Biochimica et Biophysica Acta, 1070 (1991) 474–480

BBAMEM 75445

Effect of phloretin on the carrier-mediated electrically silent ion fluxes through the bilayer lipid membrane: measurements of pH shifts near the membrane by pH microelectrode

Yuri N. Antonenko [1] and Alexander A. Bulychev [2]

[1] *A.N. Belozersky Laboratory, Moscow State University, Moscow (U.S.S.R.) and* [2] *Biophysical Department, Moscow State University, Moscow (U.S.S.R.)*

(Received 21 May 1991)

Key words: Nigericin; Tributyltin; Phloretin; Bilayer lipid membrane; Dipole potential; Ionophore

The effect of phloretin on the carrier-mediated electrically silent ion fluxes through the bilayer lipid membrane (BLM) was studied. The measurements were carried out according to our conventional technique, i.e. electrical potential recording in the presence of a protonophore, and by a new method – direct measurements of pH shifts in the unstirred layers of the BLM by pH microelectrode. Both techniques gave similar results. It was shown that the addition of phloretin increased the rate of cation/H^+ exchange induced by nigericin and decreased the rate of anion/OH^-- exchange induced by tributyltin. The effect of phloretin was higher in the presence of cholesterol in the BLM. Cholesterol decreased the nigericin- and tributyltin-induced fluxes under our experimental conditions. The application of an external voltage to the membrane had no effect on the ion fluxes thereby showing that these fluxes were electroneutral. The most probable explanation of these results bases on the effect of the membrane dipole potential on the electroneutral fluxes of ions. The possible mechanism of the dipole potential effect on the carrier-mediated electrically silent ion fluxes was discussed in terms of two competing hypotheses – the translocation through the membrane or the reactions at the membrane surface being the rate-limiting steps of the whole transport process.

Introduction

It was shown previously that phloretin dramatically increases the rate of cation and decreases the rate of anion translocation through artificial and natural membranes [1–3]. This effect of phloretin is a result of reduction of the electric potential difference between the interior of the membrane and its surfaces which is called a dipole potential [1,2,4]. The existence of the dipole potential demonstrates itself in the difference of the membrane permeabilities to cations and anions which can reach as high as 9 orders of magnitude [1]. The value of the dipole potential differs from one lipid to another and was estimated as 400 mV for phosphatidylcholine and phosphatidylethanolamine [5]. Phloretin decreased the dipole potential by 150–200 mV [1]. Cholesterol besides its effect on the membrane viscosity can increase the value of the dipole potential [6].

The nonelectrogenic ionophores such as nigericin (K^+/H^+ antiporter) or tributyltin (Cl^-/OH^- antiporter) facilitate the transfer of ions through membranes in a form of electroneutral complexes [7–9]. This implies that contrary to the electrogenic transport one does not expect the effect of phloretin in the case of nonelectrogenic transport. However, the complexes of nigericin-cation as well as of tributyltin-anion due to their structure are supposed to contain an electrical dipole moment which may interact with the dipole potential of the membrane. Therefore the transport rate of these complexes may change upon variation of the dipole potential. On the other hand, the influence

Abbreviations: TTFB, tetrachlorotrifluoromethylbenzimidazole, a protonophore; CCCP, carbonyl cyanide *m*-chlorophenyl hydrazone, a protonophore; Mes, 2-(*N*-morpholino)ethanesulfonic acid; J_H, the electrically silent hydrogen ion flux through the membrane; BLM, bilayer lipid membrane.

Correspondence: Yu.N. Antonenko, Department of Bioenergetics, A.N. Belozersky Laboratory, Moscow State University, Moscow, 119899, U.S.S.R.

of the dipole potential on the ion during association with or dissociation from the carrier at the interface may take place also.

This paper deals with the action of phloretin on the rate of the carrier-mediated electroneutral transport. The measurements of the ion transport were carried out partly in a way traditional for our laboratory [10,11] and by a new technique – direct measurements of pH shifts near the BLM by pH microelectrode. It was shown that the decrease of the dipole potential led to the decrease of the rate of Cl^-/OH^- exchange and the increase in the rate of K^+/H^+ exchange.

