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ARTICLE

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# Gravity dependency of the gramicidin A channel conductivity

## A model for gravity perception on the cellular level

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**Abstract** Theoretical investigations involving the membrane-solution interface have revealed that the density of the solution varies appreciably within interfacial layers adjacent to charged membrane surfaces. The hypothesis that gravity interacts with this configuration and modifies transport rates across horizontal and vertical membranes differently was supported by initial experiments with gramicidin A channels in phosphatidylserine (PS) membranes in 0.1 M KCl. Channel conductivity was found to be about 1.6 times higher in horizontal membranes than in vertical membranes. Here we present the results of further experiments with gramicidin A channels (incorporated into charged PS- and uncharged phosphatidylcholine (PC) membranes in KCl- and CsCl-solutions) to demonstrate that the hypothesis is more generally applicable. Again, channel conductivity was found to be higher in horizontal PS membranes by a factor of between 1.20 and 1.75 in 0.1 M CsCl. No difference in channel conductivity was found for uncharged PC membranes in 0.1 M KCl and in 0.1 M CsCl. However, for PC membranes in 0.05 M KCl the channel conductivity was significantly higher in horizontal membranes by a factor of between 1.07 and 1.14. These results are consistent with the results of our model calculations of layer density and extension, which showed that the layer formation is enhanced by increasing membrane surface charge and decreasing electrolyte ion concentration. The mechanism of gravity interaction with membrane transport processes via interface reactions might be utilized by biological systems for orientational behaviour in the gravity field, which has been observed even for cellular systems.

**Key words** Membrane · Solution interface · Interface reactions · Planar lipid bilayer

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### Introduction

Experiments with biological systems in real and simulated weightless ness have revealed that single cell organisms and cultured cells exhibit patterns of function and morphology which deviate appreciably from those on earth (Cogoli et al. 1985; de Groot et al. 1990; Planel et al. 1982; Hemmersbach-Krause et al. 1994; Block et al. 1994), indicating that gravity interacts with biological systems even on the cellular level.

The mechanism of this interaction, and especially the primary reaction, is not understood to date. The main reason is the difficulty in imaging the primary reaction to gravity in such small systems with very small masses and density variations. Gravity, by its physical nature, can only interact with masses leading to relative mass displacements in nonhomogeneous mass distributions and, generally, to tension or pressure gradients. Moreover, since gravity is a weak force, such effects should be very small on cellular systems. Consequently, there must be structures which are able to transform and amplify the very weak response into observable effects on function or morphology.

Looking for such structures, the membrane-solution interface was investigated theoretically by applying the Gouy-Chapman-Debye-Hückel (GCDH) theory to a system of a phosphatidylserine (PS) membrane (surface charge density about $-4.8 \cdot 10^{-6}$ As/cm$^2$ (Amory et al. 1985)) in contact with a 1:1 electrolyte solution. In this case the positive ions of the solution accumulate near the membrane surface, while the negative ions are more or less repelled in response to the electrostatic interaction with the membrane surface charge. Thus an interfacial layer is established where the ion distribution deviates appreciably from that of the bulk electrolyte solution (e. g. Overbeek 1952, Oshima et al. 1986; Caselli et al. 1984; Graham et al. 1986; Bockris 1970). We have demonstrated previously that, as a consequence of the modified ion distribution, the density of that layer is also different from the bulk electrolyte density (Schatz et al. 1989). Such a configuration is known from macroscopic systems to be very sensitive to

gravity. Assuming that this will be the case in the microscopic range as well, the effect of gravity should be different in vertically and horizontally oriented membranes:

1. Convection might be initiated along vertical membranes (or at least the preceding shear force). In this case gravity is perpendicular to the electrostatic force which attracts the layer to the membrane surface, so there is no competition between both forces. It should be emphasized that gravity does not interact with the single ions of the layer but with the total mass of the layer.
2. Deformation of the layers by their own weights may occur above and below horizontal membranes, compression of the layer above the membrane and extension of the layer at the lower membrane surface. In this case the membrane surface potentials should be changed inversely at the upper and lower membrane surface leading to a gravity induced trans-membrane potential difference. In both cases, transport to and across the membrane should be affected by gravity (Schatz et al. 1989).

