Gating-by-Tilt of Mechanically Sensitive Membrane Channels

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(Received 20 November 2003; published 10 September 2004)

We propose an alternative mechanism for the gating of biological membrane channels in response to membrane tension that involves a change in the slope of the membrane near the channel. Under biological membrane tensions we show that the energy difference between the closed (tilted) and open (untilted) states can far exceed k_BT and is comparable to what is available under simple dilational gating. Recent experiments demonstrate that membrane leaflet asymmetries (spontaneous curvature) can strongly affect the gating of some channels. Such a phenomenon would be easier to explain under gating-by-tilt, given its novel intrinsic sensitivity to such asymmetry.

DOI: 10.1103/PhysRevLett.93.118103 PACS numbers: 87.15.He, 87.15.Kg, 87.16.Xa

The correct biophysical function of mechanically sensitive membrane channels, such as the widely studied MscL [1–3], is vital in maintaining the viability of living cells. These channels are typically made up of a barrel-like assembly of transmembrane proteins [4]. Fluid flows through a central pore in the open (active) state but is either restricted or suppressed entirely in the closed (inactive) state. Mechanosensitive channels play important sensing roles in touch, hearing, turgor control in plant cells, etc. [5–10]. The fact that MscL functions in reconstituted membranes [2] is good evidence for a gating mechanism that depends only on membrane tension, rather than cytoskeletal effects [11] or signalling cascades.

We propose a new mechanism for the gating of biological membrane channels in response to elevated membrane tensions, which we refer to throughout as gating-by-tilt; see Fig. 1. The elevated membrane interfacial tension that acts to open the channel can be generated in several ways including via an osmotic imbalance between the cell interior and exterior or by changes in the cells morphology during adhesion, filipodia formation, etc. [12].

The scheme sketched in Fig. 1 is very similar to the transition between the open and closed structures of potassium channels that resemble a truncated cone in their closed state and splay open [13], with the transmembrane helices tilting by up to 30°. There has also been some discussion of similar schemes elsewhere [14,15]. Strikingly, there is almost negligible dilation of the exterior side of the channel, which seems to act as a hinge. Such a process strongly supports the present model and is inconsistent with the existing paradigm of simple dilation. Reference [13] is primarily interested in the role of the splay of transmembrane helices in voltage gating of the channel, but there is evidence that such conformational changes also appear to couple to the tension [16], in a way that we analyze below. It is worth noting that the correct function of ion channels is sensitive to very small structural changes, e.g., in tilt. There have also been reports of mechanosensitive response in Na [17] and Ca [18] channels.

The gating of Ms mechanosensitive channels has been probed by patch clamp experiments (see, e.g., [6] and references therein) and has been studied fairly extensively by "steered" simulations; see, e.g., Ref. [19]. However, both the experimental techniques and the simulations have limitations that make it difficult to be conclusive concerning the gating mechanism. The simulations are of very short duration, typically a few nanoseconds, while it is known that gating takes milliseconds to occur after a step change in the patch clamp pressure that controls membrane tension [6]. It is common practice in these simulations to, e.g., apply thermodynamically large artificial forces to the membrane or channel, in order to

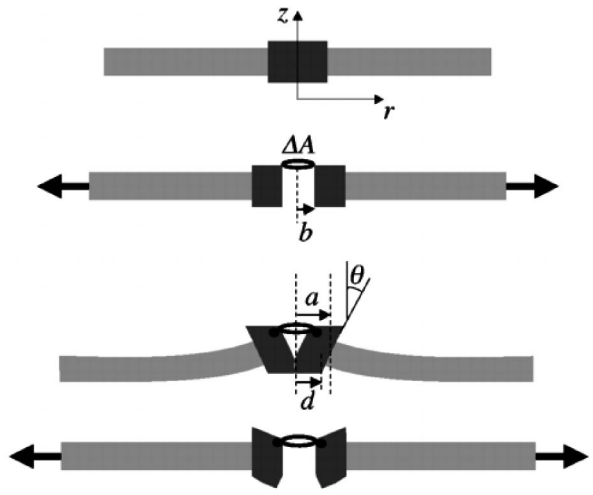

FIG. 1. Idealized sketch of a cylindrically symmetrical membrane channel showing two schemes for tension mediated gating. In both cases the conformational changes are exaggerated for clarity. The upper two images show the closed and open states of the channel under the dilational gating model and the lower two images the same for gating-by-tilt. Under both schemes the membrane tension does work by increasing the combined projected area of the membrane and channel.

induce it to open on the available time scales. It is therefore not clear that such approaches are able to differentiate between certain models for how these channels gate. An aim of this Letter is to propose a new paradigm for consideration in future simulations. Patch clamp experiments essentially measure the tension at which the channel opens and the size of its open pore by way of conductivity measurements [6,20,21]. It is not clear how these experiments can be used to differentiate between different gating models.