Materials and Methods

BLM was formed on a Teflon partition 0.4 mm in diameter, by a conventional method [12]. A membrane-forming solution contained 20 mg phosphatidylcholine from soybeans (Sigma) and 10 mg cholesterol (Serva) in 1 ml of *n*-decane unless otherwise stated. The thinning of the BLM was observed both visually and by measuring its capacity. The main element of the electrical scheme was a Keithley 301 amplifier. The experiments were carried out at room temperature (21–23 °C). Ethanol solutions of nigericin, monensin, calcimycin, ionomycin, lasalocid A (all from Calbiochem), tributyltin (Serva), valinomycin (Sigma), protonophores carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP, Sigma) and tetrachlorotrifluoromethylbenzimidazole (TTFB, a gift of Prof. E.A. Liberman) were added at both sides of the BLM. Phloretin, phlorezin and buffers were from Serva. Other chemicals were from Reachim (U.S.S.R.).

The hydrogen ion electroneutral flux (J_H) was measured by the method described earlier [10,11]. The pH gradient in the unstirred layers was determined from the difference of the electrical potentials on BLM in the presence of a protonophore in the open circuit mode. It was shown that under these conditions the J_H flux is proportional to the membrane potential [10]. J_H was calibrated against the potential by adding the increasing concentrations of sodium acetate at different pH values as described earlier [10].

In the other set of experiments the direct measurements of pH shifts near the BLM were carried out. A pH microelectrode with a tip of about 3 μm was made according to Remis, Bulychev and Kurella [13]. Typically the slope of the electrode was 50 mV/pH. This method had been applied previously for the measurements of pH shifts near the surface of an immobilized chloroplast [14]. Fig. 1A presents a scheme of mutual positions of a pH microelectrode and a membrane. The electrode was brought close to the membrane through a hole in a side window made of plexiglas. To prevent the solution leakage the empty space between the electrode shank and the hole rim was filled with

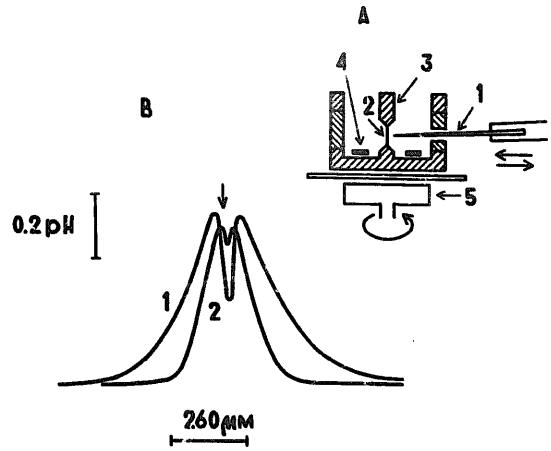

Fig. 1. (A) The scheme of mutual positions of the pH microelectrode (1) and the membrane (2). 3, A cell support; 4, a magnetic bar; 5, a rotating magnet. The directions of the electrode movements are indicated by arrows. (B) The pH profile near the BLM measured upon bringing the electrode close to it (left side till the arrow) and taking the electrode away from it (after the arrow) after the addition of 20 mM KCl at the side of the BLM opposite to the electrode and in the presence of 0.1 μM nigericin. The pH shift was alkaline. Curves 1 and 2 were measured with and without stirring the solution, respectively. The solution was 1 mM Tris, 1 mM Mes, 100 mM choline chloride (pH 6.6). The electrode movement speed was 6 μm/s.

vaseline. Fig. 1B shows a pH profile near BLM in the presence of 0.1 μM nigericin and 20 mM KCl at the side of the membrane opposite to the electrode side. Smooth movements of the electrode were performed by a hydraulic system based on the step-wise motor with a step of 1 μm. The existence of a pH shift near the surface of the BLM was claimed in a number of works (Refs. 15,10; and for a review, see Ref. 16). However, as far as we know this was the first direct experimental detection of this phenomenon. Fig. 2B shows that as the electrode approached the membrane (curve till the arrow) at a speed of 6 μm/s, the pH started to increase in accord with the scheme of K^+/H^+ exchange. However, after a certain distance the electrode movement brought about a steep change in pH electrode potential of opposite sign. Further approach of the electrode led usually to the BLM breakdown. A phase of the apparent pH decrease was observed also in the absence of KCl gradient and extended for about 10 μm from the peak of pH profile (data not shown). It may be concluded that this phase was due to the interaction of the electrode with the membrane and was not associated with a pH change near the membrane. At the moment marked by an arrow the withdrawal of the electrode started. The symmetry of the curves presented in Fig. 1B gave evidence that the movement of the electrode had a negligible effect on the unstirred layer. It is seen from curve 2, Fig. 1B that the stirring of the solution reduced the thickness of the diffusion layer significantly.