We have also shown previously that for the system [0.1 M KCl/PS membrane/gramicidin A channel], the gramicidin A channel current in horizontal membranes is about 1.6 times higher than in vertical membranes (Reitstetter et al. 1991). In 1 M KCl no difference in channel current amplitude was observed between the membrane orientations. This is consistent with our calculations of layer parameters (ion concentration, density, extension), in that there are no layers formed in 1 M electrolytes because the normal ion concentration is then sufficient to compensate the membrane surface charge (Schatz et al. 1989; Reitstetter et al. 1991).

To test whether the theory is more generally accurate, gramicidin A channel conductivity was investigated in PS- and phosphatidylcholine (PC) membranes with KCl- and CsCl solutions. PC membranes have a zero net surface charge density at pH 7, so that the ion distribution at the membrane-solution interface depends only on the electrostatic forces (image charge concept (Torrie et al. 1982)) which appear at the boundaries between media with different dielectric constants ($\varepsilon$(solution) ~ 80, $\varepsilon$(PC) ~ 2–6). If there are interfacial layers, they should be less distinct than those at boundaries with a defined charge. Consequently, the gravity effect on channel conductivity in PC membranes should be smaller than in PS membranes for the same electrolyte ion concentration.

## Materials and methods

For the experiments, planar lipid bilayers (Müller-Rudin type (Müller et al. 1962)) of L-$\alpha$-lecithin (PC) (soybean) and L-$\alpha$-phosphatidylserine (PS) (bovine brain) were used. Both lipids were purchased from SIGMA (Deisenhofen, Germany) in chloroform/methanol solution. Chloroform and methanol were removed by streaming $N_2$ gas and the lipids were redissolved in n-decane (MERCK, Darmstadt, Germany) to a 2–3% (w/v) solution for bilayer preparation. Gramicidin A (bacillus brevis) was obtained from FLUKA (Neu-Ulm, Germany) and was dissolved in ethanol for a $10^{-6}$ M stock solution. Water was twice quartz distilled. The bath solutions, 0.1 M CsCl, 0.1 M KCl and 0.05 M KCl, were buffered with 5 mM Tris-HCl to pH 7.4. To exclude interfering effects of multivalent ions, 0.5 mM EDTA was added (Apell et al. 1979). The salts were of p. a. grade.

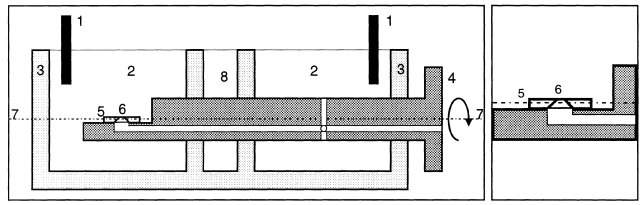


**Fig. 1** Setup for the investigation of membrane transport systems in variable membrane orientations. *1:* Ag/AgCl electrodes, *2:* compartments for both solutions, *3:* teflon block, *4:* teflon tube connecting the compartments (2), *5:* teflon sheet with *6:* aperture for bilayer deposition, *7:* axis about which the teflon tube (4) can be rotated, *8:* separation compartment to prevent capillary contact between the compartments (2). Aperture (6) is adjusted on the axis (7)

We developed a special setup to investigate channel conductivity for different bilayer orientations with respect to the gravity vector. Two chambers (about 20 ml) were cut out of a teflon block and connected by a horizontal cylindrical tube which could be turned (rotated) about its axis. One end of the tube was prepared to hold a teflon sheet (0.5 mm thick) with the aperture for the bilayer deposition (Fig. 1). In this way channel currents could be measured with the same bilayer in different orientations without changing any other experiment parameter, especially electrode position and hydrostatic pressure. Voltage was applied and current was measured via Ag/AgCl-electrodes connected to the headstage of the BLM 120 amplifier (BIOLOGIC CO, Claix, France) which allows for voltage application as well as for current measurement and bilayer capacity control. The complete setup was screened against electromagnetic and acoustic noise by a metal box (52×37×37 cm) covered with 4 cm foam.

