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Role of membrane curvature in mechanoelectrical transduction: Ion carriers nonactin and valinomycin sense changes in integral bending energy

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Abstract

We describe the phenomenon of mechanoelectrical transduction in macroscopic lipid bilayer membranes modified by two cation-selective ionophores, valinomycin and nonactin. We found that bulging these membranes, while maintaining the membrane tension constant, produced a marked supralinear increase in specific carrier-mediated conductance. Analyses of the mechanisms involved in mechanoelectrical transduction induced by the imposition of a hydrostatic pressure gradient or by an amphipathic compound chlorpromazine reveal similar changes in the charge carrier motility and carrier reaction rates at the interface(s). Furthermore, the relative change in membrane conductance was independent of membrane diameter, but was directly proportional to the square of membrane curvature, thus relating the observed phenomena to the bilayer bending energy. Extrapolated to biological membranes, these findings indicate that ion transport in cells can be influenced simply by changing shape of the membrane, without a change in membrane tension.

Keywords: Mechanosensitivity; Lipid bilayers; Liposomes; Vesicles; Ion carriers; Curvature

1. Introduction

The phenomenon of mechanosensitivity plays a key role in several fundamental physiological functions including perception of touch, sound, motion, and gravity [1–7]. On the molecular level, this property has been attributed to specialized ion channel proteins capable of transducing mechanical stimuli into electrical currents across biological membranes. These channels are thus called mechanosensitive. Besides a cytoskeletal involvement in mechanotransduction, it is implied that mechanically induced activation of these channels reflects their ability to perceive changes in the lipid bilayer elastic properties, membrane tension, thickness, equilibrium curvature, and compression and bending moduli [8–13].

In the present paper we describe the phenomenon of mechanoelectrical transduction in macroscopic Mueller–Rudin artificial planar lipid bilayer membranes (BLM) [14] that are modified with two cation-selective ionophores valinomycin and nonactin. Namely, we found that bulging carrier-modified membranes by the imposition of a hydrostatic pressure gradient, while maintaining a constant membrane tension, produced a supralinear increase in specific ion conductance. We also found that the conductance of bulged valinomycin- and nonactin-modified membranes of two different diameters followed essentially the same function of the applied hydrostatic pressure gradient, which could be linearized in a double logarithmic plot with a slope of ~2. This finding suggests a role of the bilayer bending energy

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(which is a square of membrane curvature) as a source for mechano-electrical coupling. Our analyses of the mechanisms involved in these phenomena reveal similar changes in the charge carrier motility and carrier reaction rates at the interface(s), induced in these membranes by the imposition of a hydrostatic pressure or by an amphipathic compound chlorpromazine. We also examined the role of curvature in carrier mediated ion transport in microscopic membranes of liposomes of different sizes. The results of these stopped flow experiments suggest that, under some circumstances, the curvature-dependent activation of ion transport can saturate. Based on these findings, we suggest that ion transport in cells can be influenced by changing the curvature elastic energy of the membrane, without stretching.

2. Methods

2.1. Planar lipid bilayers

Membranes were formed using the Mueller–Rudin technique [14] over the apertures of 100 or 200 μm in diameter in a partition separating two compartments (4 ml in volume) in a teflon chamber. The membrane-forming solution was composed of a 1:1 mixture (wt:wt, 25 mg of lipid per ml) of diphytanoylphosphatidyl-choline and diphytanoylphosphatidylethanolamine (Avanti Polar Lipids, Alabaster, AL) in n-octane. No pre-wetting of the aperture by membrane-forming solution was made. Bathing solutions contained 100 mM NaCl (or 100 mM KCl in the case of valinomycin), 10 mM MOPS–Tris (pH=7.4), and were made with distilled water and filter sterilized before use (Sterivex-GS, 0.22 μm filter, Millipore Corp., Bedford, MA). All chemicals were reagent grade, and were purchased from Sigma-Aldrich.

Currents through the membrane were measured using a conventional current-to-voltage converter based on an OPA-101 (Burr-Brown, Tucson, AZ) operational amplifier with a 1 GΩ feedback resistor. The current-to-voltage converter was connected to the *trans* side of the bilayer chamber using an Ag/AgCl electrode and 3 M KCl/3% agar bridges. The *cis* compartment was connected to a voltage source using an Ag/AgCl electrode and 3 M KCl/3% agar bridge. Membrane formation and stabilization was monitored by the increase in capacitive current in response to triangle voltage pulses (10 V/s) from a function generator. Upon formation, the membranes were allowed to reach steady-state conditions for 20–30 min before experimentation. The membrane capacitance was calibrated with a known capacitor. The specific capacitance of non-bulged membranes was 0.47 ± 0.07 μF/cm^2, and remained stable for more than 3 h. All experiments were conducted at 25±1 °C.

