Mechanosensitive ion channels as reporters of bilayer expansion

A theoretical model

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ABSTRACT Various amphipathic compounds have been found to activate mechanosensitive (MS) ion channels in the bacterium *Escherichia coli*. These results were interpreted qualitatively in terms of the bilayer couple hypothesis. Here we present a mathematical model that describes the results quantitatively. According to the model, the uneven partitioning of amphipaths between the monolayers of the cell membrane causes one monolayer to be compressed and the other expanded. Because the open probability (P_o) of the *E. coli* channels increased independently of which monolayer the amphipaths partitioned into, the model suggests that P_o of the MS channels is determined by the monolayer having higher tension. We derived a relation between P_o and amphipath concentration. The kinetics of P_o variation after exposure of the cell membrane to the amphipaths was calculated based on this relation. The results fit satisfactorily the experimental data obtained with the cationic amphipath chlorpromazine and with the anionic amphipath trinitrophenol. Experiments which should further test the predictions following from the model are discussed.

INTRODUCTION

Mechanosensitive (MS) channels are a special class of ion channels that have been found in a variety of cells from bacteria (1, 2) and fungi (3), to plant (4) and animal cells (5–10). How mechanical force gates the MS channels is not yet clear (11). Recent patch-clamp experiments, however, have shown that the MS channel of *E. coli* can be activated by amphipathic compounds such as chlorpromazine, trinitrophenol, or local anesthetics (12). These observations support the view that mechanical gating force can be transmitted to the channel through the surrounding lipids. These results were interpreted qualitatively on the basis of the bilayer couple hypothesis (13). According to this hypothesis, cationic amphipaths should, because of the lipid asymmetry of biological membranes (14), preferentially insert into the more negatively charged inner leaflet, whereas anionic amphipaths should incorporate into the less negative outer leaflet of the membrane lipid bilayer. Here we have developed a mathematical model that describes quantitatively the effects of amphipaths on the MS channels of *E. coli*. The model is based on the following experimental observations (1, 12).

(*a*) Suction applied to the patch pipette creates membrane tension γ^{mem}, which increases the channel open probability P_o. The free energy available for gating is linearly dependent on pressure, so that open probability follows the Boltzmann distribution given by:

$$\ln[P_o/(1 - P_o)] = m_0 \gamma^{mem} + b_0. \quad (1)$$

(*b*) Introducing amphipathic compounds into the bath solution increases the open probability of the MS channels.

(*c*) The effects of amphipaths are reversible.

(*d*) The sign of the effect is always positive, i.e., the channel open probability increases for both cationic or anionic amphipaths. This means that the effect of amphipaths does not depend on which membrane monolayer they partition into.

(*e*) Introduction of an amphipath causes a shift of the Boltzmann curve toward lower suction (increase in b_0) but does not affect the slope (m_0) of the curve.

(*f*) Cationic and anionic amphipaths are able to compensate for each other's effect.

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MODEL

Mechanics of the monolayers

Suction applied to the patch-clamp pipette (Fig. 1) creates membrane tension γ^{mem} which is distributed between the two monolayers of the membrane patch:

$$\gamma^{mem} = \gamma^{in} + \gamma^{out}, \quad (2)$$

where γ^{in} and γ^{out} is the tension present in the inner and the outer monolayer, respectively.

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When an amphipath is introduced into the bath solution, it partitions selectively into one of the membrane monolayers and expands it, creating mechanical tension in the bilayer. If the membrane patch is already under tension, resulting from suction applied to the patch pipette, the tension caused by the amphipath introduction should redistribute between the monolayers so that the sum in Eq. 2 is preserved.

Let the actual, stressed area of the inner monolayer be A^{in} and that of the outer monolayer be A^{out}, while the nonstressed areas of the monolayers are A_0^{in} and A_0^{out}, respectively. Because the diameter of the micropipette ($\sim$2 μm) is much larger than the thickness of one monolayer (2.5 nm), both the nonstressed and stressed areas can be considered to be equal:

$$A_0^{in} = A_0^{out} = A_0$$
$$A^{in} = A^{out}. \tag{3}$$

Taking into account the elastic properties of the membrane bilayer the tensions in the monolayers can be presented as:

$$\gamma^{in} = \frac{E(A^{in} - A_0)}{A_0} \tag{4}$$

$$\gamma^{out} = \frac{E(A^{out} - A_0)}{A_0}, \tag{5}$$

where E is the two-dimensional elasticity module of area expansion of one monolayer (15). When amphipaths are added to the bath solution, some of these molecules will adsorb into the membrane. Let the number of the adsorbed molecules in the inner monolayer be N^{in} and in the outer monolayer N^{out}, and the area per one amphipath molecule a^{in} and a^{out}, correspondingly. These areas will be different because of different tensions in the monolayers; in the absence of tension these areas should be the same and equal to a_0. Then the nonstressed area of the inner monolayer is $A_0^{in} + a_0N^{in}$, while the actual, stressed area is $A^{in} + a^{in}N^{in}$. The same should be valid for the outer monolayer.