Linear approximation of the pH profile in the presence of the solution stirring with the limit at the pH maximum gave the estimation of the unstirred layer thickness of 130μm. This value is in good agreement with literature data derived from permeability measurements [15]. The addition of up to 20 μM TTFB did not change the pH shift (data not shown). The measurement of the BLM potential under these conditions in the presence of TTFB protonophore gave 46 mV which corresponded to $\Delta pH = 46/58 = 0.8$ while the estimation of the pH shift at one side of the membrane from Fig. 1B, curve 2 gave $\Delta pH = 24/50 = 0.48$. The latter value is roughly two times lower than the former which summed pH shifts at two sides of the BLM. These results confirmed the conclusion previously made that the BLM potential in the presence of a protonophore was accounted for by pH changes in the unstirred layers.

Results

Fig. 2 shows the effect of phloretin on the rate of K^+/H^+ exchange induced by nigericin (Fig. 2B) and on the rate of Cl^-/OH^- exchange induced by tributyltin (Fig. 2A). The measurements were carried out by the method of the potential recording in the presence of TTFB. It is seen that the addition of phloretin increased nigericin-induced potential (stimulation 2.7 and 1.4 times in the presence and in the absence of cholesterol, respectively) and decreased the tributyltin-induced potential (the inhibition being 3.5- and 2.1-times in the presence and in the absence of cholesterol, respectively). The comparison of curves 1 and 2, Fig. 2 and curves 3 and 4 shows that in order to attain the same potential as in the presence of cholesterol, higher concentrations of ionophores should be added compared to the membrane without cholesterol. Other experiments showed that the addition of cholesterol to the membrane-forming solution (the concentration is indicated in Fig. 2) reduced tributyltin-induced flux two times and nigericin-induced flux eight times. The addition of 10 mM $CaCl_2$ had no effect on the effect of phloretin in the case of nigericin (data not shown). The electrical current measurements showed that the addition of phloretin decreased the TTFB-induced current about 100 times. This observation indicated that the BLM could lose the proton selectivity in the presence of phloretin and the potential would not reflect the electroneutral hydrogen ion flux through the membrane. To exclude the effect of phloretin on the TTFB permeability from the analysis of the data, we measured pH shifts independently with the help of pH microelectrode.

Fig. 3A shows a pH profile near the BLM which was induced by nigericin in the presence of KCl gradient. The addition of phloretin increased a pH shift four times (curve 2). Fig. 3B shows the effect of phloretin on the Cl^-/OH^- exchange. Due to the opposite direction of the H^+ flux in this system the direction of the steep electrode -membrane response was the same as

