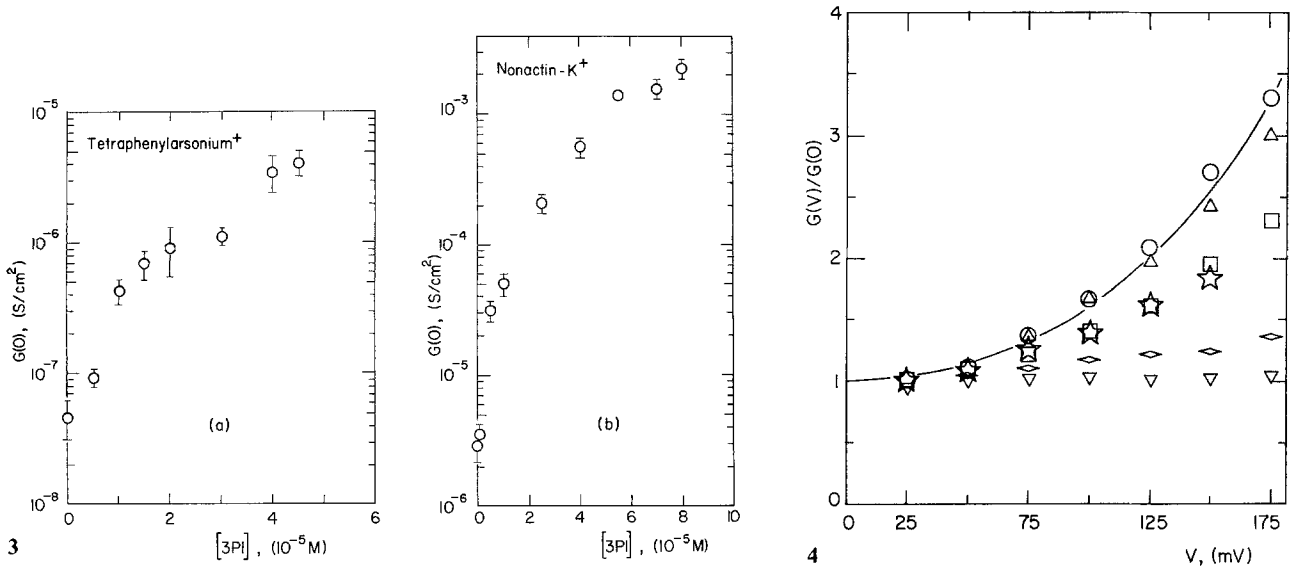

Fig. 3. Cation zero voltage conductance as a function of 3-phenylindole concentration. Aqueous solution also contained 0.1 M KCl buffered to pH 6.9 and either 5×10^{-3} M $TPhA^+$ (*a*) or 10^{-7} M nonactin (*b*)

Fig. 4. Nonactin-K^+ normalized conductance as a function of applied voltage with the indicated concentration of 3-phenylindole in the aqueous solution: o, 0 M; △, 10 μM; □, 25 μM, ☆, 40 μM; ◇, 55 μM, ▽, 80 μM. Solid line is Eq. (3) with $A=0$ and $\omega=0.007$

Table 1. Kinetic limitation parameter A for nonactin-mediated transport of K^+ and tetraphenylarsonium$^+$-induced transport, and βk_i for tetraphenylarsonium$^+$-induced transport, at various concentrations of 3-phenylindole

[3PI] (10^{-5} M)	Parameter A		βk_i for $TPhA^+$ (10^{-7} cm/sec)
	For nonactin-K^+	For $TPhA^+$	
0.0	-0.01 ± 0.02	-0.004 ± 0.012	0.024 ± 0.008
0.05	-0.02 ± 0.03		
0.5	-0.01 ± 0.02	-0.005 ± 0.011	0.048 ± 0.003
1.0	0.01 ± 0.01	-0.005 ± 0.007	0.22 ± 0.04
1.5		0.004 ± 0.010	0.36 ± 0.09
2.0		-0.006 ± 0.007	0.48 ± 0.20
2.5	0.05 ± 0.02		
3.0		0.015 ± 0.012	0.59 ± 0.06
4.0	0.07 ± 0.03	-0.003 ± 0.010	1.90 ± 0.63
4.5		0.012 ± 0.013	2.19 ± 0.46
5.5	0.18 ± 0.03		
7.0	0.20 ± 0.03		
8.0	0.30 ± 0.03		

in Fig. 3. This figure shows the zero voltage conductance of phosphatidylcholine/cholesterol bilayers as a function of aqueous 3PI concentration; error bars are the standard deviation of at least four or eight membranes for $TPhA^+$ or nonactin, respectively. Solutions containing more than 4.5×10^{-5} M 3PI together with 5×10^{-3} M $TPhA^+$, 0.1 M KCl, and buffer were noticeably opaque and thus for $TPhA^+$, the experimental conditions were restricted to 3PI concentrations $\leq 4.5 \times 10^{-5}$ M, in which range a true solution appeared to exist. The most striking feature of the data in Fig. 3 is the large increase in $G(0)$ for both cations with increasing 3PI concentration. The value of $G(0)$ for nonactin in the presence of 8.0×10^{-5} M 3PI was nearly three orders of magnitude greater than in the absence of 3PI.