The actual areas of the two monolayers with amphipaths must remain equal:

$$A^{in} + a^{in}N^{in} = A^{out} + a^{out}N^{out}, \tag{6}$$

and the tensions in two monolayers are given by:

$$\gamma^{in} = \frac{E(A^{in} + a^{in}N^{in} - A_0^{in} - a_0N^{in})}{A_0} \tag{7}$$

$$\gamma^{out} = \frac{E(A^{out} + a^{out}N^{out} - A_0^{out} - a_0N^{out})}{A_0}. \tag{8}$$

The sum of γ^{in} and γ^{out} is preserved because it is equal to membrane tension, which is determined by the constant suction to a pipette as defined by Eq. 2, while their difference, caused by amphipath adsorption, changes:

$$\gamma^{amph} = \gamma^{out} - \gamma^{in} = \frac{Ea_0(N^{in} - N^{out})}{A_0} = Ea_0(n^{in} - n^{out}) \tag{9}$$

Here we took into account condition 6 and introduced the concentrations of amphipaths in the monolayers:

$$n^{in} = N^{in}/A_0 \text{ and } n^{out} = N^{out}/A_0. \tag{10}$$

One can see that amphipaths can change the distribution of mechanical tension between monolayers only in the case of unequal distribution of these molecules between the two monolayers ($n^{in} \neq n^{out}$). In spite of this redistribution the total tension of the bilayer remains constant. With the help of Eqs. 2 and 9 we can find monolayer tensions:

$$\gamma^{in} = \frac{\gamma^{mem}}{2} - \frac{\gamma^{amph}}{2} \tag{11}$$

$$\gamma^{out} = \frac{\gamma^{mem}}{2} + \frac{\gamma^{amph}}{2}. \tag{12}$$

Now let us summarize the assumptions made and resulting mechanical picture. Before addition of amphipath molecules the membrane is in its initial (or reference) state where the areas of monolayers are equal as well as their tensions imposed by suction into pipette. The addition of molecules to one of the monolayer changes the area of this monolayer and involves the redistribution of the tensions between monolayers. For an arbitrary membrane element the change of membrane area is the sum of the area of amphipath molecules adsorbed in this element. In the absence of initial suction, the absorption of molecules into one of the monolayers will result in compression of the initial molecules in this monolayer and increasing of area per molecule in the other monolayer (when the number of molecules of initial type in an arbitrary membrane element remains constant). In our experiment the membrane was subjected to some initial tension, therefore, one of the monolayers becomes more stretched after adsorption while the other relaxes. To be specific, let us mention that if the amphipath molecules partition preferentially into the inner monolayer ($n^{in} > n^{out}$), then the tension of this monolayer decreases while the tension of the outer monolayer increases and vice versa.

Open probability

Now we have to find which monolayer is responsible for the opening of the channel. In the absence of detailed information on this subject, we restrict ourselves to the simplest suppositions using two essential experimental observations (1, 12): (*a*) the open probability P_o increases with membrane tension, and (*b*) P_o increases in the presence of an amphipath independently of which monolayer this amphipath partitions into. Because in the presence of amphipathic molecules the tension in one monolayer increases (γ^{max}) and in the other decreases (γ^{min}) one can conclude that the open probability depends on the larger tension of the two monolayers γ^{max}. γ^{max} can be presented in the same way as given by Eq. 1:

$$\ln\frac{P_o}{1 - P_o} = m_1\gamma^{max} + b_1. \tag{13}$$

m_1 and b_1 are the monolayer parameters as opposed to the bilayer parameters m_0 and b_0 in Eq. 1.

The larger monolayer tension can be found from Eqs. 2 and 9 as:

$$\gamma^{max} = \frac{\gamma^{mem}}{2} + \frac{|\gamma^{amph}|}{2}. \tag{14}$$

Therefore, the following relationship exists between the monolayer and bilayer parameters:

$$m_0 = \frac{m_1}{2} \tag{15}$$

$$b_0 = \frac{m_1|\gamma^{amph}|}{2} + b_1. \tag{16}$$

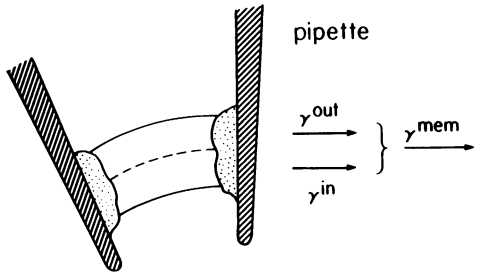

FIGURE 1 Sketch, illustrating the distribution of mechanical tension between the two monolayers of a membrane patch. In the absence of reliable information, dotted portion represents schematically the giagaseal forming region anchoring the membrane patch to the pipette wall.

