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Letter to the Editor

Models for Ion Channel Gating with Compliant States

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In a previous letter to the editor, Sachs and Lecar (1991) described a model for gating of mechanosensitive ion channels. This treatment draws on our "gating-spring" model for gating, which involves an elastic element that conveys force to the channel (Corey and Hudspeth, 1983; Howard and Hudspeth, 1988) and extends it to deal with the elasticity of the channel protein itself. The gating-spring model predicts that the difference in energy between the open and closed states of the channel depends linearly on applied force, whereas quadratic terms arise if the open and closed states of the channel do not have the same elasticity. Their letter provides a valuable reconciliation of divergent theories (Corey and Hudspeth, 1983; Howard and Hudspeth, 1988; Guhary and Sachs, 1984; Morris, 1990), but it stops short of estimating the relative importance of the linear and quadratic terms. In this letter, we show that the theoretical assumptions made by Sachs and Lecar imply that the quadratic term is small and probably negligible. An even more general model has recently been published by Lecar and Morris (1993), in which large quadratic terms are at least theoretically possible. Here we point out published experimental evidence that indicates that the quadratic term is small. We conclude that the simple gating-spring model remains an adequate description of the gating of mechanically sensitive ion channels.

To reiterate the two theories, we refer to Figs. 1 and 2 (cf. also Fig. 1 of Sachs and Lecar). In a simple two-state theory, we suppose that there is a closed state and an open state (Fig. 1 *A*). If a constant force f is applied along the reaction coordinate (Fig. 2), then the energy U of the channel in a state i is equal to the integral of the force, from an arbitrary zero to the position x_i of that state on the reaction coordinate

$$U_i = U_i^0 + \int_0^{x_i} -f\,dx \qquad (1)$$

so

$$U_c = U_c^0 - fx_c \quad \text{and} \quad U_o = U_o^0 - fx_o \qquad (2)$$

where U_i^0 is the energy of the state in the absence of an applied force. This is represented graphically by summing a straight line with slope f to the energy diagram. The energy difference between states is then

$$\Delta U = U_o - U_c = -fb + \Delta U^0 \qquad (3)$$

where b $(= x_o - x_c)$ is the movement in the direction of the force in going from closed to open; it is the swing of the gate. We can assume, without loss of generality, that the open and closed states have the same energy in the absence of an applied force ($\Delta U^0 = 0$). As usual, the probability of being open is a Boltzmann distribution between the two states, so that

$$p_o = \frac{1}{1 + e^{\Delta U/k_BT}} \qquad (4)$$

where k_BT is equal to 4.1×10^{-21} joule at room temperature. Thus, for instance, if the gate moves by $b = 4$ nm in going from closed to open, as estimated for hair cell transduction channels (Howard and Hudspeth, 1988) then a force of 1 pN causes an energy difference of 4×10^{-21} J, or about 1 k_BT. In the most sensitive range, 1 k_BT would change open probability by about 24%.

Sachs and Lecar (1991) have extended this treatment to allow each state to be elastic (Fig. 1 *B*), so that each state is described by a parabolic energy well (Fig. 2) instead of a fixed point. The principal change is that an applied force can now move the average position of the channel within a state, introducing an elastic energy term. Although Sachs and Lecar have used the Hamiltonian of a harmonic oscillator in an energy well to calculate the energies of the states, their assumption of normal temperature ($k_BT \gg h\nu$) means that the problem can be treated classically; the solution is the same as if they simply took the energy minimum of each state as the energy of that state. In Fig. 2, it can be seen that adding a constant force term shifts the profile of the well slightly, so that the energy minimum in each state moves along the reaction coordinate. Under an applied force, the position of the minimum moves by a distance

$$\Delta x_i = f/k_i \qquad (5)$$

where k_i is the spring constant of the state. A stiffer state is represented by a steeper well, as on the right in Fig. 2, and the minimum will move less under an applied force. The minimum energy of a state is then calculated as

$$U_i = \int_0^{x_i+\Delta x_i} -f\,dx + \int_{x_i}^{x_i+\Delta x_i} k_i x\,dx \qquad (6)$$