To start an experiment, the chambers were filled with the bath solution, the aperture was turned into the 30° position, covered with a drop of the lipid solution from an Eppendorf pipette, and immediately turned into the vertical orientation to accelerate bilayer formation. Two to three µl of th gramicidin stock solution were added to one side of the developing bilayer. This concentration of gramicidin A allowed for single channel observations. After 5–10 min a bilayer was formed and after 15–25 min single channels could be observed on the oscilloscope. Bilayer formation with the aperture in the horizontal position was found to be about three times retarded. The channel conductivity, $\Lambda$[pS] = $I_{channel}$ [pA]/$U_{appl}$ [V], was evaluated by measuring single channel currents, $I_{channel}$, with the BLM 120. Data were collected at a sample rate of 100 Hz and stored

on a DOS computer with patchclamp software developed by J. Dempster (Univ. of Strathclyde, UK). Programs for data analysis were developed by the authors. Since the current amplitude was found to be independent of the bilayer capacity in the range of 30–60 pF, only recordings of bilayers with a capacity in that range were used for analysis. For all experiments the temperature was 22±2 °C.

Four systems were investigated for gravity effects on gramicidin A channel conductivity in vertical and horizontal membranes: 1. PC membranes in 0.1 M CsCl, 2. PS membranes in 0.1 M CsCl, 3. PC membranes in 0.1 M KCl, 4. PC membranes in 0.05 M KCl.

For all systems, the correlated conductivities in vertical and horizontal membranes were evaluated for the same membrane in alternating vertical and horizontal orientations. The *F*-test (variance analysis) was applied to the mean single channel conductivities to evaluate the error probability, *p*, that the conductivities are significantly different in vertical and horizontal membranes.

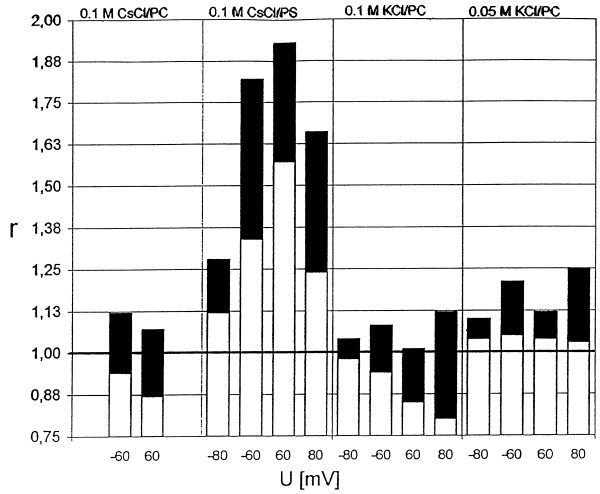


**Fig. 2** Ratio, $r = \Lambda_{hor}/\Lambda_{vert}$, of gramicidin A channel conductivities in horizontally and vertically oriented PC- and PS membranes for applied voltages, U = ±60 and ±80 mV, in 0.1 M CsCl, 0.1 M KCl, and 0.05 M KCl. Gravity effects are indicated by r≠1. The error probability, *p*, that $\Lambda_{hor}$ is significantly different from $\Lambda_{vert}$ is: for 0.1 M CsCl/PC, $p>0.06$, for 0.1 M CsCl/PS, $p<0.01$, for 0.1 M KCl/PC, $p>0.04$, and for 0.05 M KCl/PC, $p\leq0.01$

## Results

The results of the experiments are summarized in Table 1 and Fig. 2, which represent the values for the conductivities of gramicidin A in horizontal, $\Lambda_{hor}$, and in vertical membranes, $\Lambda_{vert}$, the ratio, $\Lambda_{hor}/\Lambda_{vert}$, the error probability, *p*, and the number of channels, N, for different voltages, U.

No effect was observed for PC membranes in 0.1 M CsCl or in 0.1 M KCl. The conductivities in horizontal and vertical PC membranes were not significantly different, $p>0.04$, indicating that there were no effective interfacial layers at PC membranes in 0.1 M CsCl or 0.1 M KCl. This result confirms the theory that the ion distribution in the vicinity of uncharged membranes is not modified so drastically as to create density variations sufficient for gravity interaction.

A clear effect of gravity interaction was found for PS membranes in 0.1 M CsCl. The gramicidin conductivity in horizontal membranes was significantly higher by a factor of between 1.20, $p = 0.009$, and 1.75, $p = 0.0001$, than in vertical membranes. Together with the result of earlier experiments, using PS membranes in 0.1 M KCl, which showed an effect of the same order of magnitude, we conclude that interfacial layers generally exist at charged membranes with density variations high enough for gravity interaction.