The open probability can be now presented in the final form:

$$\ln \frac{P_o}{1-P_o} = m_0 \gamma^{mem} + m_0 |\gamma^{amph}| + b_1. \tag{17}$$

We introduce here the dimensionless tensions:

$$\Gamma^{mem} = m_0 \gamma^{mem}, \tag{18}$$

$$\Gamma^{amph} = m_0 |\gamma^{amph}|, \tag{19}$$

which are normalized by characteristic tension $1/m_0$. The physical meaning of the parameter, $\gamma^* = 1/m_0$, is the tension which changes the Boltzmann factor in the open probability Eq. 17 by unity. One can see that the definition (Eq. 17) preserves all the properties of the function (1) and explicitly includes the effect of amphipaths, which create the effective membrane tension γ^{amph}. Thus, Eq. 17 contains two terms with tensions Γ^{mem} and Γ^{amph}, and the term b_1, which determines the MS channel open probability in the absence of both pipette suction and amphipaths.

Kinetics of amphipath adsorption

We will consider the kinetics of amphipath partitioning into the membrane and the consequential change in open probability. The amphipaths are introduced to (or removed from) the bath solution which is in contact with the inner monolayer of the cellular membrane (Fig. 1). Let the adsorption and desorption rate constants for the inner monolayer be k_1 and k_{-1}, and for the outer monolayer (which is in contact with pipette solution) be k_2 and k_{-2}, respectively. Also, let the rate constants of exchange of amphipaths between the monolayers be r_1 and r_{-1}, so that the flux from the inner to the outer monolayer is given by $r_1 n^{in} - r_{-1} n^{out}$. If the concentration of amphipaths is c^{in} in the bath and c^{out} in the pipette solution, then the variation of the amphipath concentration in the monolayers is given by a set of linear differential equations:

$$\frac{dn^{in}}{dt} = -(k_{-1} + r_1) n^{in} + r_{-1} n^{out} + k_1 c^{in} \tag{20}$$

$$\frac{dn^{out}}{dt} = r_1 n^{in} - (r_{-1} + k_{-2}) n^{out} + k_2 c^{out} \tag{21}$$

$$\frac{V^{out}}{A_0}\frac{dc^{out}}{dt} = k_{-2} n^{out} - k_2 c^{out}, \tag{22}$$

where V^{out} is an effective volume of the pipette solution and A_0 is, as before, the area of the patch. The volume of the bath solution is large and its concentration c^{in} is after a step-wise change kept constant. Therefore we do not need an equation for the concentration c^{in}. This set of equations can be easily solved and a solution with three exponential terms can be obtained. However, there is a good reason to simplify this solution. Because the exchange rates of phospholipid molecules in cellular membranes are known to be relatively slow and, in addition, lipid translocation processes presumably require cellular energy (16), we suppose that the exchange of amphipathic molecules between the monolayers is slow in comparison with the adsorption/desorption rate.

Then the analysis of Eq. 20–22 shows that there are three characteristic times corresponding to three distinct processes. The amphipath partitioning from bath solution to inner monolayer is fast and has characteristic time

$$\lambda_1 = \frac{1}{k_{-1}}. \tag{23}$$

Partitioning of amphipaths between the two monolayers is slow with characteristic time

$$\lambda_2 = \frac{1 + V^{out}/(A_0 K_2)}{r_{-1}}, \tag{24}$$

where $K_2 = k_2/k_{-2}$. Equilibration between outer monolayer and pipette solution is fast with characteristic time

$$\lambda_3 = \frac{1}{k_{-2} + k_2 A_0 / V^{out}}. \tag{25}$$

The slow exchange between monolayers means

$$\lambda_2 \gg \lambda_1, \lambda_3. \tag{26}$$

Given this condition, one can present the solutions in rather simple terms. If at the moment $t = 0$ amphipath concentration in the bath was made equal to c, then the variation of concentration in monolayers will be

$$n^{in}(t) = K_1 c\,[1 + (n^{in}(0)/K_1 c - 1)\, e^{-t/\lambda_1}] \tag{27}$$

$$n^{out}(t) = K_1 c\,[R + (n^{out}(0)/K_1 c - R)\, e^{-t/\lambda_2}], \tag{28}$$