Valinomycin or nonactin were added to both compartments of the bilayer chamber from stock solutions (in ethanol) to achieve final concentrations of 40 nM. The concentration of ethanol in the bathing solution after the addition of antibiotics never exceeded 0.1% (v/v). Membranes were allowed to reach steady-state conductance for 10–20 min before experimentation. Steady-state currents through the membranes modified with valinomycin or nonactin were measured at five different applied voltages including 0 mV. Slope (ohmic) conductances (λ_O) for the antibiotic-treated membranes were estimated from the lower linear (±20 mV) portion of the I/V curves. Specific conductances of flat non-modified, nonactin-treated, and valinomycin-treated membranes were $0.65\pm0.35\times10^{-8}$ S/cm^2, $1.75\pm0.35\times10^{-7}$ S/cm^2, and $4.35\pm0.55\times10^{-6}$ S/cm^2 (Mean±SD), respectively.

A hydrostatic pressure gradient (ΔP) across the membrane was established by slowly (~0.5 ml/min) lowering the level of solution in the *trans* compartment of the bilayer chamber. Removal of 2 ml of the bathing solution from the compartment of 18.8 mm in diameter and 18 mm in depth was equivalent to the hydrostatic pressure difference of 68 Pa. As long as ΔP applied did not exceed the value that resulted in doubling of membrane capacitance as compared to the initial value, bulged membranes were stable for at least 30 min. The reversal of a hydrostatic pressure gradient was made by the addition of solutions containing the same ionic and valinomycin or nonactin concentrations. Currents through bulged membranes were measured three times within 5–10 min after the establishment of ΔP. The values of specific ohmic conductances for bulged membranes were estimated with the assumption that the area of the membrane was directly proportional to its electrical capacitance. Chlorpromazine (20 mM) was dissolved in the appropriate bilayer bathing solution, and was added to *trans* compartment at least 20 min prior to collecting the data.

2.2. Kinetic analyses of carrier mediated Ion transport in BLM

Physical-chemical mechanisms of carrier-mediated ion transport involved in the observed phenomena where analyzed following the kinetic model of Läuger and Stark [15], as modified by Kuo and Bruner [16]. In this model, carrier-mediated ion transport across the membrane was analyzed in terms of three rate limiting processes: (1) the exchange of uncomplexed carrier at the membrane-solution interface (the partition coefficient of uncharged carrier); (2) formation and dissociation of carrier–ion complexes at the membrane–solution interfaces; and (3) translocation of the charged complexes across the membrane, with the assumption that only the latter was modified by an applied electric field [17]. Subsequently, the current density (J) in such modified membranes can be written as:

$$J = \frac{\beta \cdot C_M \cdot J^S \cdot \sinh\frac{FV}{2RT}}{1 + (\alpha + \beta \cdot C_M) \cdot \cosh\frac{FV}{2RT}} \quad (1)$$

where:

$$\alpha = \frac{2k_{MS}}{k_D} \quad (2)$$

$$\beta = \frac{k_{MS} \cdot N_{MS}}{k_S \cdot N_S \cdot C_M} \quad (3)$$

$$J^S = F \cdot d \cdot \gamma_S \cdot C_0 \cdot k_S \quad (4)$$

and

- k_S and k_{MS} are the rate constants of translocation of uncomplexed and complexed carriers across the membrane, respectively;
- k_D is the dissociation constant of carrier–ion complexes at the membrane/solution interfaces;
- N_{MS} and $N_S = 1/2 d\gamma_S C_O$ are the surface densities of complexed and uncomplexed carrier, respectively, in the membrane of thickness d, with the concentration of carrier in the solution of C_O, and carrier partition coefficient γ_S;
- J^S is specific (i.e., referenced to the specific ohmic conductance) current density;
- F, R, and T have their usual thermodynamic meanings;
- C_M is ion concentration in solution;
- V is applied voltage.

Solving Eq. (1) for the three limiting cases determines the limits of α and β as follows:

(1) if the translocation of the charged complexes across the membrane was the rate-limiting step, $\alpha << 1$; and $\beta C_M << 1$;
(2) if the back-diffusion of the neutral carrier across the membrane was the rate limiting step, $\beta C_M >> \alpha$; and $\beta C_M >> 1$;
(3) if the formation/dissociation of ion–carrier complexes at the membrane interface were the rate-limiting step, $\alpha >> \beta C_M$; and $\alpha >> 1$.

To distinguish these cases, two parameters such as the ratios of carrier-mediated chord (λ) and ohmic conductances (λ_O) can be derived from the

experimentally measured steady-state I/V curves [15,16], and are related to the rate-limiting parameters of ion transport as:

$$\frac{\lambda}{\lambda_0} = 2\frac{\mathrm{RT}[1+(\alpha+\beta\cdot C_\mathrm{M})]\cdot\sinh\frac{\mathrm{FV}}{2\mathrm{RT}}}{\mathrm{FV}\left[1+(\alpha+\beta\cdot C_\mathrm{M})\cdot\cosh\frac{\mathrm{FV}}{2\mathrm{RT}}\right]} \quad (5)$$

and

$$J^\mathrm{S} = \frac{2\lambda_0\mathrm{RT}}{F}\cdot\frac{1+(\alpha+\beta\cdot C_\mathrm{M})}{\beta\cdot C_\mathrm{M}} \quad (6)$$