We found that the enhancement of conductance by 3PI was pH independent over a wide range (data not shown). This result is not unexpected since 3PI is in neutral form except at very high pH.

The normalized conductance for nonactin-mediated transport of K^+ is shown in Fig. 4 as a function of applied voltage for various concentrations of 3PI in the aqueous solution. The membranes had cholesterol mole fraction of 0.22, which implies a thickness of 45 Å (Hanai, Haydon & Taylor, 1965). Using $\omega=0.007$ [based on Table I in Andersen & Fuchs (1975) relating thickness and ω], the data in Fig. 4 were fit to Eq. (3) to obtain the values of parameter A as listed in the second column of Table 1. At small concentrations of 3PI in the aqueous solution, parameter A was near zero, indicating that ion/carrier complex translocation across the membrane was the slowest step in the transport process. At 3PI concentrations of about 2.5×10^{-5} M and greater, parameter A for nonactin-K^+ transport increased, and at the highest concentration of 8.0×10^{-5} M 3PI, parameter A was 0.30, which corresponds to a voltage-

independent conductance (*see* Fig. 4). Hence, we conclude that the presence of 3PI caused strong kinetic limitations to nonactin-mediated transport of K^+ that evolved in parallel with the enhancement of transmembrane K^+ transport.

Also in Table 1 are the values of parameter A for $TPhA^+$ transport in the presence of 3PI, as determined by the best fit of the experimental $G(V)/G(0)$ versus applied voltage (data not shown) to Eq. (3). In the case of $TPhA^+$, for reasons which are still not clear, a value of $\omega = 0$ gave a better fit than a nonzero ω and was used at all 3PI concentrations. It can be seen from Table 1 that the kinetic limitation parameter A for $TPhA^+$ transport remained essentially zero, within experimental error, at all 3PI concentrations. From the definition of parameter A given in Eq. (5), this result implies that 3PI did not measureably change the ratio of the rate constants k_i/k_{ma} and that the translocation step still occurred much more slowly than the adsorption and desorption steps.

The relationship between the zero voltage conductance and the rate constants in the kinetic model of transport is particularly simple for transport for lipophilic cations. Using the zero voltage conductance for $TPhA^+$, the product βk_i was calculated from Eq. (6); the results are listed in the last column of Table 1. Note that βk_i increased from 0.024×10^{-7} cm/sec in the absence of 3PI to 2.19×10^{-7} cm/sec in the presence of 4.5×10^{-5} M 3PI.

Anion Conductance and Kinetic Parameters

The dependence of the zero voltage initial conductance on aqueous 3PI concentration is shown in Fig. 5 for both $TPhB^-$ and DPA^-; error bars are the standard deviation for 4–6 membranes. In contrast to the large changes in conductance observed for the cations, the changes in conductance for $TPhB^-$ and DPA^- can be seen to be relatively small. Furthermore, the conductance induced by the anions decreased in the presence of 3PI, whereas the conductance induced by the cations increased. Results of thin layer chromatography and UV-VIS absorption spectrophotometry on mixture of components of the membrane transport system suggest an absence of any chemical reaction or complexation products.

The transferred charge density as a function of applied voltage was fit to Eq. (7) for several membranes formed in the absence of 3PI, and it was found that the correlation was best when $b = 0.72$ for DPA^- and when $b = 0.83$ for $TPhB^-$, so these values of b were used in all curve fits. For

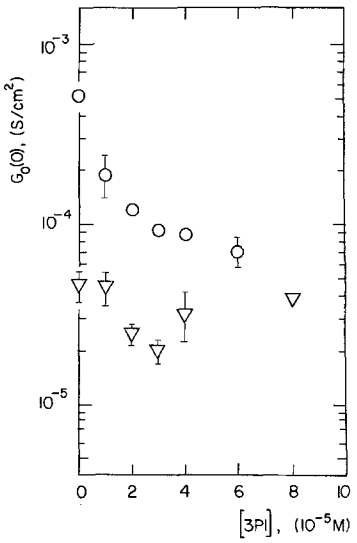

Fig. 5. Lipophilic anion zero voltage initial conductance as a function of 3-phenylindole concentration. Aqueous solution also contained 0.1 M NaCl buffered to pH 6.9 and either 10^{-7} M $TPhB^-$ (▽) or 10^{-8} M DPA^- (○)