where $K_1 = k_1/k_{-1}$ and $R = r_1/r_{-1}$ are equilibrium constants, defined so that K_1 is an equilibrium ratio of two-dimensional concentration in the inner monolayer to the three-dimensional concentration in the solution, and R is an equilibrium ratio of outer-monolayer to inner-monolayer concentration. Thus, K_1 has a dimension of length, and R is dimensionless parameter. Besides, $n^{in}(0)$ and $n^{out}(0)$ are the concentrations of amphipath in the inner and the outer monolayers, respectively, at the moment $t = 0$. Therefore, $n^{in}(t)$ varies fast, and $n^{out}(t)$ slow. The difference between the amphipath concentrations in the monolayers is:

$$\Delta n = n^{in} - n^{out}$$

$$= K_1 c\,(1 - R)\left[1 + \frac{n^{in}(0)/K_1 c - 1}{1 - R} e^{-t/\lambda_1} - \frac{n^{in}(0)/K_1 c - R}{1 - R} e^{-t/\lambda_2}\right]. \tag{29}$$

When substituted into Eq. 17, expression 29 yields:

$$\ln\frac{P_o}{1-P_o} = \Gamma^{mem} + b_1 + \zeta\left[1 + \frac{n^{in}(0)/K_1c - 1}{1-R}e^{-t/\lambda_1} - \frac{n^{out}(0)/K_1c - R}{1-R}e^{-t/\lambda_2}\right], \quad (30)$$

where we have introduced for curve-fitting purposes a dimensionless concentration of an amphipath

$$\zeta = c^{in}/c^*, \quad (31)$$

normalized to a characteristic concentration of an amphipath in the solution

$$c^* = \frac{1}{m_0 E a_0 K_1 (|1-R|)}. \quad (32)$$

The relative concentration ζ allows us a direct comparison of the effectiveness of different amphipaths on the MS channel activity independently of their individual properties. Comparison between Eqs. 30 and 17 shows that the last term in Eq. 30 is the dimensionless tension Γ^{amph} which varies with time. When an equilibrium is reached, the last time-dependent term in Eq. 30 becomes zero, so that $\Gamma^{amph} = \zeta$. Thus, the characteristic concentration c^* is a concentration that changes the equilibrium Boltzmann factor in Eq. 17 by unity. From here it follows, that by determining the relative concentration ζ from the Boltzmann fit we can easily obtain the characteristic concentration c^* for any amphipath.

Eq. 30 can be easily generalized for any system containing two types of amphipathic molecules. One has only to recall that the total concentration of amphipaths in the membrane is a sum of the two concentrations. In what follows, we will give some graphical examples of the time-dependent variation of the MS channel open probability after introducing or washing out one or two amphipaths from the bath solution. In these examples we will apply the presented theoretical model to experimental data, in which the MS channels were activated by two amphipaths, chlorpromazine and trinitrophenol (Fig. 5) (12).

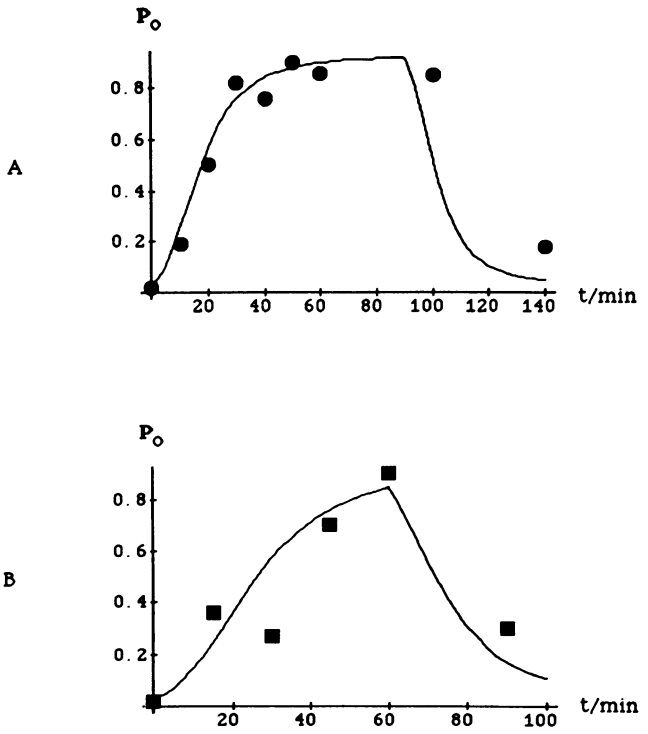

FIGURE 2 Single channel open probability as a function of time. (*A*) The cationic amphipath CPZ was introduced into the bath solution at time $t = 0$, and at $t = 90$ min was removed from the bath. These fitting parameters were used: $R \ll 1$, $\lambda_1 = 20$ min, $\Gamma^{mem} + b_1 = -3.5$, $c^* = 3.33$ μM, $c^{in} = 20$ μM and $\zeta_{CPZ} = 6$. (*B*) The anionic amphipath TNP was introduced into the bath solution at time $t = 0$ min, and at $t = 60$ min was washed out of the bath. The fitting parameters are: $R \gg 1$, $\lambda_2 = 30$ min, $\Gamma^{mem} + b_1 = -3.5$, $c^* = 83.3$ μM, $c^{in} = 500$ μM, and $\zeta_{TNP} = 6$. The experimental points (filled circles in (*A*), and filled squares in (*B*)) were taken from reference 12.