2.3. Stopped-flow fluorescence measurements in liposomes

Lipid vesicles of different diameters were prepared by extrusion through polycarbonate filters, a technique that has been shown to reproducibly yield large, unilamellar vesicles (LUV) with a mean diameter near the pore size of the filter used [18–21]. Briefly, a thin film of egg phospatidylcholine was first formed on the bottom of a round flask (50 ml in volume) by removing chloroform from the commercial lipid solution (25 mg of lipid in 2.5 ml of chloroform, Avanti Polar Lipids, Birmingham AL) under argon stream, and was left to dry under vacuum overnight. Lipids were allowed to rehydrate for 1 h in a buffer that contained 20 mM 8-amino-1,3,6-naphthalenetrisulfonate (ANTS), 50 mM K_2SO_4, 100 mM 2-(N-morpholino)-ethanesulfonic acid (MES), and 50 mM Tris–OH (pH 5.8), vortexed for 5 min, and sonicated for 10 min (33 kHz). Lipid suspensions were disrupted by ten cycles of rapid freezing and thawing to the room temperature, followed by ten consecutive extrusions through the desired pore size filters (100 nm pore size PCTE filters, Northern Lipids Inc., Vancouver, Canada V6S 2L2; or 200 nm and 1 μm pore size PCTE filters, Sterlitech Corp., Kent WA 98032). External ANTS was removed by passing the extruded liposomes through a Sephadex G-50 column. The elution buffer contained 100 mM MES, 50 mM Tris–OH (pH 5.8), 50 mM K_2SO_4, and 50 mM $NaNO_3$. The amount of entrapped ANTS in the liposomes recovered in the void volume was determined by measuring the absorbance of the suspension at 370 nm (A_{370}).

For the stopped-flow experiments, vesicles were diluted with the elution buffer to achieve an A_{370} of ~0.1. Valinomycin was added to vesicles to achieve the desired concentration from a solution prepared by at least 100-fold dilution of a 10 mM stock solution in DMSO. The mixture was allowed to equilibrate for at least 20 min. Additions of chlorpromazine to liposomes were made from the stock solution of the drug in the elution buffer. Tl^+ flux was initiated by mixing the ANTS-loaded vesicles with an equal volume of buffer that contained 50 mM $TlNO_3$, 50 mM K_2SO_4, 100 mM MES, and 50 mM Tris–OH (pH 5.8). Measurements of Tl^+ quenching of ANTS fluorescence were performed in a SF2001 stopped-flow spectrofluorometer (Kintek Corp., Austin TX). ANTS fluorescence was excited at 370 nm with a 4 nm slit width using a 75 W Xenon lamp. The emission signal was collected through a 495 nm cut-on filter or, for the experiments in the presence of chlorpromazine, a 515 nm cut-on filter (515LP, Chroma Technology, Brattleboro, VT). In each experiment, at least four individual runs were averaged *post hoc* for further analyses using the Kintek Corp. software.

3. Results

Macroscopic Mueller–Rudin BLM possesses an essentially infinite reservoir of lipids and solvent in the Plateau–Gibbs border [22–25]. This reservoir can supply bulk material for bilayer expansion following the imposition of a hydrostatic pressure gradient, thus maintaining the membrane tension constant [26]. Subsequent to the imposition of ΔP, we found that the capacitance of the membranes (C_M) increased up to 2-fold (Fig. 1). That is, following the Laplace's law ($\Delta P = 2T/r$), and assuming a membrane tension T of 2 mN/m (and rupture tension of ~5 mN/m in the absence of electrical field) for a phospatidylcholine/phosphatidylethanolamine mixture [16], the

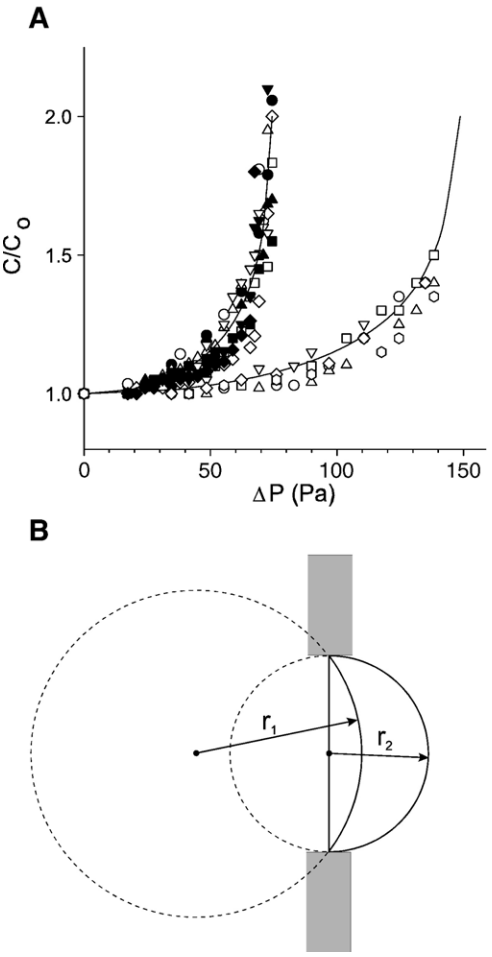

Fig. 1. Bilayer surface area as a function of a hydrostatic pressure gradient. (A) Data points represent membrane capacitance of Mueller–Rudin lipid bilayer membranes formed on the apertures of 100 and 200 μm in diameter (open and closed symbols, respectively), measured following imposition of a hydrostatic pressure gradient, and normalized for the initial membrane capacitance. Lines in the graph represent theoretical predictions for the surface area in the membranes experiencing transition from flat to hemispheric shape (shown schematically in B), following the Laplace's law.