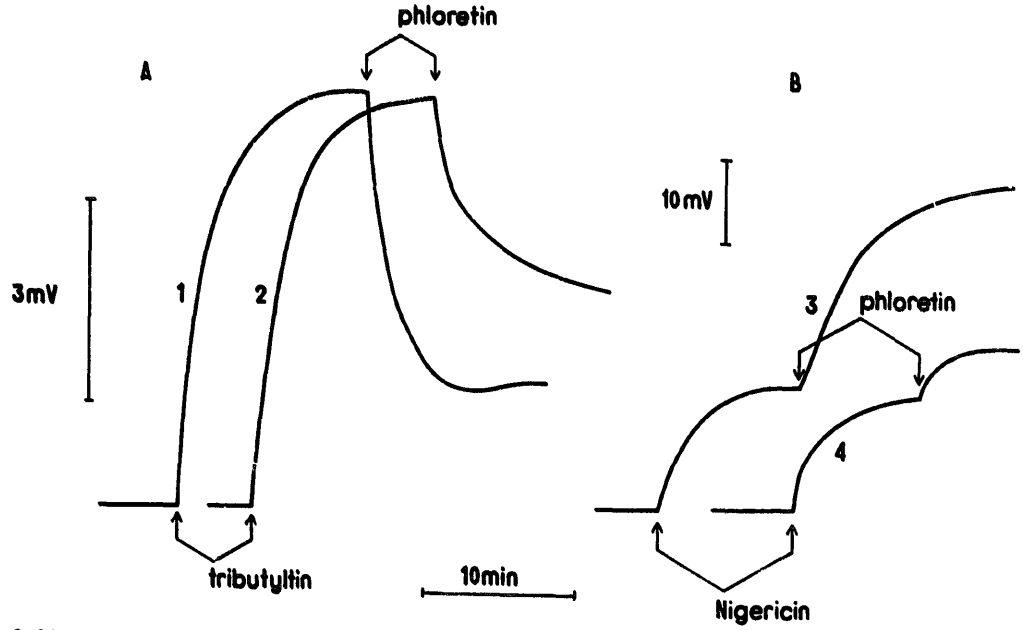

Fig. 2. The effect of phloretin (260 μM) on the rate of Cl^-/OH^- (A) and K^+/H^+ (B) exchange measured by means of the potential recording in the presence of a protonophore TTFB (10 μM). The membranes were formed from the decane solution of phosphatidylcholine and cholesterol 20 and 10 mg/ml (curves 1 and 3) and from phosphatidylcholine only (20 mg/ml, curves 2 and 4). (A) The concentration of tributyltin was 6 μM (curve 1) and 3 μM (curve 2); the solution was 1 mM Tris, 1 mM Mes, 1 mM β-alanine, (pH 7); KCl gradient was 80/50 mM; the potential had plus sign where the concentration of KCl was higher. (B) The concentration of nigericin was 66 nM (curve 3) and 10 nM (curve 4); the solution was as in the caption to Fig. 1; KCl gradient, 10/0 mM; the potential had a plus sign where the concentration of KCl was lower.

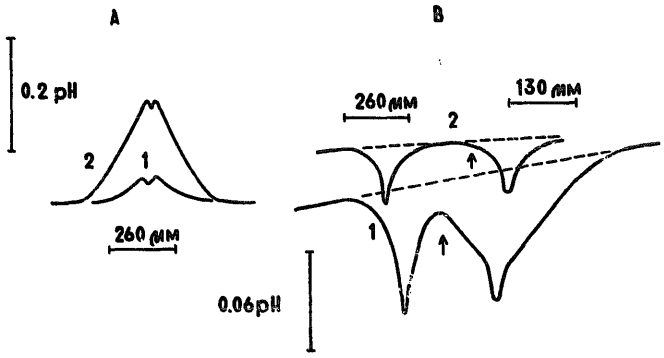

Fig. 3. The effect of phloretin (control, curves 1; 260 μM phloretin, curves 2) on the rate of Cl^-/OH^- (B) and K^+/H^- (A) exchange measured by means of pH-shift recording with a pH microelectrode. The conditions were the same as in Fig. 2, curves 1 and 3; the data presented as in the Fig. 1. The electrode movement speed was 6 μm/s, at the right side after the arrow on B the speed was 3 μm/s. The measurements were carried out under the conditions of stirring the solution.

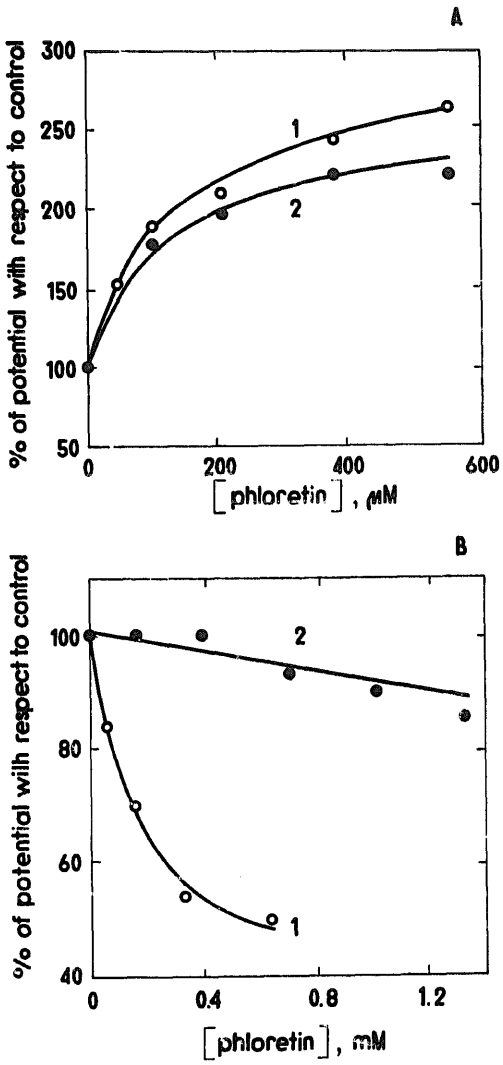

Fig. 4. The dependence of the rate of Cl^-/OH^- (A) and K^+/H^+ (B) exchange measured by means of the potential recording in the presence of a protonophore on the concentration of phloretin at two pH values, 6.4 (curve 1) and 8.0 (curve 2). The conditions were the same as in the caption to Fig. 2 (curves 1 and 3).

that of the pH shift. Fig. 3B shows two approaches to the membrane – one with usual speed and the other (after an arrow) with two times lower speed (3 μm/s) for a precise determination of the breakpoints. Phloretin reduced the pH shift about four times in agreement with the value obtained by the previous technique. These experiments confirmed the opposite directions of the effect of phloretin on cation and anion fluxes.