EXAMPLES

For the calculations of particular cases we shall need the numerical values of some parameters, such as the moments of switching different bath solutions t_1, t_2, relaxation times λ_1 and λ_2, equilibrium constant R, a dimensionless tension Γ^{mem} and b_1, and characteristic concentration c^*. They will be chosen by the curve-fitting procedure.

Introduction and subsequent removal of an amphipath

The cationic amphipath chlorpromazine (CPZ) was introduced into the bath solution at the time $t = 0$ and was washed out at 90 min (Fig. 2*A*). In accordance with the bilayer couple hypothesis (13) we supposed that CPZ partitioned mainly into the inner membrane monolayer. Hence, the term containing "slow" exponent $e^{-t/\lambda 2}$ disappears from the above equations and we are left with a single-exponential solution. Curve fitting permitted us to find the combination of tension parameters, $\Gamma^{mem} + b_1$, which in the particular case is equal to -3.5. The characteristic concentration c^* was found to be 3.33 μM, so that the relative concentration ζ in that case equals 6. The relaxation time was 20 min.

Fig. 2*A* shows that the open probability increased monotonously and then plateaued. After removing CPZ at 90 min, P_o returned close to the initial value, which demonstrates the reversibility of the effect.

A similar experiment with TNP is shown in Fig. 2*B*. According to the bilayer couple hypothesis TNP should preferentially partition into the outer monolayer, so that $R \gg 1$. This means that now the term containing "fast" exponent $e^{-t/\lambda 1}$ disappears from the above equations and

we are left with the "slow" one. TNP was introduced into the bath solution at the moment $t = 0$ and was washed out of the bath at the moment $t = 60$ min. Curve fitting gave the following parameters: $\lambda_2 = 30$ min, $\Gamma^{mem} + b_1 = -3.5$, $c^* = 83.3$ μM, and $\zeta = 6$.

Effect of amphipath concentration

Let us now consider the extent to which the time-dependent change of the channel open probability is dependent on amphipath concentration. Fig. 3 shows the variation of the open probability with time at two concentrations ($\zeta_1 = 2$ and $\zeta_2 = 10$) of an amphipath. The curves for both concentrations level off at the same plateau value. This relationship is true only if the amphipath concentration, $c > -c^* (\Gamma^{mem} + b_1)$ (Fig. 4 *A*). If $c < -c^* (\Gamma^{mem} + b_1)$, then the final plateau of the curve can be placed at different levels (Fig. 3 *B*).

The common plateau, presented in Fig. 3 *A*, corresponds to the open probability of 1. But this should not be necessarily so. If the adsorption of an amphipath is a saturable phenomenon and is governed by the Langmuir isotherm rather than by the simpler Henry isotherm used here, the plateau could be placed anywhere.