$TPhB^-$ the correlation was very good (typically correlation coefficient >0.96) even up to 250 mV, but for DPA^- the correlation was greatly decreased if all voltages were included in the fit. This occurred in spite of the fact that the steady-state current for DPA^- was measured at each voltage and subtracted from the total current so that I_o was only due to transient current relaxation. The low correlation at high voltages is probably related to the existence of some charge transfer mechanism other than lipophilic anion transport (Ketterer et al., 1971). Bruner (1975) investigated DPA^- transport across exceptionally stable synthetic lipid bilayers using large applied voltages, 200–400 mV, and found the current decay at higher voltages to be distinctly nonexponential. In determining Q_{ads} for DPA^- we have used ΔQ only for voltages less than or equal to 175 mV, in which region the fit to Eq. (7) was nearly as good as for $TPhB^-$.

Using the values of b detailed above, Q_{ads} for $TPhB^-$ and DPA^- (and consequently N_{ads}) were determined from Eq. (7) at each 3PI concentration. The partition coefficient was determined from the relationship $\beta = N_{ads}/c$, where c is the concentration of lipophilic anion in the bulk aqueous phase. These values are summarized in Table 2, along with the translocation rate constants obtained according to Eq. (9) from the experimental time constants of transient current decay. Note that the partition coefficients for both $TPhB^-$ and DPA^- are of the same order of magnitude and that they decreased by a factor of two or three in the pres-

Table 2. Partition coefficients (β) and translocation rate constants (k_i) for tetraphenylborate and dipicrylamine transport at various concentrations of 3-phenylindole

[3PI] (10^{-5} M)	β		k_i	
	For DPA^- (10^{-4} m)	For $TPhB^-$ (10^{-4} m)	For DPA^- (sec^{-1})	For $TPhB^-$ (sec^{-1})
0.0	1.53±0.11	1.07±0.22	1,300±136	14.9±1.20
1.0	0.97±0.21	1.46±0.37	800± 95	11.3±0.63
2.0	0.95±0.07	0.58±0.08	410± 59	14.0±0.66
3.0	0.84±0.09	0.36±0.07	365± 37	18.2±1.17
4.0	0.85±0.10	0.70±0.17	335± 26	16.7±0.44
6.0	0.57±0.06		385± 69	
8.0		0.52±0.05		24.5±4.56

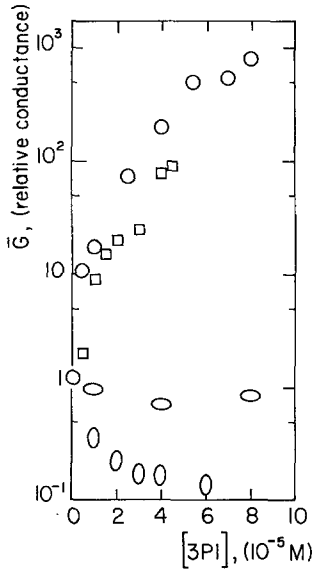

Fig. 6. Relative conductance as a function of 3-phenylindole concentration for the indicated probes: o, nonactin-K^+; □, $TPhA^+$; ⬭, $TPhB^-$; 0, DPA^-

Table 3. Electrostatic factor, $\exp(-F\Delta V_D/RT)$, and nonelectrostatic factor, $f(x)$, derived from changes of membrane conductance due to lipophilic ion transport at various concentrations of 3-phenylindole[a]

Anion	[3PI] (10^{-5} M)	$\exp(-F\Delta V_D/RT)$	$f(x)$	ΔV_D (−mV)
DPA^-	1.0	5.1	1.8	41
	2.0	9.3	2.1	56
	3.0	11.6	2.1	62
	4.0	21.6	3.6	77
$TPhB^-$	1.0	3.1	3.0	28
	2.0	6.1	3.2	46
	3.0	7.5	3.3	51
	4.0	10.6	7.4	60

[a] The factors $\exp(-F\Delta V_D/RT)$ and $f(x)$ were found by using Eqs. (11) and (12), respectively.

ence of 3PI. In contrast to the partition coefficients, the translocation rate constants of DPA^- and $TPhB^-$ differ by several orders of magnitude. Generally, k_i for $TPhB^-$ remained about the same in the presence of 3PI whereas k_i for DPA^- decreased.