The dependencies of the BLM potential on the concentration of phloretin at pH 6.4 and 8 are shown in Fig. 4. These dependencies were similar in the case of nigericin while there was a significant difference in the case of tributyltin: at pH 8 higher concentrations of phloretin are needed to decrease the potential to the same extent as at pH 6.4.

Fig. 5 shows the effect of phlorezin, an impermeable analogue of phloretin [17], on the nigericin-induced K^+/H^+ exchange. Phlorezin was most effective in the case of its addition at both sides of the membrane while the addition at one side increased the flux only by 40% irrespective of the flux direction.

The measurement of the pH shift by the pH microelectrode proved to be a convenient system for evaluation of the effect of the membrane electrical potential on the rate of Cl^-/OH^- and K^+/H^+ exchange. The traces of pH shifts near the membrane are presented

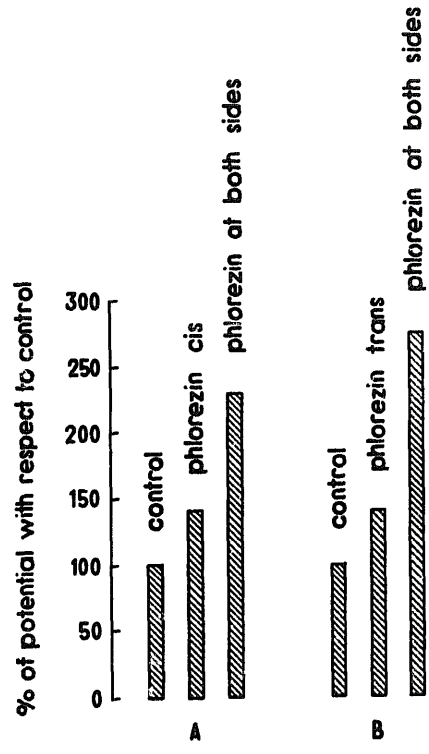

Fig. 5. The effect of phlorezin (250 μM) on the nigericin-induced (40 nM) potential on the BLM in the presence of TTFB as a percentage of the control depending on the side of the phlorezin addition. 10 mM KCl was added at the cis side. Conditions were the same as in the caption to Fig. 2, curve 3.

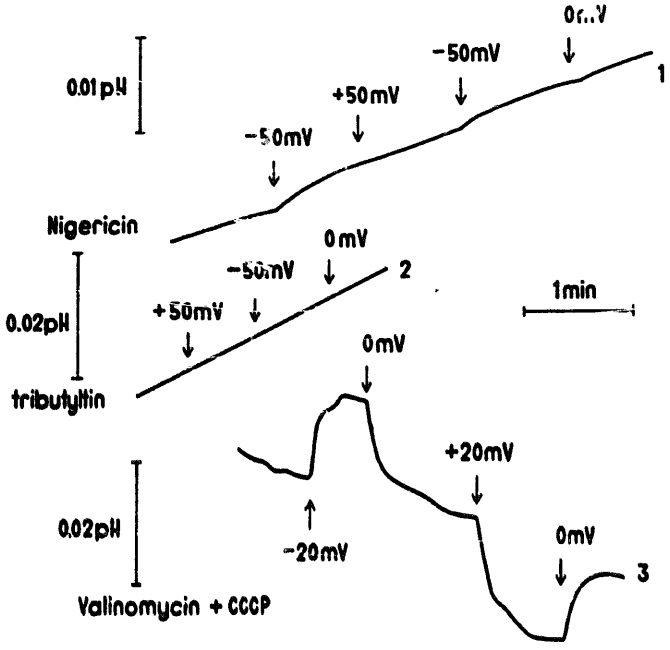

Fig. 6. The effect of the applied electric potential on the rate of K^+/H^+ exchange induced by nigericin (curve 1), Cl^-/OH^+ exchange induced by tributyltin (curve 2) and K^+/H^+ exchange induced by a couple of valinomycin (0.3 μM) and a protonophore CCCP (3 μM) measured by pH-shift recording near the BLM with a pH microelectrode. The values of pH shifts were 0.2, 0.05 and 0.27 for curves 1, 2 and 3, which were 93, 80 and 82% of the maximum pH shifts, respectively. Conditions were as in the caption to Fig. 3 except for curve 1 where the KCl gradient was 20/0 mM and the concentration of nigericin, 20 nM. The conditions on curve 3 were the same as on curve 1 except for ionophores. Recordings were obtained without mixing the solution.