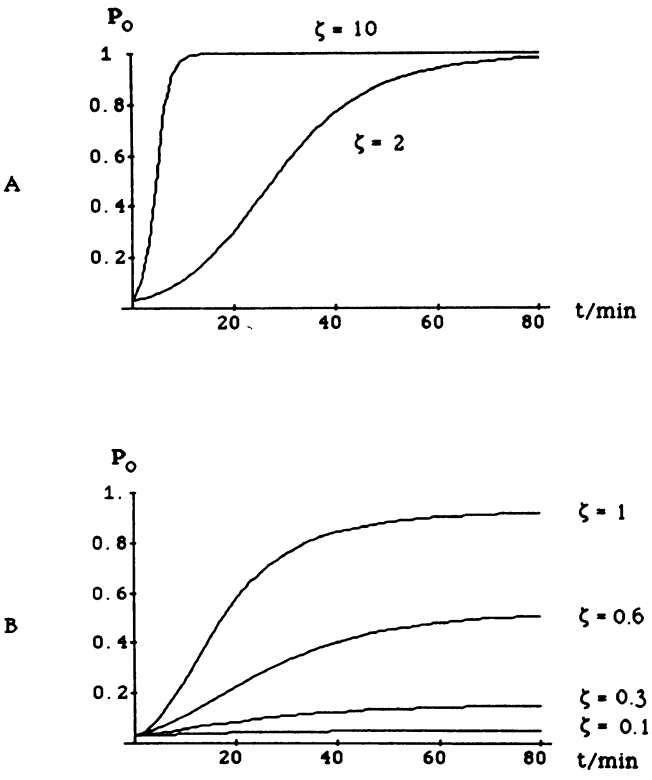

FIGURE 3 Time-dependent variation of the channel open probability at different concentrations of an amphipath. (*A*) c^{in} is large: $\zeta_1 = 10$ and $\zeta_2 = 2$. The fitting parameters are $R \ll 1$, $\lambda = 20$ min, $\Gamma^{mem} + b_1 = -3.5$. (*B*) c^{in} is small: $\zeta_3 = 1$, $\zeta_4 = 0.6$, $\zeta_5 = 0.3$, and $\zeta_6 = 0.1$. The parameters are $R \ll 1$, $\lambda = 20$ min, $\Gamma^{mem} + b_1 = -3.5$.

Compensation effect

We will now consider the effects of applying CPZ and TNP alternately or simultaneously. Our aim is to approximate the experiment shown in Fig. 5 *A*, where CPZ and TNP were applied alternately to the membrane patch containing two MS channels (12). We begin with the case where CPZ with the relative concentration $\zeta_{CPZ} = 6$ is introduced into the bath solution and then at the moment $t = 80$ min TNP is added to the solution at the same dimensionless concentration $\zeta_{TNP} = 6$ (Fig. 4 *A*). The open probability increases during the first 80 min and reaches a plateau. It decreases thereafter to the initial value. In this particular case we have a total compensation of the effects of the two amphipaths, due to their equal relative concentrations ζs. If the concentrations were different, we would expect only a partial compensation of the effects of the two amphipaths. Such a case is presented in Fig. 4 *B*, where the TNP concentration was assumed to be $\zeta_{TNP} = 2$. It would be interesting to investigate experimentally the extent of the compensation as a function of the relative amphipath concentrations.

Another program of amphipath applications is presented in Fig. 4 *C*. CPZ, with relative concentration $\zeta_{CPZ} = 6$, is introduced into the bath solution at the time $t = 0$ min and at the time $t = 80$ min is substituted by TNP at the same relative concentration $\zeta_{TNP} = 6$. As a result, the open probability increases during the first 80 min of the experiment and reaches a plateau. Thereafter, it begins to decrease, passes the minimum, and then increases again to the same plateau. In this case, however, the rate of the decrease of the open probability is larger than is the case for the examples shown in Fig. 4, *A* and *B*.

Finally, let us consider the experiment (Fig. 5 *B*) which should mimic the experimental protocol shown in Fig. 5 *A*. CPZ, with relative concentration $\zeta_{CPZ} = 6$, is introduced into the bath solution at the time $t = 0$ min, and then it is substituted by TNP with the same relative concentration $\zeta_{TNP} = 6$ at the time $t = 80$ min. Thereafter, TNP is substituted with CPZ ($\zeta_{CPZ} = 6$) at 170 min, and finally CPZ is washed out at $t = 250$ min. We obtained three oscillations of the open probability, a theoretical result, that is qualitatively the same as the experimental one in Fig. 5 *A*.