conversion of a flat bilayer of 100 μm in diameter to a hemisphere (i.e., when the radius of the membrane r becomes equal to the radius of the aperture, Fig. 1B) should be expected at pressures of 80 Pa. This is in good agreement with the experimentally determined value of 68–74 Pa (Fig. 1A), and is consistent with the tension in macroscopic BLM remaining constant during bulging.

The results of our experiments with bulging the valinomycin- and nonactin-modified membranes are shown in Fig. 2. For both valinomycin- and nonactin-modified membranes, a 1.5–2.0-fold increase in membrane capacitance (a measure of surface area) induced by the imposition of ΔP was accompanied by a 3–8-fold increase in membrane conductance (circle and triangle symbols in Fig. 2A and B, respectively). The changes were completely reversible upon removal of ΔP (see, for example data points collected in the same representative membrane at respective ΔP in Fig. 2A and B connected with lines). For both carriers, the ΔP required to reach the same relative increase in

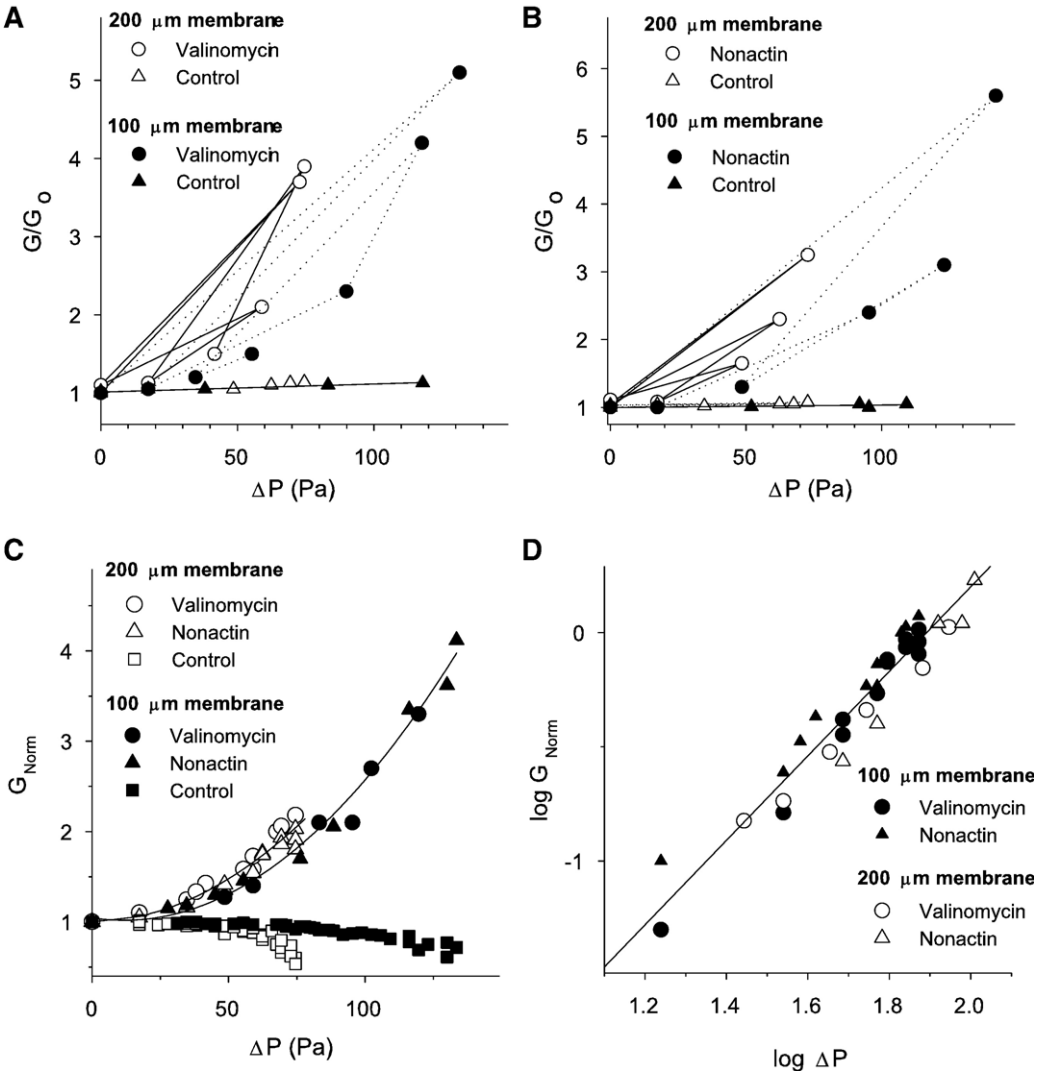

Fig. 2. Effect of bulging on the conductance of valinomycin- and nonactin- modified membranes. (A) Conductance of valinomycin-modified membranes of 100 and 200 μm in diameter (closed and open symbols, respectively) as a function of a hydrostatic pressure gradient. Lines in the graph connect data points collected in the same membrane at respective Δ*P*. (B) Conductance of nonactin-modified membranes of 100 and 200 μm in diameter (closed and open symbols, respectively) as a function of Δ*P*. Lines the graph connect data points collected in the same membrane at respective Δ*P*. (C) Summary plot of the data collected in at least 3 individual membranes as described in the legend for Panels A and B above, normalized for the change in membrane capacitance. Lines in the graph are cubic spline fits of the data collected in valinomycin- (circles) and nonactin- (triangles) modified membranes of 100 and 200 μm in diameter (closed and open symbols, respectively). (D) Linear fit of the data shown in Panel C for valinomycin- (circles) and nonactin- (triangles) modified membranes of 100 and 200 μm in diameter (closed and open symbols, respectively).