in Fig. 6 for the case of nigericin (curve 1), tributyltin (curve 2) and a couple of CCCP and valinomycin (curve 3). The slopes of the traces were due to the drift of pH electrode under the conditions of high sensitivity. The source of the potential was a simulator of the electrode potentials with an internal resistance of about 10^4 Ohm. It was shown that the application of +/− 50 mV to the BLM had no effect on the flux in the case of nigericin (less than 1%) and tributyltin (less than 3%). On the other hand the application of +/− 20 mV affected markedly the pH shift in the case of a couple of carriers – CCCP and valinomycin (curve 3). It is worth noting that the formation of the pH shift by a couple of electrogenic ionophores valinomycin and CCCP was observed for the first time as far as we know. This couple performed the antiport of potassium for hydrogen ions similarly to nigericin, however, this time cations crossed the membrane in a form of charged complexes.

A detailed analysis of the trace 1, Fig. 6 revealed a small (less than 1%) effect of the application of −50 mV on the nigericin-induced pH shift which was irreversible after turning off the potential. This observation could be accounted for by the additional incorporation of nigericin into the BLM induced by the potential under the conditions of KCl gradient on the membrane. Thus it may be concluded in general that the application of the potential to the BLM had no effect on the electroneutral fluxes through the membrane.

Phloretin was less effective in the absence of cholesterol in the BLM (Fig. 2) especially in the case of nigericin. Phloretin had no effect on the nigericin-induced potential for the BLM made from egg yolk phosphatidylcholine but was active in the case of tributyltin (data not shown).

Discussion

Our results showed that phloretin stimulated cation/H^+ exchange and inhibited anion/OH^- exchange. It was known previously that the main effect of phloretin on the BLM is the decrease of its dipole potential, which affects the rate of electrogenic fluxes induced by carriers [1,2]. However, in the case of nigericin and tributyltin, electrogenic fluxes were orders of magnitude less than the electroneutral H^+ flux and did not contribute significantly to the total H^+transport [18–21]. Our data confirmed that the ionophore-mediated H^+ flux is independent of the applied potential (Fig. 6) and showed that phloretin affected electrically silent transport of ions.

It seemed unlikely that the effect of phloretin was due to the direct interactions with the carriers for several reasons. (1) The effect depended on the membrane composition and was absent in the membrane made from phosphatidylcholine. (2) The impermeable analogue of phloretin was effective also.

Phloretin is a weak acid with pK_a 7.3 and its action on the membrane was shown to be accomplished by a neutral form [1]. Nevertheless, the anionic form of phloretin can bind to the membrane and change the surface charge in the BLM. The change of the surface potential can affect the rate of K^+/H^+ exchange [20,22] and Cl^-/OH^- exchange [23] and therefore contribute to our effect of phloretin. However, the addition of high concentration of calcium ions had no effect on the nigericin-induced transport in the absence and in the presence of phloretin under our experimental conditions (see Results) thereby indicating that the phenomenon is independent of the surface charge. Moreover, at pH 8 much higher concentrations of phloretin are needed to decrease the rate of Cl^-/OH^- exchange to the same extent as at pH 6.4 (Fig. 4). The pH independence of the effect of phloretin in the case of nigericin can be due to different sensitivity of the rate of K^+/H^+ exchange to the change in the dipole potential since at pH 8 and 6.4 different steps of ion transport may be rate-limiting [24].

The correlation of the phloretin action with the electrogenic and nonelectrogenic fluxes (the stimula-