A small effect was found for PC membranes in 0.05 M KCl. Conductivity in horizontal membranes was higher than in vertical membranes by a factor of between 1.07, $p = 0.0001$, and 1.14, $p = 0.012$, indicating that even at un-

**Table 1** Gramicidin A channel conductivities in horizontal, $\Lambda_{hor}$, and vertical, $\Lambda_{vert}$, PC- and PS membranes at voltages, U, for different electrolytes (0.1 M CsCl, 0.1 M KCl, and 0.05 M KCl). The ratio, $\Lambda_{hor}/\Lambda_{vert}$, indicates the difference of the conductivities in horizontal and vertical membranes with the error probability, *p*, that the conductivities in horizontal membranes are significantly different from those in vertical membranes. N is the number of channels for the calculations

| System | U [mV] | $\Lambda_{hor}$ [pS] | $\Lambda_{vert}$ [pS] | $\Lambda_{hor}/\Lambda_{vert}$ | *p* | N |
|---|---|---|---|---|---|---|
| 1 | 60 | 11.36 ± 0.61 | 11.72 ± 0.57 | 0.97 ± 0.10 | 0.14 | 267 |
| 0.1 M CsCl/PC | –60 | 11.10 ± 0.59 | 10.74 ± 0.41 | 1.03 ± 0.09 | 0.06 | 324 |
| 2 | 80 | 22.71 ± 0.36 | 15.70 ± 2.00 | 1.45 ± 0.21 | 0.003 | 80 |
| 0.1 M CsCl/PS | –80 | 21.67 ± 1.33 | 18.05 ± 0.12 | 1.20 ± 0.08 | 0.009 | 42 |
| | 60 | 40.43 ± 2.05 | 23.15 ± 1.23 | 1.75 ± 0.18 | 0.0001 | 138 |
| | –60 | 39.33 ± 2.22 | 24.95 ± 2.39 | 1.58 ± 0.24 | 0.0007 | 197 |
| 3 | 80 | 10.44 ± 1.37 | 10.89 ± 0.36 | 0.96 ± 0.16 | 0.49 | 122 |
| 0.1 M KCl/PC | –80 | 10.21 ± 0.26 | 10.12 ± 0.01 | 1.01 ± 0.03 | 0.42 | 132 |
| | 60 | 10.57 ± 0.32 | 11.32 ± 0.71 | 0.93 ± 0.08 | 0.04 | 94 |
| | –60 | 9.63 ± 0.30 | 9.55 ± 0.42 | 1.01 ± 0.07 | 0.32 | 97 |
| 4 | 80 | 4.89 ± 0.34 | 4.29 ± 0.12 | 1.14 ± 0.11 | 0.012 | 98 |
| 0.05 M KCl/PC | –80 | 4.44 ± 0.06 | 4.15 ± 0.07 | 1.07 ± 0.03 | 0.001 | 154 |
| | 60 | 5.09 ± 0.13 | 4.73 ± 0.08 | 1.08 ± 0.04 | 0.004 | 60 |
| | –60 | 4.82 ± 0.25 | 4.28 ± 0.09 | 1.13 ± 0.08 | 0.008 | 64 |

charged membranes there are effective interfacial layers for gravity interaction as soon as the ion concentration is low enough.

## Discussion

Our hypothesis, that gravity does affect membrane transport processes by interfacial layer interaction, was verified for ion transport through the gramicidin A channel. We showed that for both, charged PS- and uncharged PC membranes, the gramicidin A channel conductivity was higher in horizontal than in vertical membranes and that an upper limit of the electrolyte ion concentration exists beyond which this effect does not appear. For uncharged PC membranes, this limit was between 0.1 M (no effect) and 0.05 M (weak effect), and for charged PS membranes between 1 M (no effect) and 0.1 M (strong effect). This is consistent with our theoretical results for a charged PS membrane which showed that the interfacial layer density as well as the layer thickness decrease with increasing ion concentration. For 0.1 M KCl we found a layer density increase of $\Delta\rho = 0.036$ g/cm$^3$ and a layer thickness of $\Delta x = 1.6$ nm, and for 1 M KCl a $\Delta\rho = 0.021$ g/cm$^3$ and a $\Delta x = 0.6$ nm (Schatz et al. 1989). The experimental results for the uncharged PC membranes imply that we can expect similar values for $\Delta\rho$ and $\Delta x$ for 0.1 M and 0.05 M KCl.

