CHAPTER 11

Lipid Stress at Play: Mechanosensitivity of Voltage-Gated Channels

Catherine E. Morris and Peter F. Juranka

Neuroscience, Ottawa Health Research Institute, Ottawa Hospital, Ottawa, Ontario K1Y 4E9, Canada

I. Overview
II. The System Components
  A. The Channel Proteins
  B. Bilayer
  C. Accessory Proteins
III. Big Picture Issues
  A. Bilayer Mechanics and VGCs
  B. Prokaryotic VGCs as Ancestral Lipid Stress Detectors?
  C. MS VGCs and MS TRP Channels: Sharing Insights
  D. No MS "Motif" Required: Just Say HMMM
  E. An Imperturbable K-Selective Pore Surrounded by MS Voltage Sensors?
  F. Alcohol and VGCs: Binding Sites or Bilayer Mechanics?
IV. Reversible Stretch-Induced Changes in Particular VGCs
  A. Kv Channels
  B. Cav and Kv3 Channels Have Similar Stretch Responses
  C. Cav: L-Type Channels in Native Preparations
  D. Nav Channels
  E. HCN Channels
V. Irreversible Stretch-Induced Gating Changes in VGCs
VI. Technical Issues
  A. Applying a Stretching Force to Study MS Modulation of VGC Activity
  B. Gadolinium Strangeness
VII. Summary Comments
  References

Current Topics in Membranes, Volume 59 1063-5823/07 $35.00
Copyright 2007, Elsevier Inc. All right reserved.
DOI: 10.1016/S1063-5823(06)59011-8

I. OVERVIEW

Membrane stretch modulates the activity of voltage-gated channels (VGCs). These channels are nearly ubiquitous among eukaryotes and they are present, too, in prokaryotes, so the potential ramifications of VGC mechanosensitivity are diverse. *In situ*, traumatic stretch can irreversibly alter VGC activity with lethal results (Iwata *et al.*, 2004), but that is pathology. Of wider biological interest is the inherent reversible stretch sensitivity of all VGC subclasses. Evidently, voltage sensor motions feel the impact of bilayer stretch, with the consequence that reversible stretch-induced changes in ionic current can be seen in recombinant systems for at least some members of the major types of VGC (Kv, Cav, Nav, HCN). Gating current has yet to be tested with stretch, but kinetic dissection of ionic currents in Kv channels shows that the rates of both independent and concerted voltage sensor motions change with stretch, while the quantity of charge that moves stays fixed (Laitko and Morris, 2004; Laitko *et al.*, 2006). In native preparations, the mechanosensitive (MS) transitions of VGCs could contribute physiologically in mechanoelectric feedback (e.g., in cardiac and smooth muscle), neuronal mechanosensing (e.g., osmosensing, tactility), and so on, but reports on stretch effects in native cells, while tantalizing, are spotty. Experimentally, a fundamental problem is the impossibility of selectively inhibiting the MS portion of a VGC's response.

This chapter deals principally with the reversible responses of VGCs to stretch, with the general relation of stretch stimuli to other forms of lipid stress, and briefly, with some irreversible stretch effects (=stretch trauma). A working assumption throughout is that MS VGC motions (i.e., motions that respond reversibly to bilayer stretch) will be susceptible to other forms of lipid stress such as the stresses produced when amphiphilic molecules (anesthetics, lipids, alcohols, lipophilic drugs) are inserted into the bilayer. Insofar as these molecules change the bilayer's lateral pressure profile (Cantor, 1997, 1999), they can be termed bilayer mechanical reagents (BMRs).

Another aim of this chapter is to delineate MS VGC behavior against the backdrop of eukaryotic channels more widely accepted as "MS channels," namely the TRP-based MS cation channels (Kung, 2005; Maroto *et al.*, 2005). We start with some "big picture issues" then focus briefly on particular MS VGCs. A few technical items about recording conditions are inserted (some readers may wish to check this first) before a summary comment.

II. THE SYSTEM COMPONENTS

Stretch produces both elastic and plastic changes to membrane structures. Figure 1 briefly inventories the membrane constituents relevant to VGC activity and stretch; as applied to membranes, whether they be native or

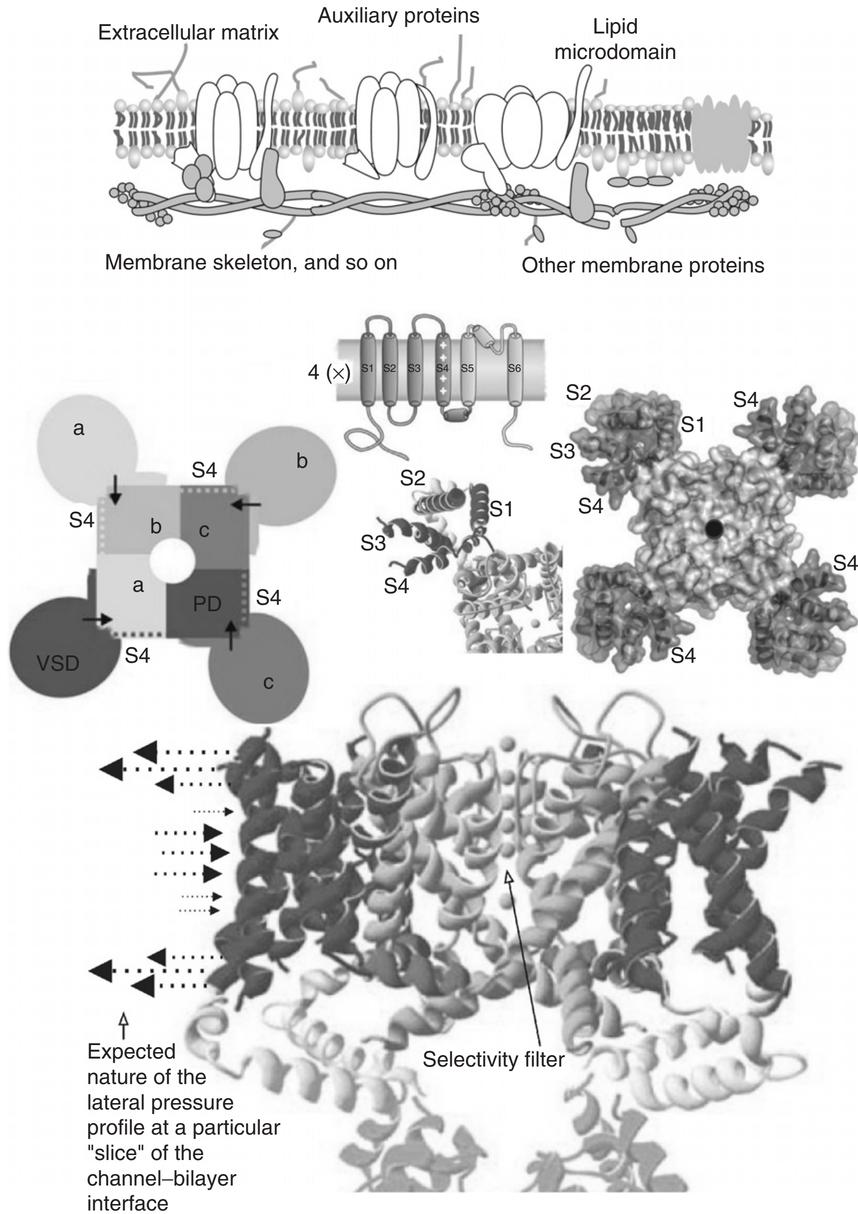

FIGURE 1 Voltage-gated channels in the bilayer. The illustration at the top of this collage serves as a reminder of the complexity of the environment experienced by VGCs in cellular bilayers. There is a large diversity of lipid species and they are not homogeneously disposed in the plane of the bilayer. Moreover, the inner and outer leaflets differ in their lipid composition. Channels interact with auxiliary proteins and regulatory proteins (e.g., kinases); various other

artificial, "stretch" is a fuzzy term for a fuzzy process. On the other hand, stretch happens.

A. The Channel Proteins

The molecular architecture and dynamics of the VGCs themselves are now known in bold outline: four somewhat loosely tethered charge-bearing sensor domains are arrayed around a central domain that houses a pore (selectivity filter and conduction path), with the sensor domains' response to voltage determining whether gates in the conduction path occlude the pore. The channels' cruciform perimeter provides a large lateral surface area where many residues of the (four) voltage sensor domains contact bilayer lipids. Even the central pore makes some contact with bilayer lipids (Long *et al*., 2005a,b; Tombola *et al*., 2006).

---

channel and nonchannel membrane proteins are present. These as well as the channels being studied can make long range mechanical connections via membrane skeleton elements and scaffolding proteins (which have their own dynamics). Not depicted are the highly structured invaginations and evaginations of bilayer (e.g., caveolae, microvilli). VGCs are composed of either four subunits (e.g., Kv and HCN channels) or four domains (Nav and Cav channels) of the Shaker-like six transmembrane structure labeled "4× S1, S2, S3, S4, S5, S6 (note that S4 is the location of most of the gating charge; note also the location of the S4–S5 linker). The homotetrameric cruciform arrangement of the Kv1.2 channel resolved by Long *et al*. (2005a,b) is illustrated at left (modified from Tombola *et al*., 2005). This is thought to be an open-like structure. Each monomer contributes a voltage sensor domain (VSD) and a pore domain (PD) (essentially S1–S4 and S5–S6, respectively) and the assembled channel exhibits a domain-swapped arrangement as shown (three of the VSDs and PDs are labeled a, b, and c). S4 is only partially sequestered by the rest of the protein; the S4–S5 linker "reaches out" along the inner surface of the bilayer (dotted lines) as part of the domain-swapping arrangement. At far right is a labeled surface presentation of Kv1.2 (from the extracellular side) and to the left of that is a fragment of the same Kv1.2 structure depicted as a ribbon diagram. At bottom is side-on (ribbon diagram) view of Kv1.2 tetramer; the cruciform channel is sectioned near one of its two widest aspects. The selectivity filter is indicated (dehydrated K ions are present in the filter) and the arrow pointing to it passes through the gating hinge region. At left is an illustration of the lateral forces that the channel in a bilayer would feel. The lateral pressure would be strongly negative where bilayer surface tension is highest (this tends to pull the protein apart) and more weakly positive (but over a wider region) near the bilayer mid region (this tends to compress the protein). Because the channel is cruciform, the lateral lipid–protein interface is extensive. For any given conformation (assuming a given lipid composition) the details of the lateral pressure profile would change continually as an imaginary pressure profile gauge swept out a 360° arc to take in the whole channel. For fully resting and fully activated states, a 90° arc would suffice for a full description, but once a subunit moved independently, the full 360° would be needed.

B. Bilayer

The three-dimensional structure of the bilayers in which VGCs reside is also understood, although only in broad outline. In the transverse (z) direction, a surface tension (from headgroups squeezing together to prevent exposure of hydrophobic lipid tails to water) at each planar interface exerts large pulling forces on any integral membrane protein (Fig. 1, bottom). These pulling forces extend only several angstroms deep (Cantor, 1999; Gullingsrud and Schulten, 2004) before giving way to counterbalancing compressive forces from the lipids' acyl chains (which, to maximize their entropy, occupy as much volume as possible). The mid-bilayer force thus compresses the mid-bilayer region of any integral membrane protein. Lipids are asymmetrically disposed in the z axis (e.g., cholesterol is more abundant in the extracellular than the intracellular leaflet) and inhomogeneous in the x–y plane where it subdivides into lipid microdomains of diverse size (usually measured in nanometers in native membranes) and composition, according to the prevailing mix of lipid and protein species (Baumgart *et al*., 2003; Devaux and Morris, 2004; Gaus *et al*., 2006; Kahya and Schwille, 2006).

C. Accessory Proteins

VGCs also bind directly to auxiliary subunits and directly or secondarily to a large collection of other proteins (e.g., intracellular membrane skeleton and scaffold proteins plus extracellular matrix proteins) (Folco *et al*., 2004; Wong and Schlichter, 2004).