conductance was larger in the smaller diameter membranes (closed symbols), whereas in the larger diameter membranes the maximal relative increase in conductance was smaller. Importantly, the specific conductance (i.e., normalized for the bilayer surface area) of valinomycin- and nonactin-modified membranes of either diameter followed essentially the same function of the applied hydrostatic pressure gradient (Fig. 2C), yielding a slope of ~2 in a double logarithmic plot of conductance versus Δ*P* (Fig. 2D). Thus, the relative change in membrane conductance is independent of membrane area, but is related to the square of membrane curvature.

To test this possibility further, we employed an amphipathic compound chlorpromazine (CPZ) which is thought to enter one leaflet of the bilayer [27,28] and induce a curvature stress [29], when added to one side of a membrane. Depicted in Fig. 3, are the results of our experiments studying the effects of chlorpromazine on carrier-mediated transport in BLM. As can be seen in the Panel A, the specific conductance of nonactin- (closed circles) and valinomycin-modified membranes (closed triangles) rose significantly in the presence of increasing concentrations of CPZ, and saturated at [CPZ]>8 μM. These findings are in contrast to the lack of an effect of chlorpromazine on specific conductance of non-modified membranes. At all concentrations examined, CPZ elicited only a small effect on membrane capacitance (Fig. 3B), demonstrating that no macroscopic changes in membrane area or shape occurred, and suggesting that the observed increases in membrane conductance for valinomycin- and nonactin-

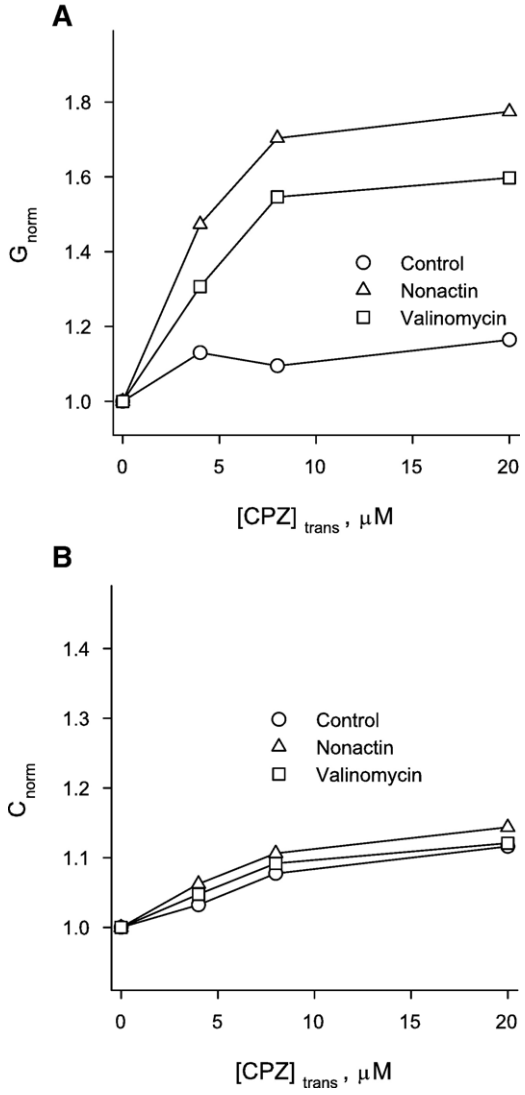

Fig. 3. Effect of chlorpromazine on nonactin- and valinomycin-mediated conductance in lipid bilayer membranes. (A) Data points represent ohmic conductances, normalized for the initial conductance and for the corresponding capacitance (shown in Panel B), measured in individual non-modified (open circle), nonactin-modified (closed circles) and valinomycin-modified (closed triangles) membranes at respective concentrations of chlorpromazine in the trans-compartment of the chamber. Results shown are characteristic for at least three separate experiments performed under each experimental condition. Initial conductance and capacitance of the membranes depicted in the graph were 3 pS and 350 pF (non-modified membrane); 69 pS and 340 pF (nonactin-modified membrane); and 171 pS and 350 pF (valinomycin-modified membrane), respectively.

modified membranes were due to some intrinsic mechanisms of ion carrier mediated transport that are influenced by changes in the membrane.