Comparison of Electrostatic and Nonelectrostatic Factors

As a summary of the experimental results, we present in Fig. 6 the relative conductance for each of the probes as a function of aqueous 3PI concentration. Figure 6 shows clearly the marked asymmetry between the effect of 3PI on positively and negatively charged probes. Although there are some stringent restrictions on the applicability of the analysis used to separate electrostatic and nonelectrostatic factors contributing to the relative conductance, we show in Table 3 the results of such an analysis as the basis for one tentative explanation of the action of 3PI. In all cases shown in Table 3, $\bar{G}^+$ was calculated from $TPhA^+$ steady-state conductance; $\bar{G}^-$ was calculated separately for $TPhB^-$ and DPA^-. The electrostatic and nonelectrostatic factors were determined from Eqs. (11) and (12), respectively. It should be kept in mind that $TPhA^+$-induced conductance in the absence of 3PI was small ($\approx 5\times10^{-8}$ S/cm^2) and the error in this measurement large ($\approx 1.5\times10^{-8}$ S/cm^2), which means that the reference conductance [$G^*(0)$ in Eq. (10)] contributed a relatively large error. For this reason the values in Table 3 should be treated only as approximations. Nevertheless, it is possible to deduce several trends in the data. Most importantly, the electrostatic factor, $\exp(-F\Delta V_D/RT)$, was the same order of magnitude as the nonelectrostatic factor, $f(x)$, for $TPhB^-$ as the lipophilic anion. With DPA^- as the anion, both factors increased with increasing 3PI concentration, but the electrostatic factor increased to a greater extent. The additional dipole potential difference, ΔV_d, according to this model, ranged from −28 to −77 mV, and was of comparable magnitude for both lipophilic anions at each 3PI concentration.

Discussion

The main result of this study is the finding that biologically active 3-phenylindole has a dramatic effect on membrane ionic permeability, *viz.* large increase of cationic and small decrease of anionic membrane conductance. A similar effect was observed with the neutral form of phloretin incorpo-

rated in cholesterol-containing phosphatidylcholine or phosphatidylethanolamine membranes (Andersen et al., 1976). Asymmetry between the results with positively and negatively charged probes has also been found when the ionic probes were used to detect the "boundary" potential produced by tetraphenylborate$^-$ and picrate$^-$ (Andersen et al., 1978) or to determine the effect of changes in structure and cholesterol content of membranes (Pickar & Benz, 1978).

The asymmetry of the action of membrane modifier on membrane conductance due to positively and negatively charged ions has been interpreted in terms of the combined effect of dipolar potential difference and bilayer fluidity changes (Andersen et al., 1976; Pickar & Benz, 1978). The results of the present study on the membrane effect of 3PI are consistent with the above interpretation. The "electrostatic" factor given in Table 3 is readily explained as due to an additional dipolar potential difference with the interior of membrane becoming more negative (with respect to the bulk aqueous phase) when 3PI is present. Although we do not have data on the dipole moment of 3PI, indole, the parent molecule of 3PI, has a dipole moment of 2.3 debye (McClellan, 1974). It is likely that neutral 3PI has a dipole moment of comparable magnitude. The applicability of the dipolar hypothesis to 3PI action in lipid membranes is supported by the results of surface potential measurements on lipid monolayers (Sinha, 1981). These measurements indicated that the electric potential of lipid monolayers decreased in the presence of 3PI, which can in part be due to the dipole moment of 3PI incorporated into the monolayer, and in part due to the decreased surface density of dipolar lipid molecules. The fact that the partition coefficient of TPhB$^-$ and DPA$^-$ decreased only slightly in the presence of 3PI (*see* Table 2) suggests that the location of the 3PI adsorption plane is on the membrane interior side with respect to the adsorption plane of lipophilic anions.

One of the major quantities determining membrane conductance is the ion translocation rate constant k_i, which in turn depends on the ion potential energy profile, membrane thickness, and the diffusion constant. Since we find the degree of membrane conductance enhancement for positive ions greater than the degree of conductance suppression for negative ions, we can assume that 3PI changes both the "electrostatic" and "nonelectrostatic" factors. It follows from Eq. (10) that a 3PI-induced dipolar change by itself would result in an equal degree of enhancement and suppression for positive and negative ions, respectively. In contrast, 3PI-induced changes of membrane thickness, diffusion constant, or change in membrane dielectric constant would alter equally and in the same direction both the cationic and anionic membrane conductance. So the assumption of existence of two competing factors is required to explain the asymmetry of action of 3PI in membranes as well as the small effect of 3PI on the translocation constant k_i of anions (Table 2). For the positive ions the effects of 3PI-induced membrane changes add up, whereas for the negative ions they subtract.