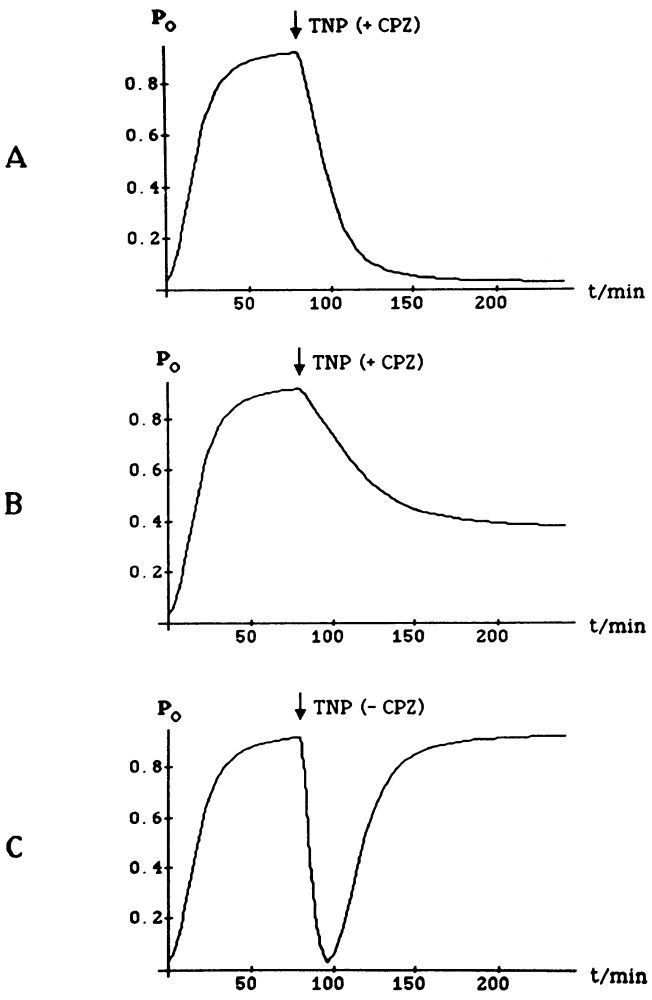

FIGURE 4 Time-dependent variation of the channel open probability in the presence of both amphipaths, CPZ and TNP. (*A*) CPZ is introduced into the bath solution at the time $t = 0$ min with the relative concentration $\zeta_{CPZ} = 6$. TNP is added to the bath solution at the time $t = 80$ min with the relative concentration $\zeta_{TNP} = 6$. (*B*) Same as in *A*, but the relative concentration of TNP is $\zeta_{TNP} = 3$. (*C*) CPZ is introduced into the bath solution at the time $t = 0$ min with the relative concentration $\zeta_{CPZ} = 6$ and is substituted by TNP at the time $t = 80$ min with the same concentration $\zeta_{TNP} = 6$.

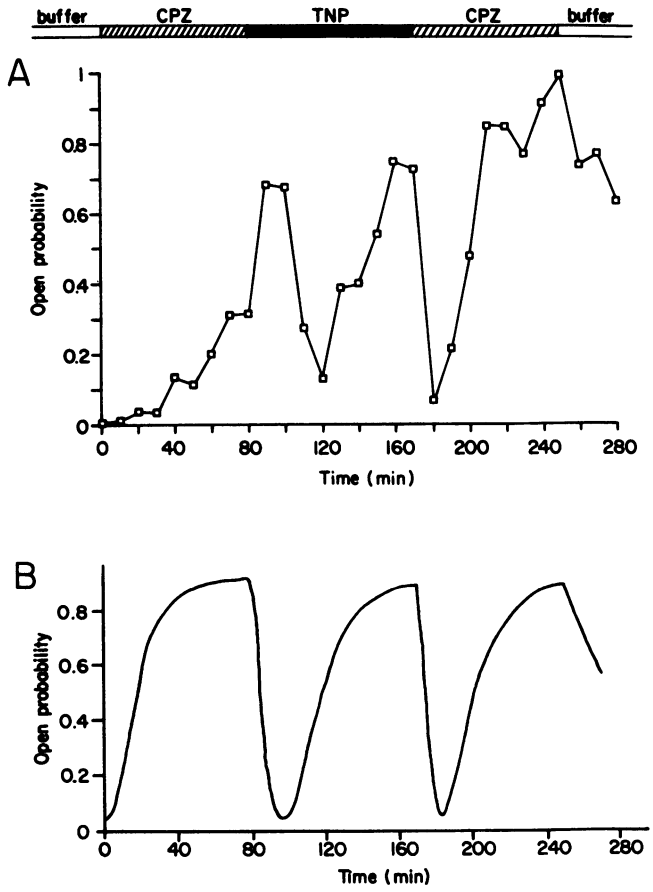

FIGURE 5 Effect of alternate exposure of the membrane patch to two amphipathic drugs with opposite electrical charges, CPZ and TNP, on time-dependent variation of the single channel open probability. (*A*) *Experiment.* The compensatory effect of 20 μM CPZ and 500 μM TNP on the open probability of the MS channel. The bath was alternately perfused either with buffer containing CPZ or TNP, or with buffer containing no drugs. Bars indicate the time points of each solution exchange. 2-min records of the channel activity were used for computation of the open probability. Two MS channels were active in the particular membrane patch. (Reproduced from reference 12.) (*B*) *Model.* CPZ is introduced into the bath solution at the time $t = 0$ min with the relative concentration $\zeta_{CPZ} = 6$. At the time $t = 80$ min CPZ is substituted by TNP with the same relative concentration $\zeta_{TNP} = 6$. Thereafter, CPZ is introduced again at the same relative concentration by substituting TNP at the time $t = 170$ min. At $t = 250$ min CPZ is washed out. The result of the applied protocol, which follows the experimental one, are three oscillations in the channel open probability.

DISCUSSION

The presented mathematical model relates the effect of amphipathic compounds on the MS channel open probability to the redistribution of mechanical tension between the two monolayers of the membrane lipid bilayer due to the uneven partitioning of the amphipaths into the monolayers. This model seems to provide the simplest hypothesis, which can account for the experimental observations reported on the effect of amphipaths on the MS channel of *E. coli* (12).