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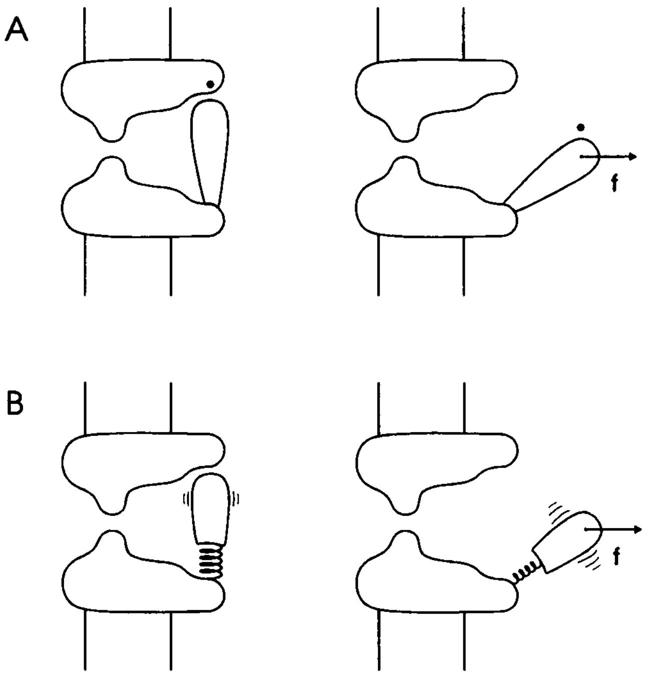

FIGURE 1 Models for gating of an ion channel by mechanical force. (*A*) The gating model of Corey and Hudspeth (1983) and Howard and Hudspeth (1988). The channel is assumed to have two conformations, closed (*left*) and open (*right*), which are in thermal equilibrium. Because the gate swings through a distance *b* upon opening, an external force (*f*) changes the energy difference between open and closed states and can bias the channel to spend more time in its open state. In hair cells the force is thought to be applied via an elastic element, the gating spring, which may correspond to the "tip links" connecting adjacent stereocilia (Assad et al. 1991). For simplicity, the gating spring is omitted. (*B*) The Sachs and Lecar model (Sachs and Lecar, 1991; Lecar and Morris, 1993). It is postulated that there are two independent differences between the closed and open states: in addition to the change in position of the gate, *b*, the channel has a different elasticity in the two states. Within a state, the gate has some freedom to move, determined by the elasticity. Although the channel elasticity is denoted as a spring in the hinge for illustration, the closed-open transition could be physically associated with the melting of a rigid coiled-coil to produce a more flexible molten globule. The application of a very large force to the channel would favor the more flexible state, whether open or closed and regardless of the direction of force.

where the first term represents the energy at the position of the minimum with an applied force, and the second term represents the elastic energy in the channel protein. Then

$$U_i = -fx_i - f\Delta x_i + \tfrac{1}{2}k_i\Delta x_i^2 \qquad (7)$$

which can be rewritten as

$$U_i = -fx_i - \frac{f^2}{2k_i}. \qquad (8)$$

The energy difference between states is then

$$\Delta U = U_o - U_c = -fb - \frac{f^2}{2}\left[\frac{1}{k_o} - \frac{1}{k_c}\right], \qquad (9)$$

which is comparable to Sachs and Lecar's Eq. 4. It can be seen that a quadratic term in force arises if the states are not of equal stiffness.