Analysis of changes in cationic transport is complicated by the observation that TPhA$^+$, in contrast to the lipophilic anions, seems to be more sensitive to changes in membrane fluidity (Pickar & Benz, 1978). In terms of the present approach the data suggest that the 3PI-related "nonelectrostatic" and "electrostatic" factors complement each other for cationic membrane transport, both of them enhancing conductivity of membrane. In connection with this viewpoint, note that the relative conductance of nonactin-K$^+$ and TPhA$^+$ were influenced to nearly the same extent by 3PI (see Fig. 6), so that it is not necessary to postulate that the adsorption plane of nonactin is in a different location than the adsorption plane of the lipophilic ions, as discussed by Benz and Gisin (1978) for the PV-K$^+$ complex. Our finding that 3PI causes a kinetic limitation for the nonactin-mediated transport of K$^+$ is of interest, and further studies would be necessary to determine which kinetic steps are involved.

The theory that the asymmetry of the effect of 3PI as deduced by positively and negatively charged probes is due to a combination of two membrane-related factors is satisfying, but it is important to realize that the theory neglects the possible significance of discrete charge and discrete dipole effects. The fact that we observed comparable effects for two structurally unrelated cations and anions suggests that the specific interactions do not dominate the effect of 3PI in our bilayer membranes.

Andersen et al. (1978) have offered an explanation for the asymmetric conductance effect observed with positively and negatively charged probes which requires only consideration of electrostatic interactions. They propose that the asymmetry is due, at least in part, to discrete charge effects. They treat adsorbed TPhB$^-$ ions as a fixed charged lattice and derive an expression for the micropotential, as distinct from macropotential measured in a typical monolayer experiment (*see also* Wang & Bruner, 1978*b*). In a bilayer, the

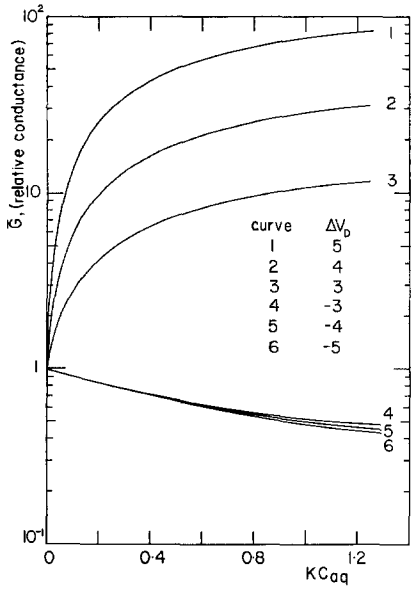

Fig. 7. Dependence of relative membrane conductance on aqueous concentration of membrane modifier according to membrane patch model. The computed results illustrate the asymmetry of the effect of membrane modifier on cationic (upper set of curves) and anionic (lower set of curves) membrane conductance. The dipolar potential difference ΔV_D measured in units of RT/F, was used as the variable parameter

change in ion potential energy along a plane in the middle of membrane, due to discreteness of charges at the membrane surface, is not uniform, which leads to differential transport of cationic and anionic probes (Andersen et al., 1978).

An alternative explanation for the asymmetry of 3PI effect on cationic and anionic conductance can be based on the assumption that 3PI induces localized regions in membrane with altered transmembrane ionic potential energy profile. These regions can be associated either with individual 3PI molecules adsorbed and oriented within the interfacial regions, or with patches, or domains of lipid bilayer enriched with 3PI. Such patches have been proposed to explain permeability changes of phosphatidylcholine vesicles in the presence of tetrachlorosalicylanilide, which is known to be an effective germicide (Barratt & Weaver, 1979). Results of ESR studies with spin-labeled fatty acids have indicated the presence of highly ordered regions, rich in tetrachlorosalicylanilide, whose boundaries were associated with the increase of vesicle permeability to paramagnetic cation tempocholine.

Suppose that the effect of 3PI is only of dipolar nature and that within each patch the transmembrane barrier height was changed by $ze\Delta V_D$. The patch conductance then becomes proportional to a Boltzmann factor, $\exp(-zF\Delta V_D/RT)$, and changes with ion polarity in opposite directions. As a result the membrane consists of two types of regions: normal and altered. With the increasing concentration of membrane modifier in the aqueous phase the area covered by patches of altered ionic permeability increases. We assume that the fractional patch area, FPA, increases according to Langmuir adsorption isotherm

$$\text{FPA} = KC_{aq}(1-\text{FPA}) \tag{13}$$

where C_{aq} is the aqueous concentration of 3PI. K is a constant whose value corresponds to the reciprocal of 3PI concentration at which half of the membrane surface is covered by patches of altered membrane permeability.