During the opening of the channel the membrane tension does work to change the channel's conformation. The conformational free energy of the channel itself (and alone) changes by an amount $\Delta G_{\text{channel}} \equiv G_{\text{open}} - G_{\text{closed}}$ which is positive, indicating that the closed state has a lower conformational free energy. The existing paradigm for tension-gated channels involves a simple dilational transition from the closed to the open state with an associated increase in total channel area ΔA [6]. This is the increase in the total effective "footprint" of the channel within the membrane. The change in free energy is proportional to this and to the membrane tension γ,

$$\Delta G_{\text{dilate}} = \gamma \Delta A, \tag{1}$$

and is the only energy that is available to overcome the change in conformational free energy of the channel $\Delta G_{\text{channel}}$.

The proposed gating-by-tilt is driven by changes in the slope of the membrane where it meets the channel. The simplest version of this involves a constant membrane slope θ in the closed state which relaxes ($\theta \to 0$) after opening; see Fig. 1. A central pore could be opened in association with such a transition. In practice a hybrid mechanism may operate in which there is both some change in tilt and some dilation. Other workers have attempted to study how changes in the lateral pressure profile of the channel might also affect gating [22,23], although, in what follows, we restrict our attention to effects that depend directly on the membrane tension.

We argue that it is *possible* for the action of the membrane tension γ to efficiently gate the channel provided the work done by the membrane ΔG_{memb} is large enough:

$$\Delta G_{\text{memb}}(\gamma) \gtrsim \Delta G_{\text{channel}} \gtrsim k_B T, \tag{2}$$

where $\Delta G_{\text{memb}} = \Delta G_{\text{dilate}}$ or ΔG_{tilt}, or a combination of the two. The criteria of Eq. (2) equate to a channel that *can* be substantially closed below tension γ and substantially open above it. It is reasonable to assume that nature has been able to evolve a channel with a configurational energy change that is roughly optimal for gating at the desired tension, in which case one expects $\Delta G_{\text{memb}} \approx \Delta G_{\text{channel}}$. If $\Delta G_{\text{memb}}/k_B T$ is smaller than unity, the energy available from the membrane tension is inadequate to overcome any activation barrier that is greater than the thermal energy scale, and so the channel is either predominantly closed, if $\Delta G_{\text{channel}} > k_B T$, or opens and closes rather randomly, if $\Delta G_{\text{channel}} < k_B T$. From Eq. (2), and the arguments above, it is possible for a channel that opens by gating-by-tilt to efficiently gate the channel at tension γ when the parameter controlling this efficiency $\Delta G_{\text{tilt}}(\gamma)/k_B T \gtrsim 1$. We now proceed to calculate the energy ΔG_{tilt}.

The Hamiltonian for membrane that is asymptotically flat, with normal parallel to the z axis, but which has a small normal deviation from flatness, of magnitude $u(\mathbf{r})$ due, e.g., to the presence of a membrane channel, is given by [24]

$$H = \frac{1}{2} \int d^2\mathbf{r} [\kappa(\nabla^2 u)^2 + \gamma(\nabla u)^2], \tag{3}$$

where κ is the membrane rigidity, $\mathbf{r}$ is the radial position with $r = |\mathbf{r}|$ (see Fig. 1), and ∇ is the two-dimensional (plane polar) version of the operator. The Gaussian rigidity $\bar{\kappa}$ may here be neglected as it contributes only a constant energy difference between the open and closed states [25]. This can be absorbed into $\Delta G_{\text{channel}}$ and, being insensitive to γ, does not affect the mechanical sensitivity of the channel. The total energy associated with distortion of the membrane can be established by a variational approach on Eq. (3) and depends on the boundary conditions for the membrane. Up to unimportant global rotations or translations of the entire frame, the displacement of membranes that are asymptotically flat at infinity is found to be

$$u = \alpha K_0(kr) + \beta \log kr \tag{4}$$

for $r \geq a$ where α and β are yet undetermined constants, $k \equiv \sqrt{\gamma/\kappa}$ is an inverse length characteristic of the membrane, and K_0 is the usual modified Bessel function of the first kind of order zero. It can be shown that solutions of the form of $u \sim \log kr$ correspond only to channels that exert a finite integrated normal force on the membrane [26], e.g., by anchoring onto the cytoskeleton or an external substrate. Thus for channels that exert no overall normal force we have $u = \alpha K_0(kr)$ where the constant α is fixed by a boundary condition corresponding to the angular tilt at the periphery of the channel $\nabla u(r = a) = -\alpha k K_1(ka) = -\theta$. Hence