The model is based on the bilayer couple hypothesis (13) which usually considers the cell membrane as a closed double shell. In our case we are dealing with a patch of this membrane so that the conditions for the

conventional analysis in the framework of this hypothesis are not completely met. Nevertheless, the main deduction from this hypothesis, i.e., that the actual areas of two monolayers should remain equal, is probably preserved by two reasons. First, the transfer of lipids from one monolayer to another is hindered at the edges of the patch where it sticks to the glass due to the gigaseal formation. The lipids in this region are probably oriented with their polar heads toward glass wall of the patch pipette. Although the exact mechanism of formation of gigaseals is not known yet, and recently it was suggested that formation of the gigaseal may involve membrane proteins (20), high gigaseals (~50 GΩ) between patch pipettes and liposomes, which may also contain functional reconstituted MS channels (21), indicate that strong lipid-glass coupling should occur. Accordingly, our first assumption should be valid. Second, if the monolayers are not free to slide along one another, which is reasonable to assume, their areas automatically should remain equal. These considerations let us analyze the role of amphipaths in the framework of the bilayer couple hypothesis.

Two characteristic parameters originated from the theory. One is a characteristic concentration $c^* = 1/[m_0 E a_0 K_1(|1 - R|)]$, and the other one is a characteristic tension $\gamma^* = 1/m_0$. Both parameters give an increment in membrane tension, which changes the Boltzmann factor determining the channel open probability by unity. Let us consider a reasonable estimate for these parameters.

The characteristic concentration c^*, which was introduced here for the first time, is equal to 3.33 μM for CPZ. From here one can calculate the value of the parameter $a_0K_1 = 1/[c^* m_0 E(|1 - R|)]$, which is the product of the area per amphipath molecule in one monolayer and the partition coefficient between the inner monolayer and the bath solution. Taking the monolayer elasticity module E being equal to 0.1 N/m (17–19) and supposing $R \ll 1$, one obtains for a_0K_1 = 2,000 nm^3.

The product $a_0K_1c_{CPZ}$ gives the ratio of the area occupied by the CPZ molecules in the inner monolayer to the total area of the patch. In our experiment with c_{CPZ} = 20 μM this product is 2.4×10^{-2}. This means that relative compression of the inner monolayer as well as the relative extension of the outer monolayer is equal to 1.2%. This is well below the maximum strain of 3%, that can be tolerated by lipid bilayers (19).

Let us now estimate the effective tension γ^{amph} created in the membrane patch by adsorption of 20 μM CPZ under the experimental conditions reported for the MS channel of *E. coli* (12). Assuming that all CPZ molecules are concentrated in the inner leaflet ($n^{out} = 0$), Eq. 9 gives a value of 2.4 mN/m for γ^{amph}. This tension would correspond to the suction of ~35 mm Hg assuming a hemispherical shape of the membrane patch and taking into account that the diameter of the patch pipettes in our experiments was ~2 μm. Suction of 35 mm Hg was sufficient for 50% activation of an average MS channel of *E. coli* (Martinac, unpublished observation). Although without knowing the exact radius of the curvature of the membrane patch, the comparison between the tension and suction may seem meaningless; it gives, however an estimate of the magnitude of mechanical force which, according to the model, can be created in the patch by adsorption of amphipatic molecules.

A few details, however, did not fit into the presented simple scheme of our model. For example, in Fig. 5 *A* the kinetic curve does not deflect down immediately after the introduction of TNP, as should be the case according to the presented version of the model, but rather pops up in the beginning and thereafter deflects in the right direction. One possible explanation is that the rate of exchange between the monolayers R is even slower than assumed in the model, and therefore it manifests itself in this observation.

An amphipath which partitions evenly into the inner and the outer monolayer would not, in the simple version of the model, produce any effects on mechanosensitivity at equilibrium, although it could have some effects in the transitive period. An example of such an amphipath is lysolecithin (L-α-lysophosphatidylcholine), which is a polar, but neutral amphipath. Because lysolecithin was also shown to activate the MS channels of *E. coli* (12) (Martinac, unpublished observation), we shall have to take into consideration some additional factors.

One of the possible effects related to the amphipaths like lysolecithin comes from the molecular shape of the amphipath. A molecule of lysolecithin has a form of a cone. Hence it produces bending deformation in the monolayer into which it partitions (20, 21). Cardiolipin, an inverted cone, is another amphipath producing in a monolayer a bending deformation of opposite sign (21). It would be very interesting to investigate if this bending deformation has anything to do with mechanosensitivity, or does only area expansion of the monolayers play a role. Future experiments will investigate such a possibility. These experiments may help us to understand the mechanism of membrane mechanosensitivity at the molecular level.

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