Membrane conductance is therefore given by the sum of the conductances due to unaltered membrane, $G_0(1-\text{FPA})$, and due to that of patches, FPA $G_0 \exp(-zF\Delta V_D/RT)$.

The relative membrane conductance $\bar{G}$ is equal to

$$\bar{G} = 1 + \text{FPA}\ [\exp(-zF\Delta V_D/RT) - 1]. \tag{14}$$

The power of this membrane patch model to explain the asymmetric effect of 3PI on cationic and anionic membrane conductance is illustrated in Fig. 7. In this figure we plot the relative membrane conductance versus KC_{aq} for positively charged ($z=1$) and negatively charged ($z=-1$) ions for ΔV_D equal to integral multiples of RT/F. The computed results exhibit the basic features of our experimental results (*compare* with Fig. 6), namely greater enhancement of cationic and smaller suppression of anionic conductance in the presence of 3PI.

It was appropriately pointed out by the reviewer that the corollary of a patch hypothesis is the existence of two relaxation processes in experiments with lipophilic anions. In Table 2 we presented data for single translocation rate constants corresponding to transients with only one relaxation time. To illuminate this apparent discrepancy we discuss below the time dependence of current transients in membranes with patches and show by means of a numerical example that our kinetic observations are also consistent with the patch hypothesis.

The transient current density $J_m(V, t)$ predicted for the patched membrane is expected to have two components, one associated with the patches and one for normal membrane regions:

$$J_m(V, t) = \text{FPA} \cdot J_p(V) \exp(-t/\tau_p) + (1-\text{FPA}) \cdot J_n(V) \exp(-t/\tau_n). \tag{15}$$

$J_p(V)$ and $J_n(V)$ are the initial current densities through the patch and through the normal mem-

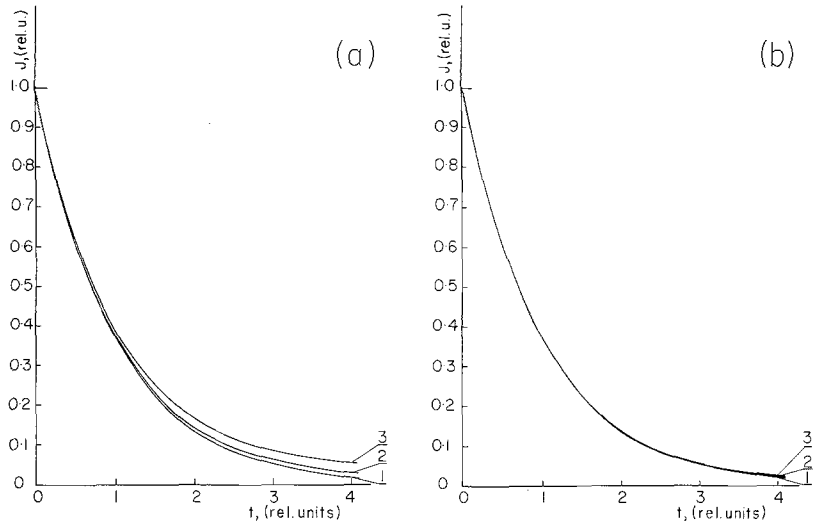

Fig. 8. Time dependence of transient membrane current predicted from membrane patch model (Eq. 17). To illustrate the effect of membrane modifier on transport kinetics we have normalized the initial current to one and used linear scales as in standard current relaxation experiments. Two cases corresponding to different ΔV_D are considered: (*a*) $\Delta V_D = 3RT/zF$, and (*b*) $\Delta V_D = 5RT/zF$. Solid curves indicate different concentrations of membrane modifier, (*1*) unmodified membrane, $KC_{aq} = 0$, (*2*) $KC_{aq} = 1/3$, and (*3*) $KC_{aq} = 1$. Time constant for the unmodified membrane regions was equal to one in all cases

brane regions, and τ_p and τ_n are the corresponding time constants. Assuming that the potential energy of an ion diffusing through the patch differs by $ze\Delta V_D$ from that in the normal region, we have

$$J_p(V) = J_n(V) \cdot \exp(-zF\Delta V_D/RT) \tag{16a}$$

and

$$\tau_p(V) = \tau_n(V) \cdot \exp(zF\Delta V_D/RT). \tag{16b}$$

Finally, the ratio of current densities is equal to

$$J_m(V, t)/J_n(V, t) = \exp(-t/\tau_n) + \mathrm{FPA}[\exp(-zF\Delta V_D/RT) \cdot \exp(-t/\tau_p) - \exp(-t/\tau_n)]. \tag{17}$$