III. BIG PICTURE ISSUES

A. Bilayer Mechanics and VGCs

That membrane stretch modulates VGC activity is evident from a glance at Fig. 2 which shows the robust MS responses of Shaker WTIR. Molecularly speaking, this channel is the prototypical VGC. At left, are Shaker currents monitored near the foot of its $g(V)$ relation (i.e., at a voltage producing a P_{open} slightly >0). Note that stretch yields "stretch-activated" (SA) Shaker channel activity just as it yields SA cation channel activity from the oocyte's endogenous TRPC1-based (Maroto *et al*., 2005) cation channels (to record the Shaker currents, these endogenous channels are usually inhibited). The figure also shows that the increase of I_{Shaker} in response to stretch is reversible, repeatable, and dose-dependent. What does this

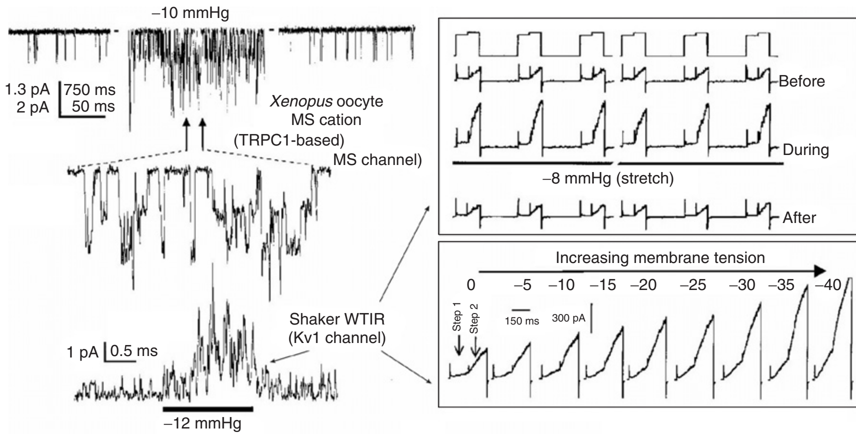

FIGURE 2 The prototypical Kv1, Shaker, is an MS VGC. Top left, typical MS cation channel activity from the TRPC1-based channels endogenous to oocytes (shown at low and high time resolution). Below that, unitary current recordings of Shaker WTIR. It too is an SA channel. At right are data sets (two different patches) showing that Shaker WTIR responses to stretch are not only reversible but repeatable, and that they show dose dependence. Modified from Gu *et al.* (2001).

general result signify in the wider context of bilayer mechanics and VGC conformation changes?

In principle, if bilayer stretch regulates VGC activity, then so should changes in bilayer lipid composition, since lipid molecules have particular bilayer mechanical properties depending on their size, shape, internal flexibility, and charge. A thorough review of this topic appeared (Tillman and Cascio, 2003). Table I provides a sampling of reports of amphiphile (including membrane lipids) modulation of VGC kinetics. No theoretical framework has emerged for such observations, which is more compelling than that of lipid stress perturbations of the conformational equilibria of the channels.

As Andersen and colleagues point out (Lundbaek *et al.*, 2004), specific lipid–protein interactions seldom appear to be involved in lipid actions of VGC gating, but rather, "hydrophobic coupling between a membrane-spanning protein and the surrounding bilayer means that protein conformational changes may be associated with a reversible, local bilayer deformation . . . the energetic cost of the bilayer deformation contributes to the total energetic cost of the protein conformational change." This group deploys gramicidin A as a molecular force-transducer for bilayer mechanics. They then alter the composition of human embryonic kidney (HEK) cell bilayer via micelle-forming amphiphiles (e.g., TX100), cholesterol depletion (Lundbaeck *et al.*, 2004), or addition of the amphiphilic drugs capsaicin and capsazepine (Lundbaeck *et al.*, 2005).

TABLE I
A Sampler: Modulation of VGCs by Bilayer Mechanical Reagents (BMRs)

Cholesterol	Kv1.3 (Hajdu *et al.*, 2003)
	HCN4 (Barbuti *et al.*, 2004)
	Nav1.4 (Lundbaek *et al.*, 2004)
	Cav (L-type) (Toselli *et al.*, 2005)
Propofol	HCN2 (Ying *et al.*, 2006)
	Nav (neuronal) (Ouyang *et al.*, 2003)
	Kv (T-lymphocytes) (Mozrzymas *et al.*, 1996)
	Cav (neuronal L-type) (Olcese *et al.*, 1994)
Phenothiazines	
Chlorpromazine	Cav (α1E) (McNaughton *et al.*, 2001)
	Nav (cardiac) (Ogata and Narahashi, 1989)
	HERG (Thomas *et al.*, 2003)
Trifluoperazine	Kv1.3 (Teisseyre and Michalak, 2003)
Mesoridazine	HERG (Su *et al.*, 2004)
Volatile anesthetics	
Isoflurane	Kv1 (Shaker) (Correa, 1998)
	Nav1.2, Nav1.4, Nav1.6 (Shiraishi and Harris, 2004)
	Cav (cardiac L- and T-type) (Camara *et al.*, 2001)
	hIK1 (Namba *et al.*, 2000)
Halothane	HERG (Li and Correa, 2002)
	Cav (L-type) (Kamatchi *et al.*, 2001)
	HCN1, HCN2 (Chen *et al.*, 2005)
1-Alkanols	Kv3 (Shahidullah *et al.*, 2003)
	BKCa (Chu and Treistman, 1997)
	HCN (neuronal I_h) (Okamoto *et al.*, 2006)
Fatty acids	BKCa channels (Clarke *et al.*, 2003)
	Nav1.5 (Xiao *et al.*, 2006)
	HERG (Guizy *et al.*, 2005)
Cannabinoids	Nav (neuronal) (Nicholson *et al.*, 2003)
	Kv1.2 (neuronal) (Poling *et al.*, 1996)
	P-type Ca channel (neuronal) (Fisyunov *et al.*, 2006)
	T-Type Ca channel (Chemin *et al.*, 2001)
Capsaicin and capsazepine	HCN1 (Gill *et al.*, 2004)
	Nav1.4 (Lundbaek *et al.*, 2005).
Lysophospholipid	HERG (Wang *et al.*, 2001)
	HCN (cardiac I_f) (Hu *et al.*, 1997)
Propanolol	HERG (Yao *et al.*, 2005)
Ceramide	Kv1.3 channels (Bock *et al.*, 2003)
Nicotine	Nav1.5 (Liu *et al.*, 2004)
Resveratrol	Nav1.5 (Wallace *et al.*, 2006)

Using this approach in conjuction with a recombinant skeletal muscle Nav channel, they have made a solid case that altered bilayer elasticity, or a parameter tightly correlated with bilayer elasticity, is what underlies the resulting kinetic modulation of the VGC.

If bilayer elasticity regulates the conformational equilibria in VGCs, then so should bilayer stretch. And as we already saw (Fig. 2), it does. The promiscuous effects of a vast number of amphiphilic drugs on VGC kinetics and the promiscuous kinetic effects of stretch on many of these same channels almost certainly share bilayer mechanical origins.

B. Prokaryotic VGCs as Ancestral Lipid Stress Detectors?

In eukaryotic Kvs structurally similar to the prokaryotic Kv channel, KvAP, bilayer stretch increases the probability of voltage sensor motion (Laitko and Morris, 2004). Electrical propagation over a long distance is clearly not what prokaryotic VGCs do for a living, but responding to bilayer stress might be part of their evolutionary raison d'etre. Osmotic safety valve channels detect near lytic bilayer stress in prokaryotes (Kung, 2005); less drastic lipid stress perturbances may be the province of MS VGCs. Benthic vent organisms (like the archaeon from which KvAP was isolated) undoubtedly lead particularly unquiet lives with respect to ambient pressure, temperature, and osmotic stress (Sako *et al.*, 1996) all of which will affect the physico-mechanical characteristics of bilayers and hence the behavior of MS VGCs.

As gauged from crystal structures (Lee *et al.*, 2005) and EPR spectroscopy-based structures (Cuello *et al.*, 2004), the thickness and elasticity of the bilayer immediately adjacent to KvAP voltage sensors might regulate the ease of voltage sensor movements. Thickness and elasticity are physical attributes of the bilayer that would vary in prokaryotes, experiencing pressure and temperature variations, osmotic stress, lipid metabolism (i.e., lipid substitutions in the bilayer), cell division, and even (for a rod-shaped organism) in the tubular vs hemispherical sectors of the cell.

Pressure, temperature, osmotic stress, and lipid composition: all these factors affect VGC kinetics as an example or two for each illustrates. *Pressure*: Hyperbaric pressure causes bilayer acyl chains to pack more densely, and this straightens them, thickening and rigidifying the bilayer (Scarlata *et al.*, 1995). Hyperbaric pressure alters the gating of Nav and Kv channels in squid axon and in vertebrate nodes of Ranvier (Conti *et al.*, 1984; Kendig, 1984), Kv1 channels in oocytes (Meyer and Heinemann, 1997) and N-type Cav channels (Etzion and Grossman, 2000). Since bilayers are more compressible than proteins, hyperbaric pressure presumably acts at least in part, via lipid stress (see discussion in Gu *et al.*, 2001), but even in a review (Macdonald, 2002)

the focus is on putative effects of hyperbaric pressure on protein volume without consideration of possible modulatory alterations at the protein–lipid interface. This view is probably due for some revision. *Temperature*: In contrast to elevated pressure, elevated temperature thins and fluidizes membranes (Pencer *et al*., 2005). Arrhenius plots for BKCa kinetics with or without cholesterol (artificial bilayers) are consistent with a model in which cholesterol exerts its modulating effect via lipid stress (Chang *et al*., 1995). In a similar line of reasoning, Kv VGC sensor motions with the highest Q10 values (i.e., closed–closed transitions far from the open state; Rodriguez *et al*., 1998) might, therefore, reflect temperature-induced changes in the microstructure of the lipid bilayer that alter lipid stresses felt by the channels, and in particular, by voltage sensors in the resting state. *Osmotic stretch*: VGC activity varies with both membrane stretch and osmotic swelling, and for Cav channels the stretch and swelling responses are similar in detail (see discussion and references in Calabrese *et al*., 2002). *Lipid-dependent bilayer mechanics*: Elevated cholesterol (e.g.) increases bilayer rigidity and thickness (Pencer *et al*., 2005; Czub and Baginski, 2006); enriching or depleting membrane cholesterol modulates various transitions (activation, slow inactivation, and/or deactivation) in Kv1.3 and HCN4 channels and shifts inactivation in Nav1.4 (Lundbaek *et al*., 2004).

The responses of archeabacterial VGCs in native lipids (these are more ordered, less flexible than eukaryotic bilayer lipids; Bartucci *et al*., 2005) to voltage and lipid stress perturbations have not been tested. Assuming that prokaryotic VGCs are responsive to bilayer mechanical perturbations (stretch, hyperbaric pressure, BMRs), then bilayer mechanics need to be included along with transbilayer voltage, in the constellation of factors responsible for the evolution of VGCs.

Interestingly, displacement currents of prestin, a voltage-sensitive protein unrelated to the VGCs, are tension sensitive (Dong and Iwasa, 2004); perhaps it is hard to build a voltage-dependent membrane protein that is not susceptible to lipid stress. Membrane proteins with recognizable "S4" voltage sensors but no pore domains [a phosphatase (Murata *et al*., 2005) and a proton channel (Ramsey *et al*., 2006)] are now known; perhaps they too will prove sensitive to lipid stress. Certainly, volatile anesthetics, which are BMRs, modulate Kv channel gating currents (Correa, 1998).

C. MS VGCs and MS TRP Channels: Sharing Insights

MS channels, including VGCs, generate both SA and stretch-inactivated (SI) currents (Fig. 3). The first reported SI current (it was a non-VGC) occurred in neuronal patches that also had SA channel activity (another

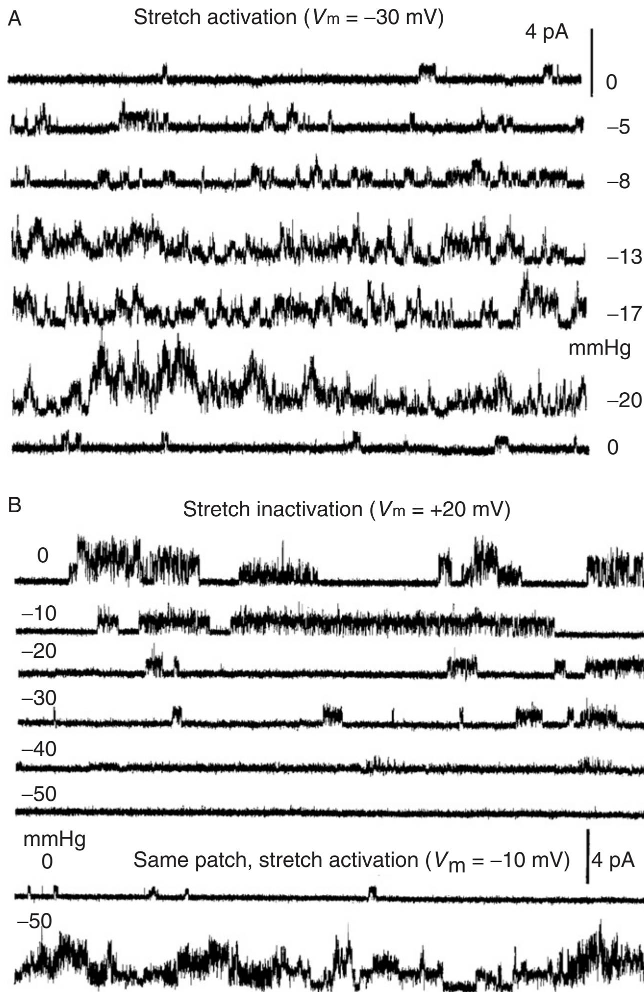

FIGURE 3 Shaker WTIR is an SA and SI channels. (A) A patch held at a voltage near the foot of the Shaker $g(V)$ relation (in the presence of gadolinium) is subjected to successively increasing pipette suction (i.e., greater membrane tension) and then back to resting tension. Dose-dependent stretch activation results. (B) Another patch held at a more depolarized voltage (which favors slow inactivation) undergoes stretch inactivation. Then, going to -10 mV (i.e., near the foot of the $g(V)$ for this patch), the same population of channels exhibit stretch activation (compare the effects of -50 mmHg at the two different voltages). Modified from Gu *et al*. (2001).

non-VGC) (Morris and Sigurdson, 1989) and with their different unitary conductances, these two were probably distinct channel types. But that need not be the case when SA and SI channels activity coexists. In skeletal muscle cells of the mdx (dystrophic) mouse (Franco-Obregon and Lansman, 2002), traumatic stretch irreversibly transforms MS channels from SA to SI channels. How this might come about is suggested by reference to our somewhat deeper understanding of reversible and irreversible responses to stretch in VGCs.