In a separate series of control experiments, we tested whether the effects of CPZ on carrier mediated conductances could arise as a consequence of it interacting with valinomycin or nonactin. We measured the absorbance and fluorescence spectra of chlorpromazine in solution in the presence of valinomycin or nonactin, and found them to be identical to those in the absence of valinomycin or nonactin ($n=4$, data not shown), suggesting that the observed effects cannot be explained by simple chemical interaction between the drugs. Furthermore, the addition of chlorpromazine to both sides of bilayer membrane, with intent to eliminate the curvature stress, induced a reversal of the increase in carrier-mediated conductance. This reversal, however, was only partial (−39.5% for valinomycin, and −34.8% for nonactin), probably due to non-uniform distribution of microcrenations induced by the drug in the two monolayers.

We next proceeded to examine the effects of curvature on carrier-mediated ion transport in microscopic membranes of liposomes of different diameters (LUV). Unlike BLMs, LUVs contain a fixed number of lipid molecules, do not contain solvent (and hence are thinner than the BLMs), and are formed in unstressed conditions (and hence have a zero or very small tension). We restricted our studies to experiments with the vesicles of 1 ìm in diameter, because freely floating giant unilamellar vesicles (>10 μm in diameter) have been demonstrated to possess excessive surface area for a given volume, and to change spontaneously their shape (swell, and even burst), especially in the presence of ionophores [30], suggesting that membrane tension and curvature cannot be controlled under these conditions. Ion translocation was assessed by means of stopped-flow fluorometry, using the initial rate of quenching of the 8-amino-1,3,6-naphthalenetrisulfonate fluorescence by Tl^+ as a measure of valinomycin-mediated conductance [31]. Ion influx was initiated by mixing the ANTS-loaded vesicles with an equal volume of Tl^+-containing buffer. The recordings of ANTS fluorescence resulting from these experiments are presented in Fig. 4. Shown in Panel A is an example of fluorescence traces in liposomes extruded through a 0.1 μm pore filter in the presence of 10 nM, 100 nM and 1 μM of valinomycin. We found that, at all valinomycin concentrations tested, the time constants of quenching ANTS fluorescence by Tl^+ (τ, estimated by fitting the experimental data to a single exponential decay function $y=y_0+a\cdot\exp(-x/\tau)$) were not significantly different ($t>0.2$) in the vesicles of different diameters (Fig. 4B, and Table 1). Moreover, addition of chlorpromazine did not further increase valinomycin-mediated conductance in these liposomes (Table 2). Taken together with our observation of curvature dependent activation of carrier-mediated ion transport in bulged macroscopic BLMs, these findings made in microscopic LUVs suggest that, under some circumstances, the effects of curvature on membrane conductance can saturate.

4. Discussion

The major observations resulting from this study are: (1) carrier-mediated conductance in bulged lipid bilayer membranes increases superlinearly, (2) the increase in ion transport in bulged membranes does not require a change in the membrane tension; (3) the relative change in membrane conductance was independent of membrane diameter, but (4) was directly proportional to the square of membrane curvature. These results were reproduced with two different ion carriers, valinomycin and nonactin, and suggest that membrane bending energy may play role in modulation of ion transport in membranes.