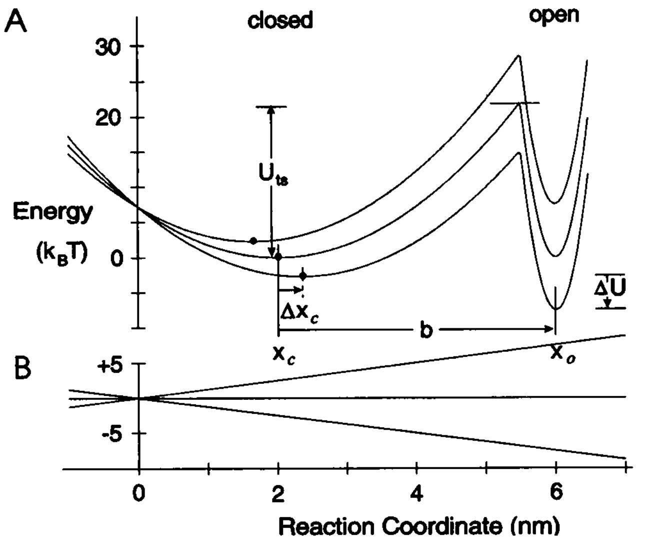

FIGURE 2 (*A*) Energy profile of a mechanically gated channel, under the influence of a force applied to the gate. The channel is assumed to have two states, closed and open, with different compliances and a single energy barrier to transitions between states. (*B*) An imposed force adds a linear energy term to the profile, which changes the energy difference between closed and open states. As drawn, the variables are k_c = 14.3 mN/m, k_o = 717 mN/m, U_{ts} = 22 k_BT, and b = 4 nm. The profiles are shown for forces of −5, 0, and +5 pN.

We now show that the assumption made by Sachs and Lecar regarding the height of the barrier or transition state implies that the quadratic term in Eq. 9 is negligible compared with the linear term. The relative importance of the quadratic term obviously depends on the elasticity of the states; a very compliant state (small k_i) will allow the minimum to move a large fraction of the gating distance *b* and will give a larger quadratic term. A rough estimate is obtained if we take Sachs and Lecar's assumption of parabolic wells, i.e., that the compliance is constant up to the transition state as in Fig. 2. Then the requirement for a reasonable transition state height U_{ts} puts a lower limit on k_i. Because

$$U_{ts} \le \frac{1}{2}k_i b^2, \qquad \text{we see} \qquad k_i \ge \frac{2U_{ts}}{b^2}. \qquad (10)$$

For instance, a transition state height of 22 k_BT, which gives open times of about a millisecond (assuming a frequency factor of k_BT/h in the rate equation; Corey and Hudspeth, 1983), requires that $k_i > 11$ mN/m if $b = 4$ nm. At the extreme, where the open state is not at all compliant and the closed state has the maximum compliance as above, Eq. 9 becomes

$$\Delta U = -fb + \frac{f^2b^2}{4U_{ts}}. \qquad (11)$$

If the first term $fb = 5\ k_BT$ (a nearly saturating energy difference) and if $U_{ts} = 22\ k_BT$, then the quadratic term is about 5% of the total. For smaller forces the quadratic term is even smaller. This is illustrated graphically in Fig. 2, where the compliance of the closed state is assumed to be 50 times greater than that of the open state. The three energy profiles represent energy differences ΔU of -5, 0, and $+5\ k_BT$. Even in this extreme example, movement of the energy minimum is not remarkable. Fig. 3 then shows the probability of being open as a function of force. The solid line shows the predictions of our gating-spring theory, without compliant states, whereas the dashed line incorporates the quadratic term. Note that the quadratic term has a very small effect on the open-probability curve; it would be nearly undetectable in experimental measurements of open probability against force or displacement.