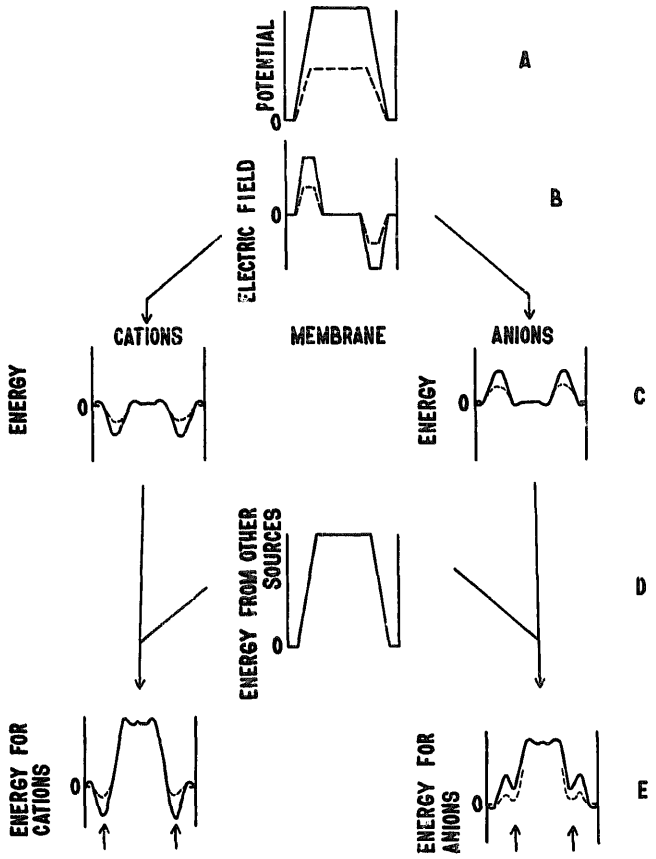

Fig. 7. The schematic distribution of the dipole potential (A) and electric field (B) across the membrane, as well as the energy of an electric dipole with certain orientations for the case of the transport of cations and anions (C). (E) The sum of the energy from the dipole moment (C) and from other sources (D). Solid lines, a control; dashed lines, in the presence of phloretin. Arrows on (E) represent the location of dipoles at the interfaces.

tion of cationic and the inhibition of anionic fluxes) indicated the common mechanism of the action i.e. through the dipole potential. Two different points of view concerning the rate-limiting step of the nonelectrogenic transport were discussed in the literature – the translocation through the membrane [20,25] and reactions with cations at membrane/water interface [26]. If one assumed that the reaction of the carrier with ions at the interface was rate-limiting, the sign of the effect (i.e. the reduction of for example anionic fluxes upon the reduction of the dipole potential) would suggest that exactly the binding of ions with carrier was the rate-limiting step. In fact, if this step proceeded in the zone of the drop of the dipole potential, then the reduction of its value should decrease the rate of anion and increase the rate of cation binding. However, the decrease of the rate of ion fluxes by cholesterol (Fig. 2) was more consistent with an alternative view that the translocation of the carrier through the membrane was rate-limiting.

Fig. 7 presents the scheme of the effect of the dipole potential on the translocation of neutral ion-ionophore complexes through the membrane. The ion-ionophore complexes were considered as dipoles whose energy was proportional to the value of electric field, dipole moment and a cosine of the angle between them. Figs. 7A and B presents the scheme of the distribution of the dipole potential (A) and the electric field (B), respectively. Thus the dipole energy depends on the dipole orientation. It is natural to suppose that the initial dipole direction is retained after the complex formation and is mainly perpendicular to the plane of the membrane. The orientation of the dipole is determined by the direction of the ion approach and the position of the oppositely charged carrier, which must be closer to the middle of the membrane. Accordingly in the case of ion dissociation, the orientation of the dipole moment should be reversed. The reorientation of the dipole may take place in the inner part of the membrane devoid of electric field. Fig. 7C shows the energy profile of the dipole taking into account its orientation. It is seen that in the case of cations the profile has two troughs. In the case of anion transport the initial orientation of the dipole should be reversed and the energy profile should have two barriers in the zones of dipole potential drops.

It may be proposed that besides the energy determined by existence of the dipole potential there is a part of energy of other origin (Fig. 7D). This form of energy profile implies the existence of the hydrophobic barrier for the complexes of ion-ionophore. Fig. 7E presents the sum of the energy profiles of Figs. 7C and D derived with the appropriate ratio of C and D. Assuming that the start and the end of the dipole translocation are located at the interface troughs (arrows on Fig. 7E) we can explain the opposite effects of phloretin on the cation and anion fluxes. In fact, the comparison of the solid and dashed curves in Fig. 7E shows that phloretin reduces the barrier in the case of cations and increases it in the case of anions. Thus the above consideration qualitatively explains our experimental data.

We can try to estimate the effect of phloretin quantitatively. Since phloretin decreased the TTFB-induced current 100 times, then the reduction of the dipole potential could be evaluated as 120 mV [6] under our conditions. Taking into account that the energy of the dipole equals to the product of the dipole moment and the electric field and assuming that the dipole size comprises 100% of the size of the zones of dipole potential drops, the change of the energy should be estimated as 120 meV. The experimentally observed change of the fluxes amounted to 3–4 which corresponded to the energy change of about 30 meV or 25% of the maximal value. Apparently, the size of the dipole was less than the size of the dipole potential drop or (and) the orientation of the dipoles was not perpendicular to the plane of the membrane.