$$u = \theta K_0(kr)/(kK_1(ka)) \quad \text{for } r \geq a, \tag{5}$$

where the resulting normal membrane deviation (a few nm or less) falls to zero over a distance $\sim k^{-1}$ from the edge of the channel. This approach therefore involves the assumption that the membrane (slope) is strongly anchored to the outside of the channel [28]. The free energy difference ΔG_{tilt} between a channel in the closed state and one in the open state ($\theta \to 0$, say) follows by substitution of Eq. (5) into Eq. (3) integrated over $r > a$.

$$\Delta G_{\text{tilt}} = \pi \kappa k a \theta^2 \frac{K_0(ka)}{K_1(ka)}. \tag{6}$$

A quantitative comparison with ΔG_{dilate} is shown in Fig. 2.

For steady state physiological tensions in the range $\gamma = 10^{-5}$–10^{-4} N/m one has $0.03 < ka < 0.1$, and the system is quantitatively inside the regime $ka \ll 1$ in which ΔG_{tilt} can be shown to have the following analytic approximation:

$$\Delta G_{\text{tilt}} \approx \pi\gamma a^2\theta^2\left[\log\frac{2}{ka} - \Gamma\right] \quad \text{for } ka \ll 1, \qquad (7)$$

where the Euler constant $\Gamma \approx 0.6$ and, at the highest gating tensions, ka can approach unity. Interestingly Eqs. (1) and (7) appear to be very similar, each being the product of an area change and the tension γ except for the factor of $\log\frac{2}{ka}$ appearing in Eq. (6).

In order to test whether gating-by-tilt is indeed a plausible mechanism that may contribute to ΔG_{memb}, we use data collected for various mechanosensitive channels [20], from which estimates for the area change ΔA, gating tension, and conformational energy change ΔG have been obtained, assuming dilational gating. This gives us enough information to calculate the corresponding value for ΔG_{tilt} from Eq. (6), assuming instead a purely gating-by-tilt scheme that occurs at the same tension. Since this energy depends on the angle θ, a sensible approach seems to be to ask instead what value of θ would be required to *entirely* account for the free energy change ΔG (previously estimated assuming dilational gating) and also what angle would be required to account for (say) 10% of it. We denote these angles θ_{100} and θ_{10}, respectively. If a channel opens, via gating-by-tilt, to expose a pore of area $\Delta A = \pi b^2$, then we impose the boundary condition that the membrane slope is θ in the closed state at radial distance $r = a = d + b/2$ from the central (z) symmetry axis of the pore, where d is the thickness of the "walls" of the channel; see Fig. 1. This is a somewhat arbitrary, although reasonable, choice for a, which is merely the radial distance to the exterior of the channel in the closed state. For the six channels for which data are available [20] we find (taking $d = 2$ nm) that $39° > \theta_{100} > 16°$, while $12° > \theta_{10} > 5°$ is correspondingly smaller. We find that the smallest values of these angles both correspond to the channel known as MscMJ, which gates at the lowest value of ΔG_{tilt}, although *not* the lowest membrane tension. This may therefore represent the best candidate for future simulation or experimental studies on channels that gate by tilt. The above angles are (all) rather small, and thus we have demonstrated that a small change in the tilt of the membrane during gating can provide a significant contribution to the free energy change ΔG_{memb}.