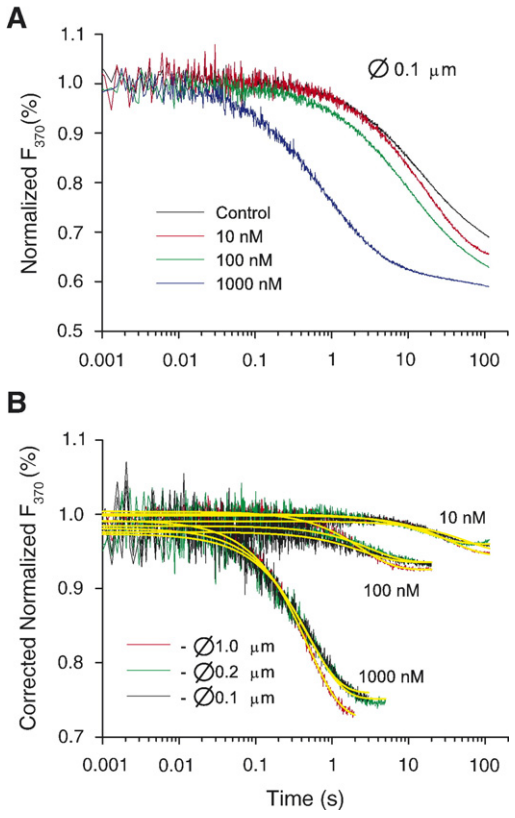

Fig. 4. Kinetics of valinomycin-mediated Tl+ influx in liposomes. Results shown are characteristic for at least four separate experiments performed with different preparations of liposomes. (A) Liposomes were extruded through the 0.1 μm pore filter. Lines in the graph represent fluorescence of ANTS entrapped in liposomes in the presence of 10 nM, 100 nM, and 1 μM of valinomycin normalized for the initial fluorescence. (B) ANTS fluorescence was measured in the presence of 10 nM, 100 nM, and 1 μM of valinomycin in liposomes that were extruded through the pores of different diameters as indicated in the graph. Lines in the graph represent the data normalized for the initial fluorescence as shown in Panel A, and corrected for the signal in the absence of valinomycin. Solid yellow lines in the graph represent fits of the data to the single exponential decay function.

There are two possible scenarios how changes in membrane tension could take place in our experiments, and these should be addressed. First, it could have occurred on a macroscopic scale if there was no flow of lipids from the Plateau–Gibbs border into the plane of the membrane. Then, at the same pressure, the magnitudes of mechanoelectrical effects should differ in the membranes of two diameters. Simple geometrical analysis shows that when both membranes are bulged to the curvature of 100 μm in radius, the larger membrane is transformed to a hemisphere and its area is increased by 100%, while the surface area of the smaller membrane is increased only by 7%. Membrane tension would be predicted to change accordingly, inducing the same relative change in membrane current, which was not the case in our experiments. Second, a change in membrane tension could arise on a microscopic scale from frictional interactions between viscously coupled monolayer leaflets, as they slide past each other in bulged membranes [32–37]. However, the macroscopic Muller–Rudin BLM are thought to have a "lubricating" layer of solvent between monolayers [38], and the viscosities being ~ two orders of magnitude lower [39] than those in solvent-free vesicles [37,40], which argues against such a possibility. Thus, it is unlikely that a change in membrane tension can account for the phenomena reported here.

The roles of some physical–chemical mechanisms of valinomycin- and nonactin mediated ion transport involved in the observed phenomena in terms of three rate limiting processes: (1) the exchange of uncomplexed carrier at the membrane–solution interface; (2) formation and dissociation of carrier–ion complexes at the membrane–solution interfaces; and (3) translocation of the charged complexes across the membrane can be considered following the kinetic model of Läuger and Stark [15], as modified by Kuo and Bruner [16]. The numerical data resulting calculations of the rate-limiting parameters of carrier-mediated ion transport are depicted in Table 3. We found that the imposition of a

Table 1
Kinetic parameters of valinomycin-mediated ion transport in liposomes

Valinomycin (μM)	Extrusion pore diameter (μM)	τ (s)	SD	n
0.01	0.1	40.61	15.14	4
	0.2	36.51	15.19	4
	1	39.18	12.36	4
0.1	0.1	2.53	0.35	4
	0.2	2.84	0.29	4
	1	2.76	0.44	4
1	0.1	0.48	0.03	4
	0.2	0.48	0.05	4
	1	0.49	0.04	4

Numerical data for τ were calculated by fitting the fluorescence data (Fig. 4) to a single exponential decay function $y=y_0+a\cdot\exp(-x/\tau)$, *n*—number of separate experiments performed with different preparations of liposomes.

Table 2
Valinomycin-mediated ion transport in liposomes in the presence and in the absence of chlorpromazine

Extrusion pore diameter (μM)	τ (Mean±SD, s)				
	Control	10 μM CPZ	*t*-test	20 μM CPZ	*t*-test
0.1	0.85±0.06	0.89±0.04	0.27	0.90±0.04	0.15
0.2	0.80±0.05	0.87±0.07	0.09	0.87±0.06	0.11
1	0.88±0.08	0.80±0.05	0.08	0.82±0.03	0.13

Numerical data for τ were calculated by fitting the fluorescence of ANTS entrapped in liposomes in the presence of 200 nM valinomycin in the external solution to a single exponential decay function $y=y_0+a\cdot\exp(-x/\tau)$.
Data shown represent at least 3 separate experiments performed with different preparations of liposomes.

Table 3
Kinetic parameters of carrier-mediated conductance in flat and bulged BLM

	ΔP (Pa)	α	β (M^{-1})	J^S ($\times10^{-8}$ A cm^{-2})
Nonactin	0	0.37	0.15	0.90
	35	0.36	0.15	1.10
	70	0.21	0.13	1.35
Valinomycin	0	0.57	0.83	5.01
	35	0.67	1.82	4.99
	70	0.67	1.45	6.45

Numerical data for α and β were calculated by fitting the voltage dependence of ratios of ohmic (λ_0) and chord conductance (λ) in antibiotic-modified bilayers of 200 μm in diameter to the Eq. (5). The value of current density J^S was estimated by the reference to the specific ohmic conductance using Eq. (6).