Equation 17 indicates that the current decay through the patched membrane is not exponential. To illustrate the effect, we present in Fig. 8*a* and *b* the time dependencies predicted from Eq. (17) for $zF\Delta V_D$ equal to $3RT$ and $5RT$. Note that for larger $|\Delta V_D|$ (Fig. 8*b*) the membrane current decay remains exponential and does not change with the increasing concentration of membrane modifier. For smaller $|\Delta V_D|$ (Fig. 8*a*) the deviations from the exponential decay become noticeable only at low current levels and may be very easily obscured by noise. In both cases the membrane current is dominated by that passing through normal regions, and thus the existence of patches results primarily in the reduction of membrane conductance. It should be noted that the meaning of ΔV_D in membrane patch model is different from that implied in the model involving "electrostatic" and "nonelectrostatic" factors. In the former, ΔV_D is associated with discrete patches, whereas in the latter it corresponds to a quantity averaged over the membrane surface.

In conclusion, we have shown that 3PI, a compound that inhibits growth of fungi and Gram-positive bacteria, has the ability to alter kinetics of ion transport in bimolecular lipid membranes and that the effects appear to be related primarily to the change of transmembrane translocation rate constant. The action of 3PI can be understood in terms of changes of 3PI-related uniform dipolar potential differences at the membrane-water interface taking place simultaneously with changes in fluidity of the membrane interior. An alternative explanation rests on the assumed existence of 3PI-related patches of altered membrane permeability due to local dipolar potential difference without the need to invoke changes of membrane fluidity. The action of 3PI in lipid bilayers is in some respects similar to that of another bactericidal agent, tetrachlorosalicylanilide, and we suggest that ESR and fluorescence depolarization studies be done to find out whether 3PI-induced clustering in lipid bilayers occurs, which would help to clarify the applicability of the patch hypothesis.

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References

Andersen, O.S., Feldberg, S., Nakadomari, H., Levy, S., McLaughlin, S. 1978. Electrostatic interactions among hydrophobic ions in lipid bilayer membranes. *Biophys. J.* **21**:35–70

Andersen, O.S., Finkelstein, A., Katz, I., Cass, A. 1976. Effect of phloretin on the permeability of thin lipid membranes. *J. Gen. Physiol.* **67**, 749–771

Andersen, O.S., Fuchs, M. 1975. Potential energy barriers to ion transport within lipid bilayers. *Biophys. J.* **15**:795–830

Barratt, M.D., Weaver, A.C. 1979. The interaction of 3,3′,4′,5-tetrachlorosalicylanilide with phosphatidylcholine bilayers. *Biochim Biophys. Acta* **555**:337–348

Benz, R., Gisin, B.F. 1978. Influence of membrane structure on ion transport through lipid bilayer membranes. *J. Membrane Biol.* **40**:293–314

Bruner, L.J. 1975. The interaction of hydrophobic ions with lipid bilayer membranes. *J. Membrane Biol.* **22**:125–141

Cousin, J. L., Motais, R. 1978. Effect of phloretin on chloride permeability: A structure-activity study. *Biochim. Biophys Acta* **507**:531–538

Dekker, W.H., Selling, H.A., Overeem, J.O. 1975. Structure-activity relationships of some antifungal indoles. *J. Agric. Food Chem.* **23**:785–791

Fischer, E., Schmidt, T. 1888. Ueber Pr. 3-Phenylindol. *Berichte Deutschen Chemischen Gesellschaft* **21**:1811–1812

Hanai, T., Haydon, D.A., Taylor, J. 1965. The variation of capacitance and conductance of biomolecular lipid membranes with area. *J. Theor. Biol.* **9**:433–443

Haydon, D.A., Myers, V.B. 1973. Surface charge, surface dipoles and membrane conductance. *Biochim. Biophys. Acta* **307**:429–443

Hladky, S.B. 1972. The steady-state theory of the carrier transport of ions. *J. Membrane Biol.* **10**:67–91

Hladky, S.B. 1973. The effect of stirring on the flux of carriers into black lipid membranes. *Biochim. Biophys. Acta* **307**:261–269

Hladky, S.B. 1975. Tests of the carrier model for ion transport by nonactin and trinactin. *Biochim. Biophys. Acta* **375**:327–349