MS cation channels like those of mdx muscle are thought to be TRP-based (Maroto *et al*., 2005) and TRP channel structure is globally similar to Shaker structure (Clapham, 2003). The kinetics of the MS VGC, Shaker, are more tractable than those of TRP channels, so insights relevant to the mdx channel "transformation" might emerge from Shaker's SA/SI behavior. TRP channels share with this Kv channel a "6TM-tetramer" body plan and they can be voltage-dependent (Brauchi *et al*., 2004; Nilius *et al*., 2005). The skeletal muscle MS "TRP" cation channel has a stretch-sensitive closed–closed transition that is weakly voltage-dependent (Guharay and Sachs, 1984, 1985); Kv channels have voltage-dependent closed–closed transitions that are stretch-sensitive (Laitko and Morris, 2004; Laitko *et al*., 2006). And—critical point—a Kv channel can generate both SA and SI currents (Gu *et al*., 2001), echoing the case of the mdx skeletal muscle channels.

What is the nature of this dual SA and SI behavior in Kv1 channels? Moving between closed, open, and slow-inactivated states, Kv1 channels undergo several voltage-dependent transitions (the voltage sensor undergoes outward movement with respect to the electric field) plus some voltage-independent transitions (mode-shift). In Shaker mutants with well-characterized rate-limiting transitions, we isolated

1. "SA transition" (i.e., a transition whose response to stretch enhances I_{Kv}) and
2. "SI transitions" (i.e., a transition whose response to stretch diminishes I_{Kv}).

The SA transition is activation—an outward motion of sensor charge, independent in each of the tetrameric channel's subunits (Laitko and Morris, 2004). The SI transitions are a preopening step and a slow inactivation step (preopening is concerted, involving all four subunits, slow inactivation is less well understood; Laitko and Morris, 2004; Laitko *et al*., 2006). We can take Shaker 5aa as an example of an SA/SI Kv channel. In 5aa, activation and slow inactivation accelerate with stretch, so 5aa channels subjected to depolarization plus stretch exhibit SA I_{Kv} followed by SI I_{Kv}. This dual SI and SA behavior in a Kv illustrates that having (at least) one open and two closed conformations allows a channel to exhibit both SA and SI behavior.

Taken alone, however, this dual behavior does not illuminate the irreversible (posttraumatic stretch) switching of mdx MS channels.

For that, we draw on the irreversible effect of traumatic stretch characterized in recombinant Nav1.4 α-subunit channels (briefly, strong membrane stretch irreversibly leads to accelerated, left-shifted activation and inactivation kinetics, the underlying molecular explanation is unknown (see the section on irreversible stretch changes, below). MS TRP channels in mdx muscles presumably experience comparable irreversible stretch-induced change in the basic rates governing transitions among (at least) three states, while retaining their inherent (reversible) MS responses. This simple scenario could explain an irreversible "SA-to-SI" switch. The Kv analogy substitutes an "irreversible voltage change" (=clamping at two different voltages) for an "irreversible mechanical change" (=stretch trauma, as in the mdx TRP or the Nav1.4 α-subunit channels) as the factor controlling the characteristic rates of the system before stretch is applied to examine the reversible MS responses. Clamped at well-separated voltages, Shaker WTIR, for example, shows SA at one voltage and SI at the other (Fig. 3). The analogy assumes that the TRP channels have (at least) three states and two MS transitions, with different transitions being rate-limiting in the conditions prevailing before and after the stretch trauma, to yield net SA behavior before and net SI behavior after. As a generic phenomenon (single population of channels with at least two MS transitions, with basic rates governed by different factors at different times), this might explain reports of the MS cation channels from a given preparation being variously SA channels or SI channels (Kirber *et al.*, 1988; Hisada *et al.*, 1993).

D. No MS "Motif" Required: Just Say HMMM

During activation, Shaker WTIR behaves as an SA channel and Shaker ILT behaves as an SI channel (Fig. 4A and C), yet except for three conservatively mutated neutral residues in the S4 voltage sensor, these channels are identical. Should we label these residues a "stretch motif"? Of course not. Should we assume the three residues contact the bilayer? Again, no. These two Shaker variants behave as SI or SA channel by virtue of

a. the identity of their rate-limiting voltage-dependent (and stretch-sensitive) steps and
b. the particular effect of stretch on that step.

Stretch, it happens, *decelerates* the rate-limiting voltage-dependent step of Shaker ILT. By contrast, it *accelerates* the (different) rate-limiting voltage-dependent step of Shaker WTIR.

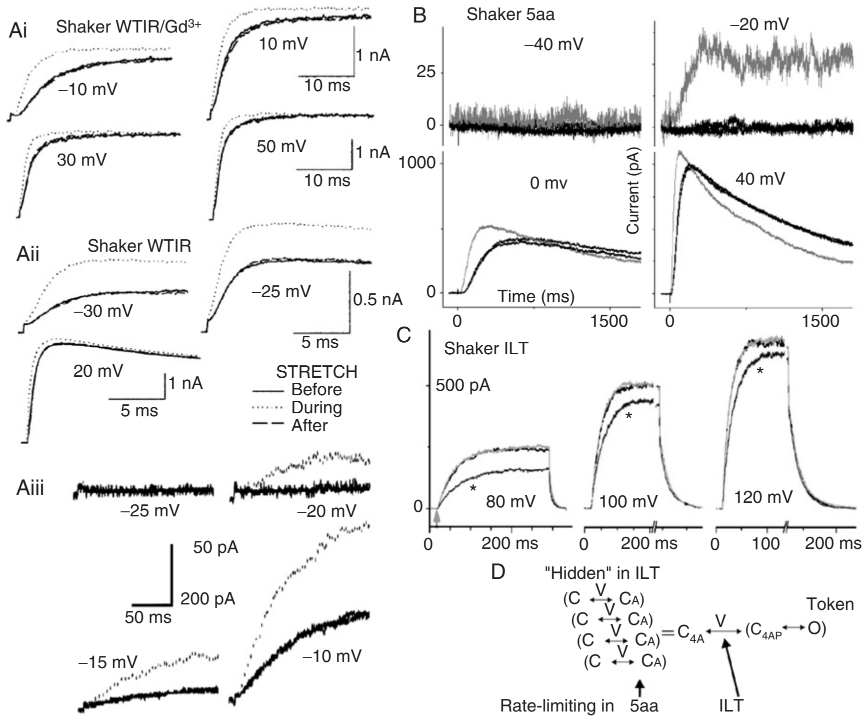

FIGURE 4 SA of Shaker WTIR and Shaker 5aa, SI of Shaker ILT. (Ai) Shaker activation before, during (dotted lines), and after stretch in the presence of gadolinium. (Aii) Likewise in another patch but with no gadolinium added. (Aiii) There is a narrow range of voltage below the foot of the $g(V)$ relation where no current flows unless the membrane is stretched (in this patch, that range included −20 mV) (from Tabarean and Morris 2002). (B) For Shaker 5aa, in this example, the comparable range extended at least from −40 to −20 mV. With larger depolarizations, it is clear that stretch (gray traces) accelerates both activation and slow inactivation (from Laitko and Morris 2004). (C) For Shaker ILT, stretch (asterisks) decelerates activation. The consequence is a right shift of the $g(V)$ curve with no change in the amount of charge moved; for Shaker WTIR, by contrast, stretch left shifts the $g(V)$ (from Laitko *et al.*, 2006). (D) A kinetic scheme for Shaker channels (Laitko *et al.*, 2006). The major motions of the voltage sensor are independent and then resolve in a concerted final voltage-dependent motion, at which point an additional concerted motion opens the channel. For current turn on (the Markov model "token" in our experiments) during depolarizing step, the independent motion is slow enough in Shaker 5aa to be the rate-limiting step, whereas in Shaker ILT the concerted voltage-dependent motion is rate-limiting. In Shaker WTIR, the two transitions have comparable rates over a wide voltage range. The independent sensor motions are "hidden" in Shaker ILT when ionic current is the token, although not when gating current is the token. Modified from Laikto *et al.* (2006).

This motif-free way of describing SA and SI gating could be called "HMMM" for Hidden Markov Model Mechanosensitivity (hidden Markov model theory is well described in Wikipedia). Ion channels kinetic schemes are Markov models; formally, in a Markov model, *all* states are directly visible to the observer, so state transition rates (probabilities) are the *only* parameters that need to be considered. In a Hidden Markov Model, by contrast, some states are not directly visible, but—and this is the critical point—variables influenced by those states are visible. In reality, this applies for most kinetic analyzes of ion channel behavior. If the MS activity of a VGC (or a ligand-gated channel, and so on) was monitored

by optically determined protein motions (e.g., Isacoff) and
by chemically determined protein motions (e.g., Yellen) and
by gating currents (Ledwell and Aldrich, 1999) and
by ionic current,

our understanding of which motions are "stretch-sensitive" (in say, Shaker WTIR or Cav or Kv3 channels) would not be restricted (as currently) to motions limiting for ionic current flow. In a multistate channel, when ionic current alone is used as the token ("token" is Hidden Markov Model terminology) for MS channel activity (or for "molecule-X"-modulated activity), a picture of reduced dimensions emerges. Like the two-dimensional shadow of, say, a rotating three-dimensional helix, this picture can be misleading.

Stretch globally alters bilayer structure (Gullingsrud and Schulten, 2004), so it will globally affect the membrane-embedded regions of membrane proteins. Even for an ideal two-state MS channel, multiple aspects of the bilayer–channel interaction (Wiggins and Phillips, 2005) would, therefore, contribute to the free energy of MS gating. Mutations enhancing MS gating could be said to belong to "an MS motif," but in all likelihood such residues would be found scattered about the protein as in the case of MscL gain-of-function mutations (Ou *et al*., 1998). Given the multiple sources of free energy itemized by Wiggins and Phillips (2005), a "global MS motif" makes sense but "global motif" seems almost oxymoronic.

For any given VGC, speculations about what structural features cause stretch to accelerate transition X and decelerate transition Y would be idle. However, it may eventuate that the independent depolarization-induced transitions of *all* VGCs (these transitions move voltage sensor charge outward—for example activation in Shaker, deactivation in the sea urchin HCN channel (Mannikko *et al*., 2002) accelerate with stretch (and slow with hyperbaric pressure and cholesterol). Our preliminary evidence on HCN channels (Lin *et al*., 2007) would support this view. If so, then some robust property of the independent voltage sensor motion, a property retained in all

VGCs, may underlie a universal lipid stress effect on that motion. Two (not mutually exclusive) possibilities are:

1. The outward movement of voltage sensor charge requires a locally thinned membrane.
2. Expansion of the sensor array in the plane of the bilayer (Tabarean and Morris, 2002) occurs during outward movement of voltage sensor charge.

In Shaker WTIR and Shaker ILT, overlapping $Q(V)$ curves (Ledwell and Aldrich, 1999) show that the activation motions are not different. Yet WTIR is SA and ILT is SI. Why? Because the next step, after activation, toward the open state—a concerted voltage-dependent motion—is fast in WTIR but ultraslow (and rate-limiting) in ILT. And in ILT, it turns out that stretch retards that concerted motion (Laitko *et al.*, 2006). It presumably does so too in WTIR, but evidently not enough to slow the onset of the token we monitor, namely ionic current. Because ionic current is our only monitored parameter, a reduced dimension picture emerges—in other words, we are dealing with HMMM.

It is self-evident that all conformation changes are inherently thermal and thus, to some degree, temperature-sensitive. In the same way, any conformation changes occurring *in the plane of the bilayer* must be inherently lipid stress-sensitive. Special "MS motifs" would be needed only for specialized MS tuning (e.g., for directional or frequency sensitivity or to create a threshold at a specific tension). These same conformation changes (in the plane of the bilayer) should also be susceptible to BMRs (i.e., molecules that change the shape of the lateral pressure profile). No specific "BMR"-binding site ("motif") in the protein would be required (except, again, for very special cases where the lipid can be shown to be a ligand for a specific part of the channel such as the domain responsible for fast inactivation; Oliver *et al.*, 2004). And what if Kv channel-X but not Kv channel-Y responds to, say, butanol or cholesterol? The parsimonious assumption is that lipid stress-sensitive rate-limiting transitions in a HMM dominate the measured response, not that the response is dominated by the presence or absence of butanol or cholesterol or so on binding sites. In other words, the parsimonious assumption invokes a lipid stress version of HMMM.