Riddell and co-workers [26] did not observe the effect of cholesterol on the nigericin-induced transport and concluded that the rate-limiting step was the reaction of potassium ion dissociation from the carrier at the membrane surface. Such an interpretation was inconsistent with the effect of cholesterol in our experiments and failed to explain the requirement of phlorezin at both sides of the membrane for the stimulation of nigericin-mediated ion transport. It follows from this discussion that the rate-limiting step of the nigericin transport process may differ depending on the experimental conditions (especially the lipid composition of the BLM). This might be the cause of the absence of the effect of phloretin in the case of egg yolk phosphatidylcholine membrane.

Besides the action on the membrane viscosity cholesterol is known to increase the dipole potential of the BLM [6]. This factor may account for the stronger inhibition of K^+/H^+ compared to Cl^-/OH^- exchange by cholesterol (see Results) in agreement with the sign of the effect of phloretin.

Summing up this discussion we may conclude that the effect of phloretin on the rate of the electrically silent ion transport can be accounted for by the reduction of the dipole potential. The most probable scheme of this phenomenon implies that the dipole potential affects the rate of the translocation of the ion-carrier complex across the membrane including the zones of the dipole potential drops. The most important element of this scheme is the orientation of the electrical dipole moments of the complexes near the interface which depends on the type of transporting ions and carriers.

References

1 Andersen, O.S., Finkelstein, A., Katz, I. and Cass, A. (1976) J. Gen. Physiol. 67, 749–771.
2 Melnik, E., Lattorre, R., Hall, J.E. and Tosteson, D.C. (1977) J. Gen. Physiol. 69, 243–257.
3 Tsybulskaya, M.V., Antonenko, Yu.N., Tropsha, A.E. and Yaguzhinsky, L.S. (1984) Biofizika 29, 801–805 (in Russian).
4 Liberman, E.A. and Topaly, V.P. (1969) Biofizika 11, 452–460 (English translation in Biophysics 14, 477).
5 Hladky, S.B. (1974) Biochim. Biophys. Acta 356, 71–85.
6 Szabo, G. (1974) Nature 252, 47–49.
7 Pressman, B.C. (1976) Annu. Rev. Biochem. 45, 501–531.
8 Ovchinnikov, Yu.A., Ivanov, V.T. and Shkrob, A.M. (1974) Membrane active complexones, B.B.A. Library Vol. 12, Elsevier, Amsterdam.
9 Tosteson, M.T. and Wieth, J.O. (1979) J. Gen. Physiol. 73, 789–800.
10 Antonenko, Yu.N. and Yaguzhinsky, L.S. (1990) Biochim. Biophys. Acta 1026, 236–240.
11 Antonenko, Yu.N. (1990) J. Membr. Biol. 113, 109–113.
12 Mueller, P., Rudin, D.O., Tien, H.T. and Wescott, W.C. (1963) J. Phys. Chem. 67, 534–535.
13 Remis, D., Bulychev, A.A. and Kurella, G.A. (1986) Biochim. Biophys. Acta 852, 68–73.
14 Remis, D., Bulychev, A.A. and Rubin, A.B. (1990) Biol. Membr. 7, 382–389 (in Russian).
15 Walter, A. and Gutknecht, J. (1986) J. Membr. Biol. 90, 207–217.
16 Barry, P.H. and Diamond, J.M. (1984) Physiol. Rev. 64, 763–872.
17 Sokolov, V.S., Cherny, V.V. and Markin, V.S. (1984) Biofizika 29, 424–429 (in Russian).
18 Markin, V.S., Sokolov, V.S., Boguslavsky, L.I. and Yaguzhinsky, L.S. (1975) J. Membr. Biol. 25, 23–45.
19 Toro, M., Arst, E., Cerbon, J., Algeria, G., Alva, R., Meds, Y. and Estrada-O, S. (1987) J. Membr. Biol. 95, 1–8.
20 Amblard, G., Sandeaux, R., Sandeaux, J. and Gavach, C. (1985) J. Membr. Biol. 88, 15–23.
21 Kafka, M.S. and Holz, R.W. (1976) Biochim. Biophys. Acta 426, 131–137.
22 Riddell, F.G. and Arumugam, S. (1988) Biochim. Biophys. Acta 945, 65–72.
23 Ivanov, A.S. (1977) Ph.D. Thesis, 2nd Pirogov Medical Institute.
24 Antonenko, Yu.N. and Yaguzhinsky, L.S. (1988) Biol. Membr. 5, 718–727 (in Russian).
25 Antonenko, Yu.N. and Yaguzhinsky, L.S. (1988) Biochim. Biophys. Acta 938, 125–130.
26 Riddell, F.G., Arumugam, S., Braphy, R.J., Cox, B.G., Payne, M.C.H. and Southon, T.E. (1988) J. Am. Chem. Soc. 110, 734–738.