Hladky, S.B. 1979. The carrier mechanism. *In:* Current Topics in Membranes and Transport. F. Bronner and A. Kleinzeller, editors. pp. 53–164. Academic Press, New York

Hoppe, H.H., Kerkenaar, A., Sijpesteijn, A.K. 1976*a*. On the mode of action of 3-phenylindole towards *Aspergillus niger*. *Pest. Biochem. Physiol.* **6**:413–421

Hoppe, H.H., Kerkenaar, A., Sijpesteijn, A.K. 1976*b*. Interaction with phospholipids as a possible mode of action of 3-phenylindole on *Aspergillus niger*. *Pest Biochem. Physiol.* **6**:422–429

Ketterer, B., Neumcke, B., Läuger, P. 1971. Transport mechanism of hydrophobic ions through lipid bilayer membranes. *J. Membrane Biol.* **5**:225–245

Liberman, Y.A., Topaly, V.P. 1969. Permeability of bimolecular phospholipid membranes for fat-soluble ions. *Biophysics* **14**:477–487

McClellan, A.L. 1974. Tables of Experimental Dipole Moments. Vol. 2. p. 288. Rahara Enterprises, El Cerrito (Calif.)

McLaughlin, S. 1973. Salicylates and phospholipid bilayer membranes. *Nature (London)* **243**:234–236

McLaughlin, S., Eisenberg, M. 1975. Antibiotics and membrane biology. *Annu. Rev. Biophys. Bioeng.* **4**:335–366

McLaughlin, S., Dilger, J.P. 1980. Transport of protons across membranes by weak acids. *Physiol. Rev.* **60**:825–863

Mueller, P., Rudin, D.O., Tien, H., Wescott, W.C. 1963. Methods for the formation of single bimolecular lipid membranes in aqueous solution. *J. Phys. Chem.* **67**:534–535

Owen, J.D. 1974. The effect of phloretin on the potassium conductance in *Aplysia* giant neurons. *J. Membrane Biol.* **16**:65–78

Pickar, A.D., Benz, R. 1978. Transport of oppositely charged lipophilic probe ions in lipid bilayer membranes having various structures. *J. Membrane Biol.* **44**:353–376

Sinha, B. 1981. Physical effects of 3-phenylindole on ion transport across bilayer lipid membranes. Ph.D. Dissertation. Portland State University, Portland, Oregon

Smejtek, P., Paulis-Illangasekare, M. 1979*a*. Modification of ion transport in lipid bilayer membranes in the presence of 2,4-dichlorophenoxyacetic acid (2,4-D). I. Enhancement of cationic conductance and changes of the kinetics of nonactin-mediated transport of potassium. *Biophys. J.* **26**:441–466

Smejtek, P., Paulis-Illangasekare, M. 1979*b*. Modification of ion transport in lipid bilayer membranes in the presence of 2,4-dichlorophenoxyacetic acid (2,4-D). II. Suppression of tetraphenylborate conductance and changes of interfacial potentials. *Biophys. J.* **26**:467–488

Stark, G., Benz, R. 1971. The transport of potassium through lipid bilayer membranes by the neutral carriers valinomycin and monactin. *J. Membrane Biol.* **5**:133–153

Szabo, G. 1974. Dual mechanism for the action of cholesterol on membrane permeability. *Nature (London)* **257**:47–49

Szabo, G. 1976. The influence of dipole potentials on the magnitude and the kinetics of ion transport in lipid bilayer membranes. *In:* Extreme Environments: Mechanisms of Microbial Adaptation. M.R. Heinrich, editor. pp. 321–348. Academic Press, New York

Szabo, G., Eisenman, G., McLaughlin, S.G.A., Krasne, S. 1972. Ionic probes of membrane structures. *Ann. N. Y. Acad. Sci.* **193**:273–290

Wang, C.C., Bruner, L.J. 1978a. Dielectric saturation of the aqueous boundary layer adjacent to charged bilayer membranes. *J. Membrane Biol.* **38**:311–331

Wang, C.C., Bruner, L.J. 1978*b*. Evidence for a discrete charge effect within lipid bilayer membranes. *Biophys. J.* **24**:749–764

Wieth, J.O., Dalmark, M., Gunn, R.Z., Tosteson, D.C. 1973. The transfer of monovalent inorganic anions through the red cell membrane. *In:* Erythrocytes, Thrombocytes, Leucocytes. E. Gerlach, K. Moses, E. Deutsch, and W. Wilmanns, editors. pp. 71–76. Georg Thieme Verlag KG, Stuttgart

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