In summary, in Kv channels, SI and SA effects are not about "MS motifs" but rather, they reflect the stretch-perturbed operation of particular multistate kinetic schemes. It is likely that this assertion applies, too, for MS TRP channels. The same line of reasoning says that for amphiphilic molecules (BMRs) exerting actions on VGCs via lipid stress, it is probably not "binding motifs" that underlie the action but multistate kinetic schemes with "hidden" transitions. We return to this in the alkanol section below.

E. An Imperturbable K-Selective Pore Surrounded by MS Voltage Sensors?

The voltage-dependent conformation changes of Kv proteins are susceptible to membrane stretch but the selectivity filter at the heart of the assembled Kv tetramer seems inured to stretch. This can be said for a Kv1 (ShakerWTIR and Shaker ILT), Kv3 (Shaw) (Fig. 5), and BKCa channels (Dopico *et al.*, 1994) as well as for Cav channel (Fig. 6). The evidence: (1) when NP_{open} increases with stretch in Shaker WTIR, in the Shaw channel, or in BKCa, single-channel amplitude with or without stretch is identical (Dopico *et al.*, 1994; Gu *et al.*, 2001; Laitko *et al.*, 2006) and (2) in both Shaker WTIR and ILT, $g(V)_{max}$ is unaffected by stretch.

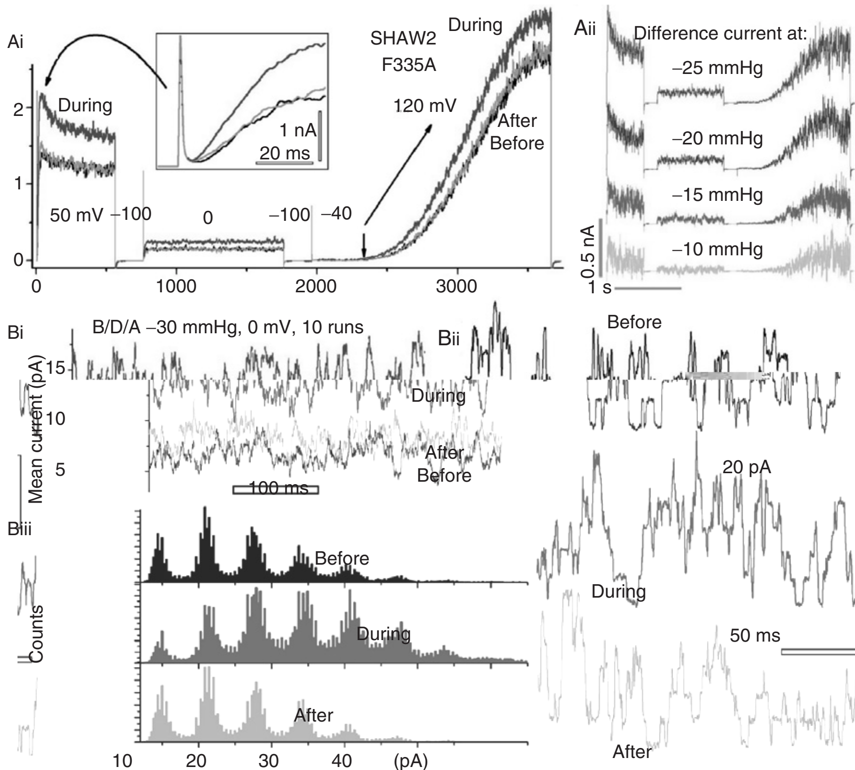

FIGURE 5 A Kv3 channel current is enhanced by stretch. This same Kv3 channel is *inhibited* by alkanols (Shahidullah *et al.*, 2003). Alkanols lower surface tension whereas stretch increases it, but it is not yet clear if these surface tension effects converge on the same transition. Nevertheless, as the figure shows, stretch reversibly (Ai) and in a dose-dependent manner (Aii) increase the channels activity. Recordings at 0 mV (Bi) rule out any possibility of interference from the endogenous MS channel. Unitary currents at 0 mV before, during, and after stretch (Bii) show no evidence for increased unitary current (Biii) during stretch. Modified from Laitko *et al.* (2006).

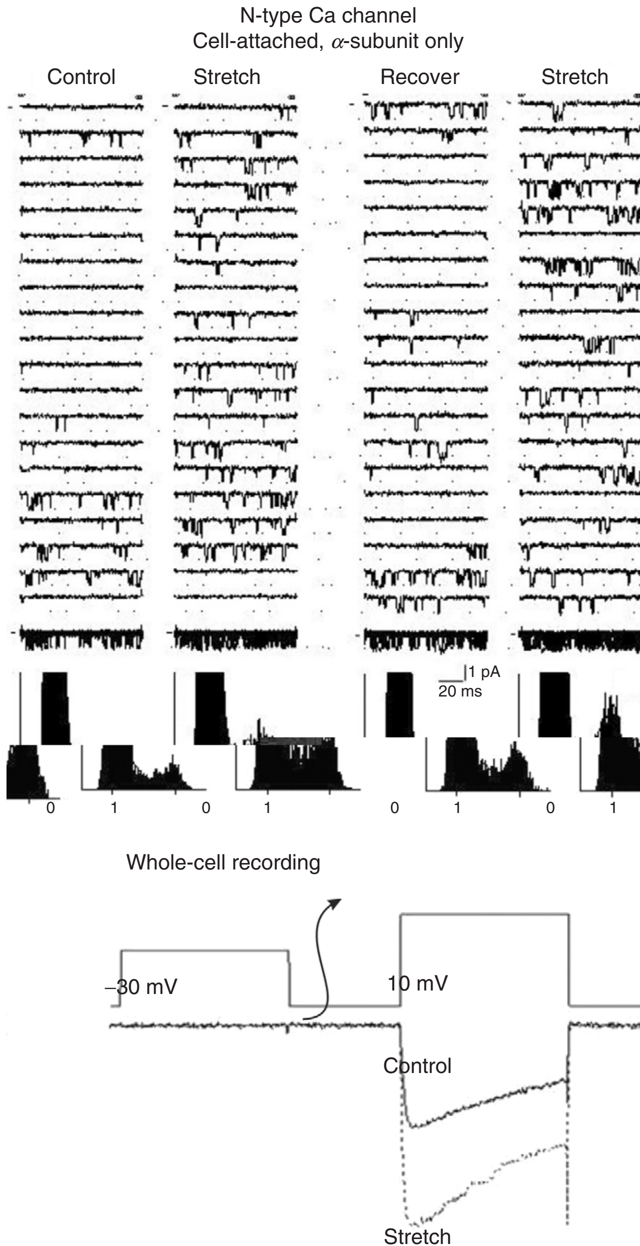

FIGURE 6 Cav channel activity increases reversibly with stretch. Single-channel (α-subunit only) and whole-cell recordings (full complement of subunits) of recombinant N-type (high voltage activated) Cav channels illustrate that stretch increases the peak and steady state current levels and accelerates inactivation from the open state but has no evident effect on unitary current and no effect on the rate of current onset. Modified from Calabrese *et al.* (2002).

Perhaps the circular domain-swapping arrangement noted by Long *et al*. (2005b) in the Kv1.2 tetramer contributes to the mechanical stability (Riechmann *et al*., 2005) of Kv selectivity filters. Other eukaryotic MS channels (e.g., two-pore-domain-K and TRP channels) have stretch-insensitive unitary conductances; since the TRPs are 6TM channels, it will be interesting to learn if they too have Kv-like domain-swapping arrangements.

In planar bilayers, BKCa channel activity is modulated by BMRs without changing unitary conductance since cholesterol reduces BKCa activity (Chang *et al*., 1995) and this is antagonized by ethanol (Crowley *et al*., 2003). Since cholesterol and ethanol have opposing effects on surface tension (and line tension) in bilayers, these certainly look like lipid stress-mediated phenomena. In oocyte membrane, the BMRs ethanol, butanol, hexanol, and heptanol reversibly enhance BKCa current (Chu and Treistman, 1997). They do not, however, detectably perturb the selectivity filter (Chu *et al*., 1998).

But under some conditions, the pathway in series with the selectivity filter—or perhaps even the selectivity filter itself—may succumb to lipid stress. Chang *et al*. (1995) detected a small (~5%) decrease in BKCa channel conductance when cholesterol was increased by ~10%. Moczydlowski and colleagues studied BKCa in a barebones artificial bilayer regime (Park *et al*., 2003) by way of retesting their own longstanding hypothesis that anionic lipid enhances BKCa unitary conductance via surface charge effects. The original hypothesis did not hold, and they now postulate that "lipid modulation of K^+ conductance is preferentially coupled through conformational changes of the selectivity filter region ... [and they] ... discuss possible mechanisms for the effect of anionic lipids in the context of ... general membrane physical properties proposed to regulate membrane protein conformation via energetics of bilayer stress." Ultrasimple artificial bilayers, having fewer degrees of freedom for energy minimization, may allow some amphiphiles more latitude to exert pressure effects at the channel–protein interface than when present at the same concentration in complex bilayers.

F. Alcohol and VGCs: Binding Sites or Bilayer Mechanics?

Alkanols partition into the bilayer headgroup region, being stabilized by hydrogen bonding to carbonyl oxygens of the phospholipid's glycerol backbone; partitioning increases with increased chain length (Feller *et al*., 2002). The impact of short chain alkanols on the bilayer lateral pressure profile can be powerful enough to destabilize the tetrameric arrangements of ion channels (Van den Brink-van der Laan *et al*., 2004). A short chain alkanol series has become an even better experimental tool for bilayer mechanics now that Ly and Longo (2004), using pipette aspiration of giant unilamellar

vesicles, confirmed experimentally for bilayers a fact long established for monolayers . . .

> Area compressibility, which is proportional to interfacial (=surface) tension, follows Traube's rule of interfacial tension reduction: for each additional alcohol CH_2 group the concentration required to reach the same area compressibility falls ~3-fold.

Whereas stretch increases bilayer surface tension (Gullingsrud and Schulten, 2004), alkanols lower it. Stretch and alkanols are similar, however, in that both thin the bilayer and decompress its acyl chains.

To monitor reversible modulation of VGCs by stretch or by "BMR X," currents obtained "before, during, and after" exposure are needed and while this is readily achieved for membrane patches subjected to stretch, this is not so for slowly exchanging BMR molecules like cholesterol. Short chain alcohols, by contrast, are experimentally tractable, as attested by the Covarrubias group's concentration jump experiments on Kv3 channels (Shahidullah *et al.*, 2003).

Strikingly, this group interprets their extensive data as evidence that bilayer mechanics do not mediate alkanol effects on channels. Before looking at that work, therefore, it seems germane to look at alkanol actions on two non-VGC proteins. The first is rhodopsin (a prototypical G-protein–coupled receptor). In rod outer disk segments, Mitchell *et al.* (1996) monitored effects of an alkanol series on a photoactivatable rhodopsin conformation change and found the pattern of altered rates conforms to a model of lipid-mediated action (in effect, as if Traube's rule is the operative factor). Their rhodopsin studies with other BMRs also support an interpretation of lipid mediation, as do their findings (a) that at physiologically relevant levels of osmolality and ethanol, ~90% of ethanol's effect arises from disordered acyl chain packing (Mitchell and Litman, 2000) and (b) that cholesterol and alkanols have opposite-going effects, indicative of reduced acyl chain packing free volume, not of specific cholesterol–rhodopsin interactions (Niu *et al.*, 2002). For rhodopsin reconstituted into vesicles (rhodopsin/lipid ratio from 1:422 to 1:40), they reported that elevated rhodopsin-packing density minimally impacts rhodopsin's structural stability yet markedly reduces its activation (Niu and Mitchell, 2005).

Extrapolated to VGCs, such a scenario would predict "lipid tuning" of channel dynamics in densely packed lipid rafts and ethanol interference with that tuning (Crowley *et al.*, 2003). This scenario is appealing, obviating as it does, the need to postulate hosts of VGC-specific binding sites for various lipophilic reagents. Nonetheless, the view that alkanols act on channels (ligand-gated channels as well as VGCs) via protein sites has considerable currency. The second system to consider, then, is the recombinant glycine

receptor channel for which Davies *et al*. (2004) find that ethanol and butanol actions are antagonized by hyperbaric pressure. The word "bilayer" does not appear in the chapter; results are interpreted as evidence that alkanols target a pressure-antagonism-sensitive mechanism in glycine receptors, with "the mutant α1(A52S) GlyR findings suggest[ing] ... the N-terminus as a potential target for ethanol action." The ionic current (their "token": see HMMM section above) alteration in a point-mutated channel is seen as evidence that the mutated residue is part of a "motif" (=binding target).