Lecar and Morris (1993) generalized the Sachs and Lecar model to remove the transition state assumption. In this new, more complex model, a large or even dominating quadratic term is formally possible, though the gating mechanism is obscure. The best way to test this model is to compare it with data. It happens that, at least for hair-cell transduction channel gating, three independent lines of evidence show that the quadratic term for these channels is small. First, if the closed state is more compliant, increasing the force would open channels, to a point, but with further increase of force the energy minimum of the closed state would actually move past the minimum of the open state, and channels would close again (Eqs. 4 and 9). Such behavior has not been observed for any mechanically sensitive channel. Also, the open probability would never reach 100%. In fact, Holton and Hudspeth (1986) showed that large negative displacements left less than 1% of the channels open, whereas large positive displacements opened at least 99% of the channels. We can calculate that the quadratic term must be less than 28% of the total, at least for reasonable forces ($fb < 5k_BT$). Second, Howard and Hudspeth (1988) directly measured the stiffness of the hair bundle when almost all the channels were closed and also when almost all the channels were open. The stiffnesses differed by less than 10%. Moreover, this difference was less than 20% of the stiffness change associated with the gating of the transduction channels. For all stimuli up to saturation ($fb = 5k_BT$) this implies that the quadratic term is less than 13% of the total even under the assumption that all the bundle's compliance resides in the channels themselves. Finally, Howard and Hudspeth (1988) showed that the position of minimum stiffness of a hair bundle is nearly equal to the position of maximum sensitivity (measured as the slope of $P_{open}(x)$). The difference of these two positions corresponds to a difference in open probability of less than 0.1, which in turn implies that the quadratic term is less than 25% of the linear term (for $fb < 5k_BT$).

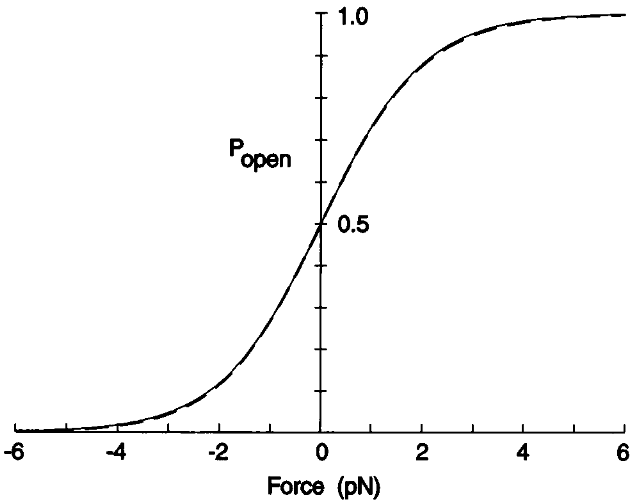

FIGURE 3 Open probability as a function of imposed force, for the simple gating-spring model (———) and for the compliant state model (— —). Variables are as in Fig. 2.

Recently, Markin et al. (1993) and Jaramillo et al. (1993) have found that the stiffness of the saccular hair bundle at large positive displacements is less than that at large negative displacements, by as much as 35%. Russell et al. (1992) have also described an increased stiffness at negative displacements that does not correspond to a channel-opening transition. Markin et al. have interpreted their data by postulating the existence of a second, "latched" closed state with a smaller compliance than either the unlatched closed state or the open state. But in their model, the gating transition between the unlatched closed state and the open state is still driven by the gating displacement ($b = x_o - x_c$) rather than a difference in compliance. That is, the linear term is more important for the opening transition. A different interpretation is that there are two open states with different gating displacements and that the positive displacements used were not large enough to pull the channels completely into the second open state. A third interpretation is that the lower stiffness in the positive direction is an artifact that is the result of poor coupling between the probe and the bundle. In no case is there strong reason to think that differences in channel compliance play a major role in gating.

Thus for hair cells, the quadratic models of Sachs and Lecar (1991) and Lecar and Morris (1993) are unnecessary. The quadratic term is certainly less than 25% of the linear term, even for forces that would open or close essentially all (> 99%) of the channels. For smaller forces, the quadratic term is much smaller. For mechanically gated channels in general the quadratic term is negligible even for large differences in compliance of the open and closed states. Hence, what is crucial for gating is the swing of the gate (b) rather than the difference in state compliance. A compliance difference will have only small effects on the estimates of the swing of the channel's gate (Howard and Hudspeth, 1988). Finally, this analysis holds as well for voltage-gated channels, where the force results from the electric field acting on a charged portion of the channel protein.

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