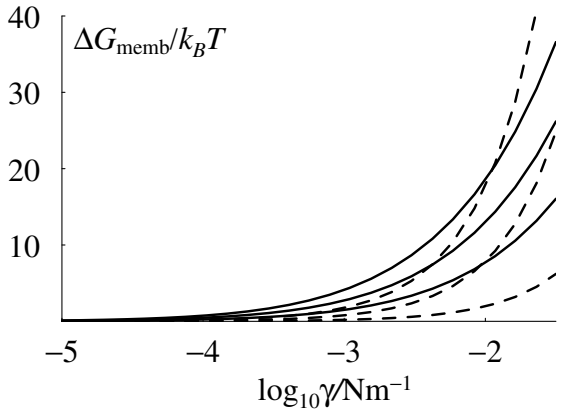

FIG. 2. The work available from the coupling of the membrane tension to a conformational change of a membrane channel ΔG_{memb} is plotted against the logarithm of the membrane tension γ. The dashed lines correspond to the energy change ΔG_{dilate} available from dilational opening (1) and the solid lines the energy ΔG_{tilt} available from gating-by-tilt (6). Three curves are shown in each case corresponding to different values of the channel size. For dilational gating (dashed curves) the radius of the central pore in the open configuration is, from upper curve to lower curve, $b = 1.5, 1, 0.5$ nm, while for gating-by-tilt the radial distance of the exterior of the closed channel is, from upper curve to lower curve, $a = 4, 3, 2$ nm. These represent biologically reasonable sizes [20,31]. In this illustrative example we have taken the change in tilt to be $\theta = \pi/6 = 30°$ and the membrane rigidity $\kappa = 20k_BT$. The viability of the gating process relies on the value of the parameter $\Delta G_{\text{memb}}/k_BT$ being larger than unity, as discussed in the text. Steady state biological membrane tensions are in the 10^{-5} to 10^{-4} N/m range [32] while Msc channels can gate at tensions up to $\gtrsim 10^{-2}$ N/m [20].

It has also been observed that the composition of lipids in the membrane can have a significant effect on the gating of MscL channels [22]. It is possible to construct arguments that can explain a dependence on membrane composition in terms of mismatches between the membrane (thickness) and the channel geometry, or the pressure distribution exerted by the membrane interior on the edge of the channel. However, the dramatic effects recently observed after addition of "conical" lysophophatidylcholine (LPC) lipids to one leaflet of the membrane [22] are generically difficult to explain within such models. In these experiments it was observed that the channels open, even under small applied tensions, only if the conical lipids are localized in one leaflet, giving rise to an asymmetry that tends to make the membrane prefer to bend into a convex shape, away from the LPC rich face, rather than the opposite concave one. It is possible to analyze this difference within a model that has the LPC homogeneously distributed across the membrane or, experimentally, the membrane patch that is clamped. In this case the Hamiltonian for the membrane includes the so-called spontaneous curvature c_o, which is a function of the difference in LPC concentrations across the inner and outer leaflets of the membrane. If the LPC is localized in the upper leaflet, $c_o < 0$. When the spontaneous curvature is small, $c_o^2 \ll k^2$, one obtains [24]

$$H = \frac{1}{2}\int d^2\mathbf{r}[\kappa(\nabla^2 u - c_o)^2 + \gamma(\nabla u)^2]. \qquad (8)$$

The corresponding minimum energy solution Eq. (5) for

the displacement is unchanged under these conditions. However, the total free energy of the membrane can be shown to be $\Delta G_{\mathrm{memb}} = \Delta G_{\mathrm{tilt}} + \Delta G_{c_o}$ with ΔG_{tilt} as before (6) and

$$\Delta G_{c_o} = 2\pi c_o \kappa \theta a. \tag{9}$$

This free energy difference between the open and closed states can be positive, favoring opening, or negative, helping to maintain a closed channel if $\theta > 0$ or $\theta < 0$, respectively. The sign of θ depends on whether the channel is oriented either "up" or "down." In experiments on reconstituted membranes it is very likely that the channels are in both orientations. Equation (9) suggests that for moderate spontaneous curvatures $c_o = 1/(10a)$ the up channels (say) are driven to open by an energy difference $\Delta G_{c_o} \approx 7k_BT$, even in the absence of any tension. This gives a Boltzmann weight of about 1000, with a corresponding enhancement in the number of open channels. This could help to explain why some channels isolated in a patch clamped membrane are observed to remain open under these conditions, even under small tensions (pressure differences) [22]. Indeed, these authors independently suggest that, "The asymmetry of the lateral pressure profile between the two leaflets of the bilayer is what actually initiates the sequence of mechanical transduction steps that leads to the open state." We argue that such effects are highly suggestive of a role of the gating-by-tilt mechanism proposed here.

In summary, we propose that gating-by-tilt should be considered a viable mechanism for channel gating, particularly in view of its similarity with, e.g., the gating of certain K channels [13]. Under gating-by-tilt, the tilting walls of the channel can be attached by a hinge to a fairly rigid frame, from which they swing open. This suggests that gating-by-tilt may have further inherent design advantages over dilational gating in which the *entire* channel must move (dilate).

Note added.—Since preparing this work we have learned of an independent model [33] in which channel tilt remains fixed throughout channel opening, in which case the tilt uniformly favors the closed (undilated) state. This is fundamentally different from the present work in which we propose tilt *variation* as the gating mechanism.

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