By analogy to the SA–SI story for Shaker WTIR and Shaker ILT, a more parsimonious possibility would be that these ligand-gated channels have hidden kinetic processes, and that lipid stress acts on a hidden transition(s) in a way that affects the token (ionic current). In other words, this has all the hallmarks of HMMM creating the illusion of a motif (=binding target).

With these alkanol stories in mind, we return to Kv3 (Shaw2) channels. Shortly after the molecular cloning of Shaker, Shaw, Shal, and Shab, in a seminal paper, Covarrubias and Rubin (1993) wrote:

> There is presently a debate regarding the relative merits of lipid-based and protein-based theories of anesthesia and the action of ethanol ... of four structurally homologous cloned K^+ channels ... only the Shaw2 channel ... is rapidly and reversibly blocked by ethanol in a concentration-dependent manner ... [this] ... can be explained by assuming a bimolecular interaction between ethanol and the channel. ... also ... [these] channels were selectively blocked by halothane (1 mM). Our results support the "protein hypothesis" of ethanol and anesthetic action.

Others (Chu and Treistman, 1997) found that octanol and decanol potently inhibit Shaw2 but not BKCa and took this channel specificity as further support for the protein theory. When Correa (1998) tested volatile anesthetics on Shaker-gating current (a different "token," note, than ionic current), she found that Shaker does, after all, respond to halothane and other BMRs (alkanols were not tested). The steps closest to opening (the steps dominating Shaw2 ionic current onset) are most affected, and this she took as support for direct protein–BMR interactions. It is now known, however, from the Shaker ILT response to stretch (Laitko *et al*., 2006), that lipid stress acts on these preopening voltage-dependent steps.

Since Shaw2 kinetics were thought to approximate a "two-state" situation (Shahidullah *et al*., 2003), our view was that if BMR actions on Shaw2 are lipid-mediated, stretch and alkanol effects could be directly compared. Clearly, Shaw2 is an MS channel; during depolarizing steps, it is an SA channel (Fig. 5). However, Shaw2 kinetics are not "two-state" and compounding that, the dominant MS transition is not rate-limiting for activation (Laitko *et al*., 2006). Consequently, direct comparisons of the stretch (which

thins the bilayer, lowers mid-bilayer lateral pressure, and increases the surface tension) with the bilayer mechanical effects of short chain alcohols (which share the first two effects, but decrease the surface tension) were not feasible.

What is clear, however, is that alkanols decrease steady state I_{Kv3} and stretch increases it. If both outcomes do arise from lipid stress, one possibility is that high surface tension (from stretch) slows Kv3 pore closing and low surface tension (from alkanols) accelerates the same transition. Unfortunately, effects of stretch on Kv3 pore closing still need to be tested; we were discouraged by finding that tail current rates tested before, during and after stretch in other Kvs are oocyte batch dependent (Laitko *et al.*, 2006).

Nevertheless, a direct slowing action of stretch on Kv3 pore closing is one possible outcome that could reconcile a bilayer mechanical model with the Covarrubias "protein" model (Harris *et al.*, 2000). In that model, alkanols stabilize the Kv3 channel-closed conformation by a direct interaction at a crevice formed by a 13-amino acid cytoplasmic S4–S5 loop. Covarrubias' group has provided several strong lines of evidence supporting this view. They monitored steady state current during fast (1 ms rise time) concentration jumps on excised patches (Shahidullah *et al.*, 2003), finding that "on binding" rates (i.e., onset of inhibition of steady state ionic current) changed ~3-fold per alkanol carbon (they took this to reflect "productive collisions at an alkanol binding site"). Butanol's inhibitory action is >1000 faster from the intracellular than the extracellular face (Harris *et al.*, 2000), a result seen as reflecting a cytoplasmic binding site (an alternate view is that the asymmetrically lowered surface tension can perturb the lateral pressure profile to favor a closed state). Circular dichroism spectroscopy on a peptide that forms the S4–S5 loop (a different token from the ionic current data) shows alkanol chain length-dependent binding (Shahidullah *et al.*, 2003). Chimeras of alkanol-sensitive and an alcohol-insensitive Kv3s and site-directed mutagenesis data further implicate the S4–S5 loop in the inhibition of channels by alkanols. Strikingly, a proline to alanine point mutation in the S6-gating hinge region changes the Kv3 channel from one whose steady state current decreases with butanol to one whose steady state current increases with butanol (Harris *et al.*, 2000). Recalling the Shaker WTIR/ILT story (a three-point mutation changes the channel from SA to SI), we wonder if this Shaw2 mutant is hinting at a lipid stress-mediated example of HMMM.

Alkanols reduce the line tension of a bilayer pore (Dan and Safran, 1998; Ly and Longo, 2004) and thus, for any irregular-shaped channel, they inevitably perturb the lateral pressure profile (Wiggins and Phillips, 2005). As we pointed out (Laitko *et al.*, 2006, Fig. 9C), the diffusional mobility of certain BMRs (including an alkanol) may drop at a channel's perimeter, and

if so their effects on the lateral pressure profile could exceed expectation (i.e., expectation from the BMR's line tension effect). Whenever a BMR's concentration at the perimeter of channels exceeds the "bulk" bilayer concentration, moreover, distinctions between "low affinity BMR-binding sites" vs lipid stress-mediated BMR effects could be largely semantic. In the Kv3 channel, Shaw2, the location of the S4–S5 loop is conjectural, but in Kv1.2 channels the equivalent loop "runs parallel to the intracellular membrane surface just at the level of the inner helix bundle crossing" (Long *et al.*, 2005a,b). Since this is precisely adjacent to where alkanols preferentially locate and since they lower bilayer surface tension by partitioning in the headgroup region (with acyl chains aligned normal to the bilayer plane), they could hardly avoid lateral contact with an S4–S5 loop located just there.

Two decades ago, Treistman and Wilson (1987) testing an alkanol series and temperature on the *Aplysia* neurons potassium current, I_A, found it "unlikely [an alkanol] exerts its actions on I_A via perturbation of a bulk lipid phase." However, they also followed alcohol effects on fluorescent lipid probes and from this pointed out that their I_A results could "be consistent with [lipid] domain-specific actions within a heterogeneously organized lipid environment." In turn, we could add, this could be consistent with alkanol-modulated changes in the channel-specific lateral pressure profile at the protein–lipid interface.

The "protein hypothesis" gained ground at a time when (1) bulk bilayer lipid effects like "fluidity" were being ruled out and (2) the kinetically important motions of VGCs were seen as sequestered from the bilayer. Combining updated structural information with information of the effects of stretch and BMRs on recombinant VGCs suggests that these two extreme views ("protein" vs "bulk bilayer") can be reconciled by an intermediate view that focuses on channel-specific motions at the complex-shaped and conformation-dependent lateral interface between bilayer lipids and the channel protein.

The wider issue of the interaction between VGC conformations and bilayer mechanics would be well served by a full description of the kinetic effects of stretch, hyperbaric pressure, temperature, and BMRs (e.g., an alkanol series and cholesterol) on each of several identified transitions in one structurally characterized VGC. A Kv1 channel would be the best candidate because of emerging Kv1.2 structural information and because MS transitions in a Kv1 (Shaker) are already known to include activation, prepore opening, and slow inactivation. Use of defined bilayers in this endeavor would facilitate computational probing of the findings as is being done for bacterial MS channels (Meyer *et al.*, 2006).

IV. REVERSIBLE STRETCH-INDUCED CHANGES IN PARTICULAR VGCs

A. Kv Channels

The Kv1, Shaker, is a robustly MS channel whether in cell-attached or excised patches, and whether membrane tension is generated by negative or positive pipette pressure (Gu *et al.*, 2001). Monitored at a fixed voltage, Shaker can behave as either an SA or an SI channel (Fig. 3). Stretch increases the rate of Shaker activation at the foot of the $g(V)$ curve, and slightly (~10 mV) left shifts the entire curve at lytic tension (Tabarean and Morris, 2002). When stretch is applied during a voltage step, both rise and decay times accelerate and peak current increases, a pattern particularly evident in the S3–S4 deletion mutants, including Shaker 5aa. In Shaker 5aa, activation and slow inactivation speeds are similar, allowing effects of stretch on both to be monitored simultaneously and modeled. Shaker 5aa behavior was described within the framework of a linear "Aldrich" model (appropriately scaled for Shaker 5aa and with slow inactivation added) where, over a wide voltage range, 1.5- to 2-fold rate changes (forward and backward rates for closed–closed transitions changed reciprocally, yielding <10-fold increase in overall forward rate along the activation path) could account for the observed effects of moderate stretch over a wide range of voltages.

Subsequently (Laitko and Morris, 2004), we focused on the simple kinetics of Shaker 5aa (using the voltage range where 5aa kinetics obey a scheme in which four independent forward steps resolve in a cooperative C—O transition (Fig. 4D). Stretch did not affect the voltage dependence of the key activation parameter in Shaker 5aa, only the basic rates. Time- and amplitude-scaling transformations showed that while voltage changes the rates of activation and inactivation of Shaker 5aa differently, stretch had exactly the same effect (~1.5- to 2-fold acceleration) on the characteristic times of both processes (Fig. 7). This suggests that the protein motions limiting 5aa activation and slow inactivation are similar enough physically to "feel" the stretch-altered bilayer similarly (e.g., both might involve motions over a substantial fraction of the channel–lipid interface). Interestingly, Pathak *et al.* (2006) have subsequently reported preliminary fluorescence data showing that slow inactivation in Shaker involves (in addition to the pore domain) a motion that projects throughout the voltage-sensing domain.

As already explained, we used Shaker ILT to examine the concerted voltage-dependent closed–closed motion just prior to the concerted opening step (Laitko *et al.*, 2006). We found that this motion, which may entail some

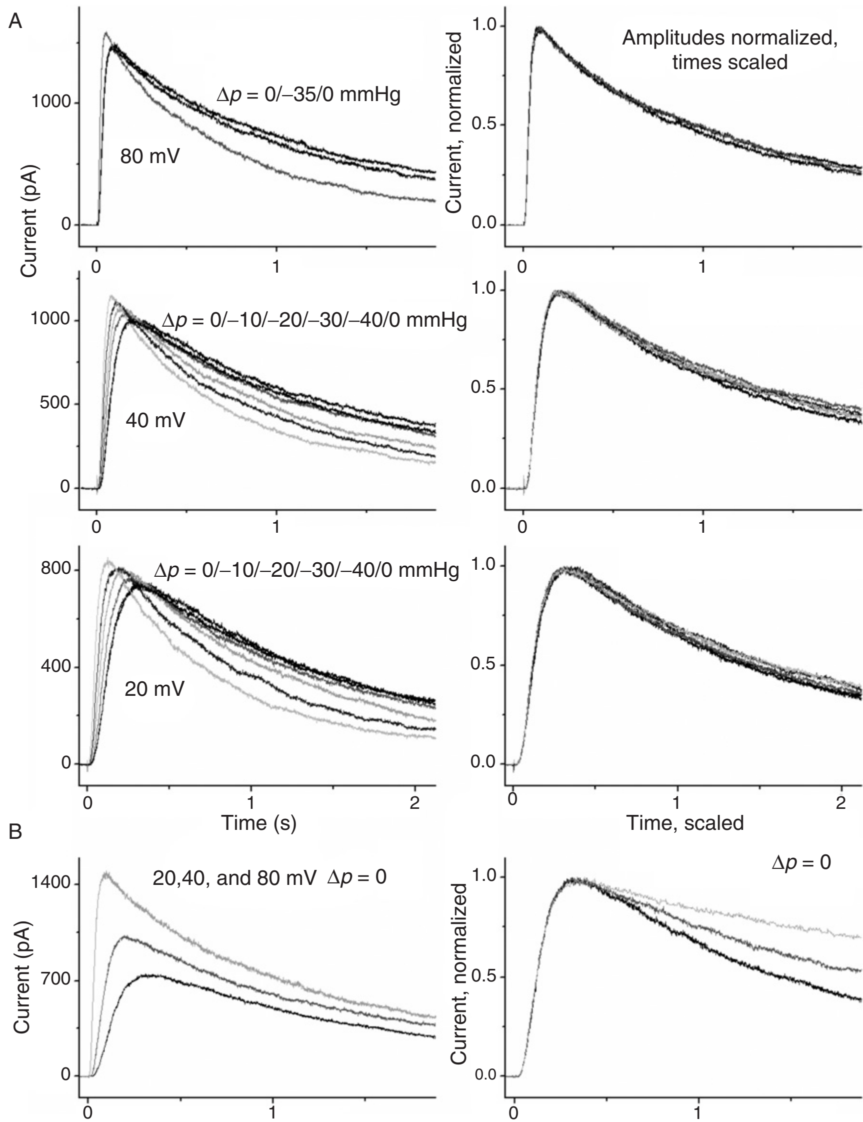

FIGURE 7 Activation and slow inactivation transition rates are equally stretch sensitive in Shaker 5aa. This finding is illustrated (all traces from one patch) by comparing the effect of rescaling currents for different stretch intensities at any given voltage (A) vs rescaling for different voltages at a given stretch intensity (B). (A) First, at one voltage (80 mV), currents before/during/after stretch; at right these are amplitude and time scaled. Next, at 40 mV then at 20 mV, a stretch dose response; at right, amplitude and time scaled (the procedure is to rescale for activation only. The outcome is that inactivation trajectories all overlap if they are all

expansion in the plane of the bilayer, nevertheless decelerates with stretch, possibly for entropic reasons (stretch may interfere with the tidy orchestration of eight moving parts of the channel). Pore closing in both Shaker ILT and Shaker WTIR (as monitored by tail current rates) showed a consistent effect of stretch within a batch of oocytes but not between batches (acceleration, no effect, and deceleration were all obtained). It may be that oocyte bilayer lipid composition differs enough among frogs to confound direct lipid stress (i.e., stretch) effects.