EDTA was used to eliminate membrane surface potential compensation by divalent cations. To exclude interfering effects of gravity interaction with the EDTA molecules (e. g. layering), we calculated the sedimentation velocity of EDTA molecules in the solution after volumetric determination of the molecular volume. Sedimentation velocity was found to be about $1.66 \cdot 10^{-11}$ cm/s $= 5.97 \cdot 10^{-8}$ cm/h, indicating that no effect has to be expected. Additionally, some experiments without EDTA showed no effect on channel conductivity in vertical and horizontal membranes.

The question of whether the conductivities are increased in horizontal membranes or decreased in vertical membranes cannot be answered definitively by our experiments. However, comparing the results with available data of gramicidin A conductivities in DOPS (dioleoyl-PS)- and DOPC (dioleoyl-PC) membranes (11.5 pS in DOPC and 52.6 pS in DOPS, both for 0.1 M CsCl, (Beitinger 1991)), it looks more like a reduction of conductivity in vertical membranes.

Additionally, if gravity is assumed to interact with the horizontal membrane configuration, channel conductivity should be changed when the applied voltage is reverse (Schatz et al. 1989). Looking at the conductivity values for horizontal membranes in Table 1, there seems to be a weak rectification at horizontal membranes with a smaller conductivity for negative applied voltages. From the theory of the interfacial layers it follows: a) that the surface potentials, $-U_0$, have the same value on both sides of a vertical membrane with identical solutions on both sides, when no voltage is applied, and b) that for horizontal membranes under the same conditions the surface potential at the upper side, $U_u$, may be changed by layer compression to $U_u = -U_{u0} + \Delta U_u$ and at the lower side by layer extension to $U_l = -U_{l0} - \Delta U_l$. In this way a transmembrane potential difference, $\Delta U_{m0}$, is created by gravity, $\Delta U_{m0} = U_u - U_l = \Delta U_u + \Delta U_l$. With the active electrode facing the upper membrane surface the effective voltage across the membrane is $\Delta U_{m1} = -U_a + U_u - U_l = -U_a + \Delta U_{m0}$, when the electrode has a negative potential, $U_a$, (negative applied voltage) and $\Delta U_{m2} = U_a + \Delta U_{m0}$, when the applied voltage is reversed. Since $\Delta U_{m2} > \Delta U_{m1}$, the conductivity should be greater for positive applied voltages. So the polarity of the bias to smaller conductivities for negative voltages in horizontal membranes is of the predicted direction, but the significance of this effect is very low, $p > 0.07$.

Therefore gravity induced shear forces or convection along the surface of vertical membranes might reduce the ion transport through the channel. The gramicidin A channel is assumed to consist of two monomers with a total length of 2.5 – 3.0 nm (Urry 1971), just to bridge the hydrocarbon core of a phospholipid bilayer (Läuger 1986), so that the ions have to pass an aqueous convergence region, surrounded by the polar headgroups of the lipid, before they can enter the channel. The channel conductivity is then limited by the ion mobility within the channel and by the rate at which they enter the channel (Apell et al. 1979). There is no reason to assume that the ion mobility within the channel depends on membrane orientation, but, if the density deviation within the interfacial layer is sufficient to initiate convection along the vertical membrane surface, ion diffusion across the layer will be reduced according to the diffusion equation for that case, $(\partial c/\partial t) = D(\partial^2 c/\partial x^2) - v_{conv}\,(\partial c/\partial x)$, ($D$ = diffusion coefficient, $c$ = ion concentration, $t$ = time, $v_{conv}$ = convection velocity, $x$ = distance to the membrane surface). If the density deviation does not initiate convection, then the preceding shear stress upon the membrane surface might reduce the cross section of the convergence region leading to the channel entrance and, in this way, obstruct the ions in reaching the channel mouth.

Recently, pore formation of the polypeptide antibiotic alamethicin in artificial membranes was reported to depend significantly on membrane orientation and applied centrifugal forces (Hanke 1995). The number of pore states as well as the alamethicin induced membrane current was found to be reversibly reduced when the acceleration parallel to the membrane was increased, indicating that here the pore formation kinetics were affected by similar interface reactions as was the gramicidin A channel conductivity in our experiments.

Therefore, we conclude that the model of gravity interaction with membrane transport processes via interface reactions is not restricted to the special system of the gramicidin A channel in PC- and PS membranes, but should be relevant for membrane transport processes in general. This interaction might enable biological systems such as cells to sense gravity and make use of it for orientation behaviour.

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