Table 4
Effect of chlorpromazine on kinetic parameters of carrier-mediated conductance in BLM

	[Chlorpromazine] ($\times 10^{-6}$ M)	α	β (M^{-1})	J^S ($\times 10^{-8}$ A cm^{-2})
Nonactin	0	0.39	0.14	1.01
	4	0.31	0.14	1.38
	20	0.15	0.16	1.31
Valinomycin	0	0.52	0.63	7.06
	4	0.61	0.91	10.05
	20	0.65	1.21	6.57

Numerical data for α and β were calculated by fitting the voltage dependence of ratios of ohmic (λ_O) and chord conductance (λ) in antibiotic-modified bilayers of 200 μm in diameter to the Eq. (5). The value of current density J^S was estimated by the reference to the specific ohmic conductance using Eq. (6).

hydrostatic pressure gradient ΔP led to changes in both α and β in carrier-modified membranes suggesting that bulging affected the translocation of charged complex across the membrane, which is the main rate-limiting step for membrane conductance in the presence of these carriers [15–17].

The effects of bulging the membrane on the translocation of charged carrier–ion complexes can be considered in terms of energy barrier for transport:

$$g = g_0 \cdot \exp\left(\frac{\Delta E_{\text{barrier}}}{kT}\right) \quad (7)$$

Then, the ~4-fold changes in conductance that we observe in our experiments in BLM correspond to the decrease in equal to 1.39 kT, a value comparable to the estimates of other energies involved in the phenomenon of mechanoelectrical coupling in biological membranes [12,41–43]. On the other hand, our finding that the relative change in membrane conductance was independent of membrane diameter but was directly proportional to the square of membrane curvature may relate the observed phenomena to the bilayer bending energy

$$E_{\text{bend}} = 2 \cdot k_B \cdot R^{-2} \quad (8)$$

where κ_B stands for the bilayer bending modulus, and R—for the bilayer radius of curvature.

If the bilayer bending modulus is a sum of bending moduli for two monolayers ($\kappa_M = 4.2 \cdot 10^{-20}$J, [44]), then $\kappa_B = 8.4 \cdot 10^{-20}$J = 20 kT, where kT stands for the product of Boltzmann constant and absolute temperature, and the bending energy of a hemisphere is $E^{HS}_{\text{bend}} = 4 \cdot \pi \cdot \kappa_B = 250\ kT$. If the area occupied by one lipid molecule in a monolayer (a) is equal to 0.5 nm^2, then the number of lipid molecules in the hemispheric BLM with a radius of curvature of 50 μm is $n = 2\pi R^2 / a = 3 \times 10^{10}$. That makes the contribution of a single lipid molecule to the overall change in bending energy equal to a minuscule $\sim 10^{-8}\ kT$. Therefore, the effects of bulging on the energy barrier for translocation of charged carrier–ion complexes can be explained by changes in integral bending energy that result from the collective properties of multiple lipid molecules in a curved bilayer.

The exact molecular mechanisms of how changes in integral bending energy impact physiologically relevant ion transporters and channels (which are thought to be modulated via interactions with a limited subset of annular lipid molecules in the membranes of cells and organelles) are yet to be established. Our finding of nearly identical rates of ion translocation mediated by valinomycin in microscopic membranes of liposomes of different diameters (0.1–1.0 μm, three orders of magnitude smaller than our macroscopic BLM), which could not be further increased by the amphipathic compound chlorpromazine, suggests that these effects may saturate at some limiting curvature. The substantial body of evidence that amphipaths/lysophospholipids can open a variety of initially closed ion channels [27,45–53], presumably generating local curvatures even greater than those ever seen in the smallest possible vesicle, however, argue against such saturation. Notably, the results of our analyses of kinetic parameters of carrier mediated conductance in the presence of chlorpromazine in macroscopic BLM (Table 4) were similar to those induced by bulging of these membranes (Table 3). We conclude that modification of carrier mediated ion transport in membranes due to chemically induced microscopic curvature stress and by macroscopic bulging may involve some similar mechanism(s) related to the translocation of charged complex across the membrane which is the main rate-limiting step for membrane conductance in the presence of these carriers [15–17]. Alternatively, it is plausible to hypothesize that sensitivities of different channels and transporters to changes in membrane curvature may be different, intrinsic to their nature, or to their environment.

To conclude, in the present paper we report an observation of a marked supralinear increase in carrier-mediated conductance induced by bulging artificial lipid bilayer membranes by the imposition of a hydrostatic pressure gradient, while maintaining constant membrane tension. The relative change in membrane conductance was independent of membrane diameter, but was directly proportional to the square of membrane curvature, suggesting a role of the bilayer bending energy as a source for mechano-electrical coupling. Our analyses of the mechanisms involved in observed phenomena reveal similar changes in the charge carrier motility and carrier reaction rates at the interface(s), induced in these membranes by the imposition of a hydrostatic pressure, or by an amphipathic compound chlorpromazine. Taken together, and extrapolated to biological membranes, these findings may suggest that ion transport in cells can be influenced simply by changing shape of the membrane, without a change in membrane tension.

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