Kv3 and BKCa channels were discussed in earlier sections and Kv3 is discussed in comparison to Cav channel stretch responses in the next section.

Assorted accounts have appeared on swelling-activated Kv conductances in native preparations, but none test the possibility that membrane stretch is involved. For example, in gastric myocytes, hypoosmotic solutions markedly increase I_{KCa} and a 4-aminopyridine sensitive I_{Kv} (Piao *et al.*, 2001). A trigeminal nerve Kv conductance is described as MS (Piao *et al.*, 2006) based on responses to osmotic stimuli. The actin cytoskeleton is necessary for the response and membrane stretch was not tested.

In cardiac cells, swelling enhances a slowly activating delayed rectifier current (I_{Ks}) (Kubota *et al.*, 2002). Two proteins constitute I_{Ks} (KCNQ1 and KCNE1) {KCNQ1 = KvLQT1; KCNE1 = minK} but recombinant homomeric KCNQ1 channels on their own respond to swelling (increased conductance, no change in kinetics) and protein tyrosine kinase activity is not involved. Grunnet *et al.* (2003) found that recombinant KCNQ1 and KCNQ4 (but not KCNQ2 and KCNQ3) channels are tightly regulated by cell volume changes. Current amplitude increases with no change in kinetics and here too the actin cytoskeleton is implicated (a similar result was obtained by the same group for HNC2 channels; Calloe *et al.*, 2005). The swelling-induced effect does not require the auxiliary subunits (KCNE1–3). Stretch was not tested.

B. Cav and Kv3 Channels Have Similar Stretch Responses

Historically, the first indication that VGCs are included among the MS channels was a careful report on a smooth muscle L-type Ca current in which whole-cell inflation was used to stress the membrane (Langton, 1993).

---

accelerated the same-fold as activation). (B) Same patch, when the same voltages are tested with no stretch and currents normalized to activation, the inactivation trajectories do not overlap. Thus, activation and slow inactivation have different voltage dependences but the same stretch dependence. For more details, see Fig. 8 and related text of Laitko and Morris (2004).

Many native myocytes exhibit the pattern of responses described in that paper and comparable responses have been confirmed for recombinant L-type (Lyford *et al.*, 2002) and N-type (Calabrese *et al.*, 2002) Cav channels (Fig. 6). In both recombinants, mechanosensitivity resides with the α- (pore) subunit. The demonstrable tension-sensitivity (as opposed to "pressure"-sensitivity) of N-type channels allowed us to argue that Cav channels respond directly to bilayer stretch. Whether stretch is applied under whole-cell conditions (inflation or osmotic swelling), under perforated patch (osmotic swelling), or via stretch of cell-attached patches, the following stretch effects are seen for both N- and L-type Cav channels:

1. Stretch reversibly increases peak and steady state I_{Cav} ~1.5-fold.
2. Stretch does not change the rate of I_{Cav} onset.
3. Stretch reversibly increases g_{max} with no shift in the midpoint of $g(V)$ (g_{max} and $g(V)$ midpoint are determined from I/V plots; effectively stretch scales up the I/V relation).
4. Stretch increases single-channel activity without changing the unitary conductance.

New membrane insertion does not account for the increased g_{max} for either recombinant N-type (Calabrese *et al.*, 2002) or native L-type current (Ben-Tabou De-Leon *et al.*, 2006) since membrane capacitance is unaffected. In the case of N-type channels (but not L-type), inactivation from the open state accelerates with stretch.

A T-type channel stably transfected in HEK cells, into which were co-transfected N-type channels, exhibited no response to the same stretch stimuli that enhanced N-type current (Calabrese *et al.*, 2002). On the other hand, native pituitary cell T-type current responds like L-type current in the same cells albeit more weakly (Ben-Tabou De-Leon *et al.*, 2003, 2006).

When reporting the action of stretch on N-type current (Calabrese *et al.*, 2002), we were struck by a qualitative difference between Cav and Kv (Shaker) responses: the rate of current onset does not change with stretch and activation does not left shift. We felt that stretch might not affect voltage sensor motions in Cav channels as it does in Shaker channels. We have now, however, observed Cav-channel-like responses in a Kv3 channel (Laitko *et al.*, 2006): in both, stretch reversibly and repeatably increases the peak and steady state current, with an apparent increase in g_{max} and with no evidence (from the current onset rate) that activation accelerates with stretch (Fig. 5). Taking an Occam's razor approach, it seems simplest therefore, to use for the Cav channels a generic working hypothesis consistent with what is known from Shaker (and mutants): That hypothesis is that the net effect of stretch on I_{Cav} results from

1. stretch acceleration of an MS voltage-dependent forward transition (not the rate-limiting transition for onset of I_{Cav}) and
2. stretch deceleration of an MS-concerted voltage-sensitive forward transition (simply to be consistent with Shaker ILT) and
3. in the N-type Cav channel, stretch-accelerated inactivation.

These putative MS transitions in Cav channels would be modulated by stretch in such a way that, very robustly, the net result is increased peak and steady state I_{Cav}, with no evidence of shift in the $g(V)$ curve.

We hoped that activation kinetics in T-type Cav channels would be more "accessible" than in N- or L-type, but inexplicably the recombinant T-type isoform we tested (Calabrese *et al.*, 2002) showed no stretch sensitivity in HEK cells. However, Ben-Tabou De-Leon *et al.* (2003, 2006) find that in pituitary cells, T-type currents are mildly responsive to swell/shrink stimuli, and although they made no kinetic analysis, their traces suggest that activation and inactivation rates respond. This would be worth a closer look, since access to MS activation could help clarify whether the "Occam's razor" hypothesis is warranted.

C. Cav: L-Type Channels in Native Preparations

L-type Cav channel mechanosensitivity has been tested in more native myocytes using more types of mechanostimuli than for any other VGC. When the channels respond, the response is stereotyped (as described above: increased peak and steady state I_{Cav}, with no evidence of shift in the $g(V)$ curve). Nevertheless, the substantial collection of reports is full of idiosyncrasies, contradictions, and quirks, some of which we reviewed (Morris and Laitko, 2005; see also references in Calabrese *et al.*, 2002). A few additional items are worthy of mention.

For hypo- and hyperosmotic stimuli, Ben-Tabou De-Leon *et al.* (2003, 2006) showed that F-actin reagents (cytochalsin D and phalloidin, efficacy monitored via microscopy) have no effect on the swell/shrink modulation of L- and T-type I_{Cav} in pituitary cells. In ventricular myocytes, by contrast, Pascarel *et al.* (2001) found that F-actin is needed for T-type current to respond to swelling.

Amano *et al.* (2005) observed that both hypoosmotic solution and fast bath flow modulate L-type current in smooth muscle myocytes. It is unclear if the flow-induced enhancement (which is transient except when cells are thapsigargin-treated) is a membrane mechanical effect.

Xu *et al.* (2000) tested several stretch stimuli on smooth muscle I_{Ca} using whole-cell clamp. Hypoosmotic bath solution increases I_{Ca} as does cell

inflation, but direct longitudinal stretch (up to 130%) using two electrodes does not. Belus and White (2003) using axial stretch on ventricular myocytes observed no effect of stretch on L-type current, although the same stimulus activated a streptomycin sensitive linear current. Perhaps, in the ventricular myocytes, the plasma membrane regions where L-type Ca channels reside can avoid lipid stress during a directional mechanical stimulus; cardiac muscle L-type Ca channels are expressed in longitudinally arrayed puncta (Grabner *et al.*, 1998). Since flow is a directional mechanostimulus, the transience of the flow effect noted by Amano *et al.* (2005) might echo the ineffectiveness of directional stretch.

D. Nav Channels

Gastrointestinal interstitial cells of Cajal express a Nav1.5 current that appears to be modulated by shear stress and stretch, but this evidently depends on membrane skeleton (Strege *et al.*, 2003). Squid axon excitability is reversibly susceptible to axonal inflation (Terakawa and Nakayama, 1985) and squid axon gating and ionic current are modulated (slowed) by hyperbaric pressure (Conti *et al.*, 1984). Immunolabeling reveals that Nav channels are present in the sensory neurite of Pacinian corpuscles (Pawson and Bolanowski, 2002). TTX-sensitive Na entry into vascular smooth muscle cells and into Nav1.2 transfected CHO cells inhibits shear stress-mediated activation of a signaling kinase (Traub *et al.*, 1999). In Nav1.4 channels expressed in oocytes, where there is such an overwhelming irreversible effect of stretch (a switch to a fast-gating mode; Tabarean *et al.*, 1999) we did not rigorously test the fast Nav1.4 currents for reversible MS responses. However, this same Nav channel, expressed in HEK cells shows left shifts in its inactivation curves when bilayer elasticity is elevated by adding various amphiphiles or by depleting cholesterol (Lundbaek *et al.*, 2004). We have therefore begun testing Nav1.5 α-subunit channels in cell-attached oocyte patches subjected to stretch. Unlike the Nav1.4 α-subunit, these do not show irreversible changes in response to stretch and our preliminary finding is that both the onset and decline phases of current transients elicited by voltage steps accelerate reversibly with stretch (CEM, unpublished observation).

E. HCN Channels

Recombinant sea urchin and mammalian HCN channels (spHCN and HCN2) expressed in oocytes respond directly and reversibly to membrane stretch (Lin *et al.*, 2007); our preliminary findings are that both

hyperpolarization-induced turn on of I_{HCN2} and depolarization-induced turn off of I_{HCN2} accelerate with stretch.

It remains to be seen whether these MS responses bear any relation to the responses of HCN2 channels coexpressed with aquaporin in *Xenopus laevis* oocytes and monitored via two-electrode voltage-clamp during swelling (Calloe *et al.*, 2005). The hyperpolarization-activated current increases ~30% with no change in activation kinetics. This response was abolished by cytochalasin D. The only indication that membrane stretch triggers the response is the fact that inflating oocytes with oil mimics the effect of osmotic swelling.

V. IRREVERSIBLE STRETCH-INDUCED GATING CHANGES IN VGCs

Stretched membrane patches experience not only elastic changes (idealized as reversible far-field tension in the plane of the membrane) but also plastic (irreversible) changes in patch microstructure (these changes are not characterized but could involve damage to the integrity of the membrane skeleton, protein clusters, caveolae, microvilli, and any organized lipid microdomains). The impact of plastic change becomes self-evident whenever the responses of channels to stretch depend on a patch's mechanical "history." The physical nature of the microstructural changes will vary among cell types.

Recombinant Nav1.4 in oocyte patches (Shcherbatko *et al.*, 1999; Tabarean *et al.*, 1999) and N-type Cav studied under whole-cell clamp (Calabrese *et al.*, 2002) both exhibit history-dependent activity changes due to unidentified cumulative mechanical trauma of the membrane. Figure 8A shows, from a single oocyte, Nav1.4 current families from four patches, two of which formed without applied stretch; stretch irreversibly alters the channel's gating characteristics. Surprisingly, the effect of this "trauma" on Nav1.4 expressed in *Xenopus* oocytes is like the effect of the channel's auxiliary subunit. In oocytes, Nav1.4 α- (pore) subunits show anomalously slow, right-shifted kinetics because oocytes lack auxiliary β1-subunits. Coexpression of α- and β-subunits leads to normal gating behavior. Surprisingly, however, applying stretch to patches with only the α-subunit makes the Nav1.4 α channels switch irreversibly into a gating mode that is not only fast and left-shifted but indistinguishable from what is seen with the β1-subunit present (Shcherbatko *et al.*, 1999; Tabarean *et al.*, 1999). How stretch cumulatively and irreversibly alters the α-subunit environment to achieve the same effect as having β1-subunits is unknown. Interestingly, the auxiliary β1-subunit (which spans the bilayer only once) has an extracellular domain that acts as an adhesion molecule (McEwen and Isom, 2004). On the basis of effects of

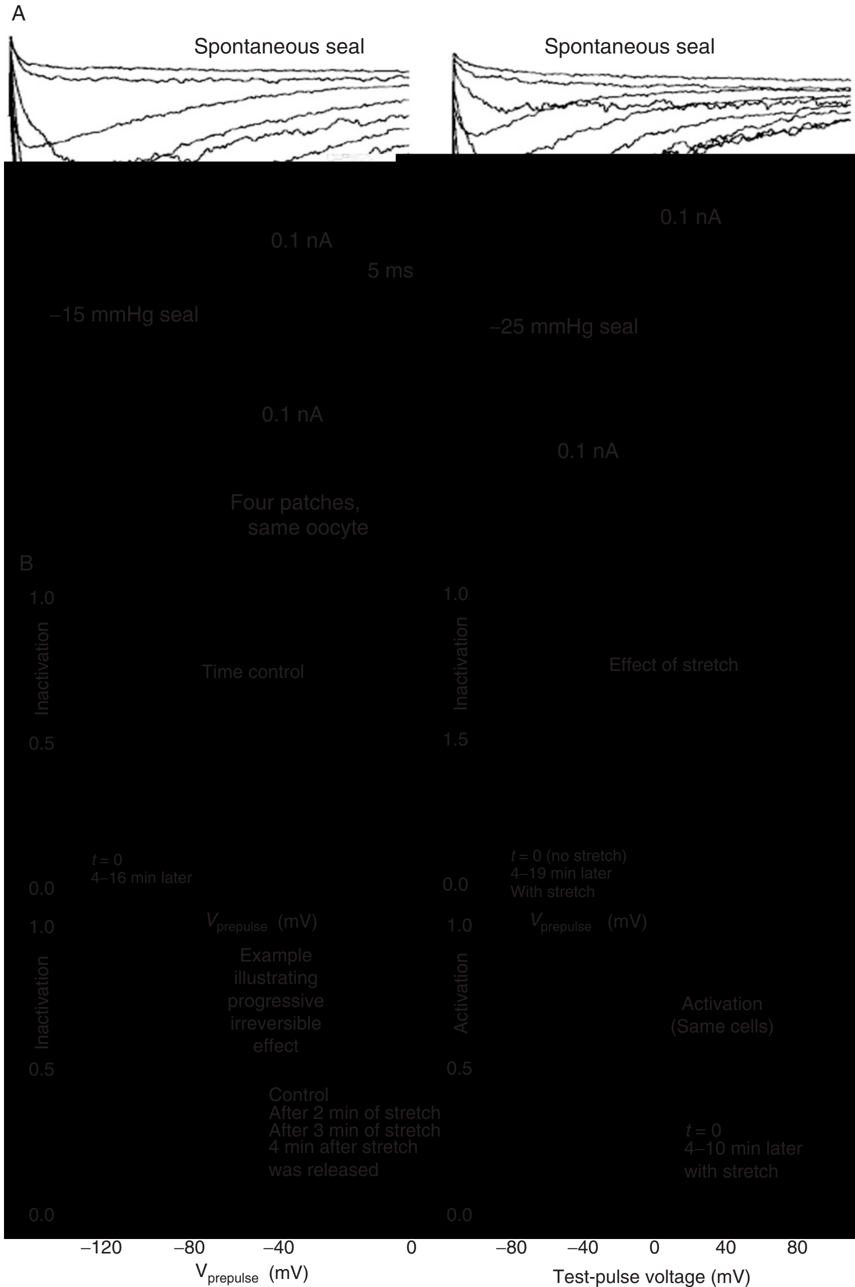

FIGURE 8 Irreversible effects of stretch on Nav1.4 and N-type Cav channels. (A) Even "unintended" stretch experienced during seal formation causes an irreversible switch from anomalous slow mode to fast mode gating in Nav1.4 (α-subunit only) channels expressed in oocytes.

nocadazole, it was suggested (Shcherbatko *et al.*, 1999) that stretch might disrupt kinetically important interactions of Nav1.4 channels with microtubules. This seems unpersuasive (see Fig. 1B and related discussion of Morris *et al.*, 2006); moreover, in mammalian cells Nav1.4 gating is unaffected by cytoskeletal reagents (Moran *et al.*, 2000).

N-type Cav channels in the whole-cell configuration show run down and time-dependent left shift of their inactivation curve (Fig. 8B). In this configuration, stretch that increases I_{Cav} (while having no effect on the voltage dependence of activation) causes a further irreversible left shift of the inactivation curve (see Fig. 6 in Calabrese *et al.*, 2002 for details). Meanwhile, the same channels do exhibit reversible stretch acceleration of open state inactivation.

Kinetically, discrete irreversible stretch changes in VGC behavior such as these may correspond to (1) changes that, in an intact cell, would be reversed via enzyme-mediated processes or (2) changes that in an intact cell would be avoided by virtue of mechanoprotective arrangements. Membrane skeleton is undoubtedly important for mechanoprotection (Morris, 2001) and sequestration of channels in specialized membrane subdomains may also contribute. In skeletal muscle, caveolae are exceedingly difficult to flatten with stretch (Dulhunty and Franzini-Armstrong, 1975); perhaps residence in caveolae affords VGCs a degree of mechanoprotection from both reversible stretch effects and irreversible stretch damage.

VI. TECHNICAL ISSUES

A. Applying a Stretching Force to Study MS Modulation of VGC Activity

Elevating the tension in a membrane by applying stretch to a patch of membrane or an intact cell is not a precision process. Various techniques have been used in the context of VGCs and are briefly itemized here. This topic has been reviewed (Morris *et al.*, 2006).

1. Gigaseal Patch Recording

Pipette suction (negative pressure) is used to apply stretch to either cell-attached or excised patches. Usually, we apply suction ~1 s before step pulses begin. When *P*/*N* linear subtraction is used, suction is present during

---

Modified from Tabarean *et al.* (1999). (B) For N-type Cav channels recorded under whole-cell clamp in HEK cells, stretch exacerbates a time-dependent left shift of inactivation (as in the Nav1.4 irreversible effect, the effect is progressive, as illustrated), even though, in the same cells, the voltage dependence of activation is entirely unaffected. Modified from Calabrese *et al.* (2002).

the subtraction pulses. Positive pipette pressure also elevates the membrane tension with the membrane at opposite curvature. Since patches are more durable with suction than with positive pressure, suction is the stimulus most used. Irreversible changes that occur with stretch must inevitably include changes in the lipid organization of the bilayer. We suspect this accounts for some of the nonstationary behavior seen in many patches. Stretch-induced disruption of the membrane skeleton probably exacerbates the previously mentioned effect. Sometimes, a patch will show entirely reversible stretch effects with the first stimulus, and other times, there will be a settling in period. Dose response data are hard to obtain. We deal with this by routinely checking for reversibility (testing the response to a voltage step or ramp before, during, and after a stretch stimulus).

2. Hydrostatic Inflation

Positive pipette pressure applied under whole-cell clamp inflates the cell. This stimulus is not a strictly reversible stimulus. Cell debris can move into the tip during a pressure-off step. Applied pressure is an unreliable gauge of membrane tension because mechanical resistances develop in the pipette (Langton, 1993; Calabrese *et al*., 2002). In HEK cells, elevated membrane tension develops only when a frank increase in cell volume occurs (Calabrese *et al*., 2002). Long range structures (membrane skeleton?) may disorganize or rupture at this point, causing load to transfer to the bilayer. Inflation may also be coincident with flattening of membrane irregularities, at which point the microdomain organization as well as the local curvatures in the VGC-bearing bilayer may change (similar events presumably occur in patches on gigaseal formation). For various MS VGCs, whole-cell inflation and osmotic swelling may have the same effect on channel activity, but it is never certain that membrane stress is the source of gating energy in the case of osmotic swelling.

3. Osmotic Swelling

For mammalian cell lines and several native cells, both perforated patch recordings (downshock of bath solution) and whole-cell recording (pipette solution at elevated osmotic pressure or, more commonly, external solution at reduced osmotic pressure) have been used. As with hydrostatic inflation, monitoring cell volume during recordings is critical. *Xenopus* oocytes, being adapted for prolonged sojourns in pond water, swell very little with downshock, but will swell if the cells are made to express aquaporin (Calloe *et al*., 2005); under those conditions, VGC currents can be recorded under two microelectrode clamps in conjunction with transient (and visually measureable) osmotic-swelling stimuli.

4. Inflation by Oil Injection

During two microelectrode voltage clamps, *Xenopus* oocytes can be inflated by injecting about 50 nl of oil using the same device used to inject cRNA. This procedure detectably increases oocyte volume without dilution of any cytoplasmic molecules (Calloe *et al*., 2005).

5. Stretching Native Myocytes Using Two Pipettes

See Xu *et al*., 2000; Belus and White, 2003.

6. Shear Flow

This complex mechanical stimulus needs careful verification (Levitan *et al*., 2000); flow effects can be confounded with mechanochemical signals (Maroto and Hamill, 2001).

B. Gadolinium Strangeness

To cleanly measure MS VGC currents, other MS currents need to be eliminated. Gadolinium inhibits some SA cation channels (Yang and Sachs, 1989; although see Hurwitz *et al*., 2002) making it useful in some biophysical studies. We have found it highly problematic used in connection with recombinant VGCs expressed in *Xenopus* oocytes (Gu *et al*., 2001) and so prefer 1-mM lanthanum chloride whenever possible). Gadolinium right-shifts voltage-dependent gating parameters (thought be a surface charge shielding effect) and reduces maximal conductances (Elinder and Arhem, 1994; Gu *et al*., 2001). Given that lanthanides also rigidify the exposed bilayer leaflet (Tanaka *et al*., 2002), they probably exert lipid stress effect too, but no-gadolinium controls (Tabarean and Morris, 2002) showed that the effects of stretch on Shaker are evident with or without a lanthanide.

Gadolinium's side effects are generally acceptable in biophysical studies but its use is not warranted to ‘‘selectively'' block SA cation channels in native cell where the final outputs are action potentials or a background cation conductance. At vanishingly small concentrations, carbonate, bicarbonate, phosphate, and hypophosphate ions precipitate gadolinium; when gadolinium is used to block SA cation channels in native cells exposed to CO_2-bicarbonate buffered solutions, it is difficult to know what to make of the findings. Do cells become coated with a microprecipitate of gadolinium-(bi)carbonate-(bi)phosphate that somehow produces the observed effects?

VII. SUMMARY COMMENTS

The conformational motions of VGCs respond to changes in lipid stress. Fields as distinct as sensory physiology and anesthesiology and evolutionary biology would be well served by a more precise understanding of this fact and its implications. What is ideally needed at this juncture is to pick one well-characterized VGC and subject it to a range of the biophysically important physical and chemical lipid stress agents. These include stretch, hyperbaric pressure, and temperature, plus an array of BMRs (e.g., an alkanol series, cholesterol). Kv1 channels are the obvious candidate, given the amassed structure–function information available for them. Already, it has been shown that activation, prepore opening, and slow inactivation transitions in the prototypical Kv1, Shaker, are MS. To facilitate computational probing, bilayer-based studies would be ideal (KvAP, though not a Kv1—more a "Kv0"—could be used here), but preparations that allow for gating current measurements and other means (optical, chemical) of monitoring channel motions will also be needed since many transitions affected by physical and chemical lipid stressors will not be directly accessible through measurements of ionic current. Our experience with Kv channels, using stretch as the lipid stressor, illustrates that the behavior of "hidden transitions" can be critical; finessing the hidden transitions as well as focusing on the rate-limiting ones should greatly clarify the actions of amphiphilic molecules on VGCs.

Many VGCs (like some TRP channels) are "MS channels," and what seems likely to bear most fruit now is to dissect the behavior of 6TM channels in terms of their diverse inherent susceptibilities to the physical and chemical agents of lipid stress.

Acknowledgments

Work in CE Morris' laboratory is supported by grants from the Canadian Institutes of Health Research.

References

Amano, S., Ishikawa, T., and Nakayama, K. (2005). Facilitation of L-type Ca2+ currents by fluid flow in rabbit cerebral artery myocytes. *J. Pharmacol. Sci.* **98,** 425–429.

Barbuti, A., Gravante, B., Riolfo, M., Milanesi, R., Terragni, B., and DiFrancesco, D. (2004). Localization of pacemaker channels in lipid rafts regulates channel kinetics. *Circ. Res.* **94,** 1325–1331.

Bartucci, R., Gambacorta, A., Gliozzi, A., Marsh, D., and Sportelli, L. (2005). Bipolar tetraether lipids: Chain flexibility and membrane polarity gradients from spin-label electron spin resonance. *Biochemistry* **44,** 15017–15023.

Baumgart, T., Hess, S. T., and Webb, W. W. (2003). Imaging coexisting fluid domains in biomembrane models coupling curvature and line tension. *Nature* **425,** 821–824.

Belus, A., and White, E. (2003). Streptomycin and intracel, lular calcium modulate the response of single guinea-pig ventricular myocytes to axial stretch. *J. Physiol.* **546,** 501–509.

Ben-Tabou De-Leon, E., Blotnick, E., and Nussinovitch, I. (2003). Effects of osmotic swelling on voltage-gated calcium channel currents in rat anterior pituitary cells. *Am. J. Physiol. Cell Physiol.* **285,** C840–C852.

Ben-Tabou De-Leon, S., Ben-Zeev, G., and Nussinovitch, I. (2006). Effects of osmotic shrinkage on voltage-gated Ca2+ channel currents in rat anterior pituitary cells. *Am. J. Physiol. Cell Physiol.* **290,** C222–C232.

Bock, J., Szabo, I., Gamper, N., Adams, C., and Gulbins, E. (2003). Ceramide inhibits the potassium channel Kv1. 3 by the formation of membrane platforms. *Biochem. Biophys. Res. Commun.* **305,** 890–897.

Brauchi, S., Orio, P., and Latorre, R. (2004). Clues to understanding cold sensation: Thermodynamics and electrophysiological analysis of the cold receptor TRPM8. *Proc. Natl. Acad. Sci. USA* **101,** 15494–15499.

Calabrese, B., Tabarean, I. V., Juranka, P., and Morris, C. E. (2002). Mechanosensitivity of N-type calcium channel currents. *Biophys. J.* **83,** 2560–2574.

Calloe, K., Elmedyb, P., Olesen, S. P., Jorgensen, N. K., and Grunnet, M. (2005). Hypoosmotic cell swelling as a novel mechanism for modulation of cloned HCN2 channels. *Biophys. J.* **89,** 2159–2169.

Camara, A. K., Begic, Z., Kwok, W. M., and Bosnjak, Z. J. (2001). Differential modulation of the cardiac L- and T-type calcium channel currents by isoflurane. *Anesthesiology* **95,** 515–524.

Cantor, R. S. (1997). The lateral pressure profile in membranes: A physical mechanism of general anesthesia. *Biochemistry* **36,** 2339–2344.

Cantor, R. S. (1999). Lipid composition and the lateral pressure profile in bilayers. *Biophys. J.* **76,** 2625–2639.

Chang, H. M., Reitstetter, R., Mason, R. P., and Gruener, R. (1995). Attenuation of channel kinetics and conductance by cholesterol: An interpretation using structural stress as a unifying concept. *J. Membr. Biol.* **143,** 51–63.

Chemin, J., Monteil, A., Perez-Reyes, E., Nargeot, J., and Lory, P. (2001). Direct inhibition of T-type calcium channels by the endogenous cannabinoid anandamide. *EMBO J.* **20,** 7033–7040.

Chen, X., Sirois, J. E., Lei, Q., Talley, E. M., Lynch, C., III, and Bayliss, D. A. (2005). HCN subunit-specific and cAMP-modulated effects of anesthetics on neuronal pacemaker currents. *J. Neurosci.* **25,** 5803–5814.

Chu, B., and Treistman, S. N. (1997). Modulation of two cloned potassium channels by 1-alkanols demonstrates different cutoffs. *Alcohol Clin. Exp. Res.* **21,** 1103–1107.

Chu, B., Dopico, A. M., Lemos, J. R., and Treistman, S. N. (1998). Ethanol potentiation of calcium-activated potassium channels reconstituted into planar lipid bilayers. *Mol. Pharmacol.* **54,** 397–406.

Clapham, D. E. (2003). TRP channels as cellular sensors. *Nature* **426,** 517–524.

Clarke, A. L., Petrou, S., Walsh, J. V., Jr., and Singer, J. J. (2003). Site of action of fatty acids and other charged lipids on BKCa channels from arterial smooth muscle cells. *Am. J. Physiol. Cell Physiol.* **284,** C607–C619.

Conti, F., Inoue, I., Kukita, F., and Stuhmer, W. (1984). Pressure dependence of sodium gating currents in the squid giant axon. *Eur. Biophys. J.* **11,** 137–147.

Correa, A. M. (1998). Gating kinetics of Shaker K+ channels are differentially modified by general anesthetics. *Am. J. Physiol.* **275,** C1009–C1021.

Covarrubias, M., and Rubin, E. (1993). Ethanol selectively blocks a noninactivating K+ current expressed in Xenopus oocytes. *Proc. Natl. Acad. Sci. USA* **90,** 6957–6960.

Crowley, J. J., Treistman, S. N., and Dopico, A. M. (2003). Cholesterol antagonizes ethanol potentiation of human brain BKCa channels reconstituted into phospholipid bilayers. *Mol. Pharmacol.* **64,** 365–372.

Cuello, L. G., Cortes, D. M., and Perozo, E. (2004). Molecular architecture of the KvAP voltage-dependent K+ channel in a lipid bilayer. *Science* **306,** 491–495.

Czub, J., and Baginski, M. (2006). Comparative molecular dynamics study of lipid membranes containing cholesterol and ergosterol. *Biophys. J.* **90,** 2368–2382.

Dan, N., and Safran, S. A. (1998). Effect of lipid characteristics on the structure of transmembrane proteins. *Biophys. J.* **75,** 1410–1414.

Davies, D. L., Crawford, D. K., Trudell, J. R., Mihic, S. J., and Alkana, R. L. (2004). Multiple sites of ethanol action in alpha1 and alpha2 glycine receptors suggested by sensitivity to pressure antagonism. *J. Neurochem.* **89,** 1175–1185.

Devaux, P. F., and Morris, R. (2004). Transmembrane asymmetry and lateral domains in biological membranes. *Traffic* **5,** 241–246.

Dong, X. X., and Iwasa, K. H. (2004). Tension sensitivity of prestin: Comparison with the membrane motor in outer hair cells. *Biophys. J.* **86,** 1201–1208.

Dopico, A. M., Kirber, M. T., Singer, J. J., and Walsh, J. V., Jr. (1994). Membrane stretch directly activates large conductance Ca(2+)-activated K+ channels in mesenteric artery smooth muscle cells. *Am. J. Hypertens.* **7,** 82–89.

Dulhunty, A. F., and Franzini-Armstrong, C. (1975). The relative contributions of the folds and caveolae to the surface membrane of frog skeletal muscle fibres at different sarcomere lengths. *J. Physiol.* **250,** 513–539.

Elinder, F., and Arhem, P. (1994). Effects of gadolinium on ion channels in the myelinated axon of Xenopus laevis: Four sites of action. *Biophys. J.* **67,** 71–83.

Etzion, Y., and Grossman, Y. (2000). Pressure-induced depression of synaptic transmission in the cerebellar parallel fibre synapse involves suppression of presynaptic N-type Ca2+ channels. *Eur. J. Neurosci.* **12,** 4007–4016.

Feller, S. E., Brown, C. A., Nizza, D. T., and Gawrisch, K. (2002). Nuclear overhauser enhancement spectroscopy cross-relaxation rates and ethanol distribution across membranes. *Biophys. J.* **82,** 1396–1404.

Fisyunov, A., Tsintsadze, V., Min, R., Burnashev, N., and Lozovaya, N. A. (2006). Cannabinoids modulate the P-type high voltage-activated calcium currents in Purkinje neurons. *J. Neurophysiol.* **96,** 1267–1277.

Folco, E. J., Liu, G. X., and Koren, G. (2004). Caveolin-3 and SAP97 form a scaffolding protein complex that regulates the voltage-gated potassium channel Kv1. 5. *Am. J. Physiol. Heart Circ. Physiol.* **287,** H681–H690.

Franco-Obregon, A., and Lansman, J. B. (2002). Changes in mechanosensitive channel gating following mechanical stimulation in skeletal muscle myotubes from the mdx mouse. *J. Physiol.* **539,** 391–407.

Gaus, K., Zech, T., and Harder, T. (2006). Visualizing membrane microdomains by Laurdan 2-photon microscopy. *Mol. Membr. Biol.* **23,** 41–48.

Gill, C. H., Randall, A., Bates, S. A., Hill, K., Owen, D., Larkman, P. M., Cairns, W., Yusaf, S. P., Murdock, P. R., Strijbos, P. J., Powell, A. J., Benham, C. D., *et al.* (2004). Characterization of the human HCN1 channel and its inhibition by capsazepine. *Br. J. Pharmacol.* **143,** 411–421.

Grabner, M., Dirksen, R. T., and Beam, K. G. (1998). Tagging with green fluorescent protein reveals a distinct subcellular distribution of L-type and non-L-type Ca2+ channels expressed in dysgenic myotubes. *Proc. Natl. Acad. Sci. USA* **95,** 1903–1908.

Grunnet, M., Jespersen, T., MacAulay, N., Jorgensen, N. K., Schmitt, N., Pongs, O., Olesen, S. P., and Klaerke, D. A. (2003). KCNQ1 channels sense small changes in cell volume. *J. Physiol.* **549,** 419–427.

Gu, C. X., Juranka, P. F., and Morris, C. E. (2001). Stretch-activation and stretch-inactivation of Shaker-IR, a voltage-gated K+ channel. *Biophys. J.* **80,** 2678–2693.

Guharay, F., and Sachs, F. (1984). Stretch-activated single ion channel currents in tissue-cultured embryonic chick skeletal muscle. *J. Physiol.* **352,** 685–701.

Guharay, F., and Sachs, F. (1985). Mechanotransducer ion channels in chick skeletal muscle: The effects of extracellular pH. *J. Physiol.* **363,** 119–134.

Guizy, M., Arias, C., David, M., Gonzalez, T., and Valenzuela, C. (2005). {Omega}-3 and {omega}-6 polyunsaturated fatty acids block HERG channels. *Am. J. Physiol. Cell Physiol.* **289,** C1251–C1260.

Gullingsrud, J., and Schulten, K. (2004). Lipid bilayer pressure profiles and mechanosensitive channel gating. *Biophys. J.* **86,** 3496–3509.

Hajdu, P., Varga, Z., Pieri, C., Panyi, G., and Gaspar, R., Jr. (2003). Cholesterol modifies the gating of Kv1. 3 in human T lymphocytes. *Pflugers Arch.* **445,** 674–682.

Harris, T., Shahidullah, M., Ellingson, J. S., and Covarrubias, M. (2000). General anesthetic action at an internal protein site involving the S4-S5 cytoplasmic loop of a neuronal K(+) channel. *J. Biol. Chem.* **275,** 4928–4936.

Hisada, T., Walsh, J. V. Jr, and Singer, J. J. (1993). Stretch-inactivated cationic channels in single smooth muscle cells. *Pflugers Arch.* **422,** 393–396.

Hu, Y. M., Zhang, Z., Gao, R. B., and Xu, Y. Q. (1997). Effect of lysophosphatidylcholine on the pacemaker current I(f) of sheep cardiac Purkinje fibers in ischemia-like condition. *Sheng Li Xue Bao* **49,** 513–520.

Hurwitz, C. G., Hu, V. Y., and Segal, A. S. (2002). A mechanogated nonselective cation channel in proximal tubule that is ATP sensitive. *Am. J. Physiol. Renal Physiol.* **283,** F93–F104.

Iwata, A., Stys, P. K., Wolf, J. A., Chen, X.H, Taylor, A. G., Meaney, D. F., and Smith, D. H. (2004). Traumatic axonal injury induces proteolytic cleavage of the voltage-gated sodium channels modulated by tetrodotoxin and protease inhibitors. *J. Neurosci.* **24,** 4605–4613.

Kahya, N., and Schwille, P. (2006). Fluorescence correlation studies of lipid domains in model membranes. *Mol. Membr. Biol.* **23,** 29–39.

Kamatchi, G. L., Durieux, M. E., and Lynch, C., III (2001). Differential sensitivity of expressed L-type calcium channels and muscarinic M(1) receptors to volatile anesthetics in Xenopus oocytes. *J. Pharmacol. Exp. Ther.* **297,** 981–990.

Kendig, J. J. (1984). Nitrogen narcosis and pressure reversal of anesthetic effects in node of Ranvier. *Am. J. Physiol.* **246,** C91–C95.

Kirber, M. T., Walsh, J. V., Jr., and Singer, J. J. (1988). Stretch-activated ion channels in smooth muscle: A mechanism for the initiation of stretch-induced contraction. *Pflugers Arch.* **412,** 339–345.

Kubota, T., Horie, M., Takano, M., Yoshida, H., Otani, H., and Sasayama, S. (2002). Role of KCNQ1 in the cell swelling-induced enhancement of the slowly activating delayed rectifier K(+) current. *Jpn. J. Physiol.* **52,** 31–39.

Kung, C. (2005). A possible unifying principle for mechanosensation. *Nature* **436,** 647–654.

Laitko, U., and Morris, C. E. (2004). Membrane tension accelerates rate-limiting voltage-dependent activation and slow inactivation steps in a Shaker channel. *J. Gen. Physiol.* **123,** 135–154.

Laitko, U., Juranka, P. F., and Morris, C. E. (2006). Membrane stretch slows the concerted step prior to opening in a Kv channel. *J. Gen. Physiol.* **127,** 687–701.

Langton, P. D. (1993). Calcium channel currents recorded from isolated myocytes of rat basilar artery are stretch sensitive. *J. Physiol.* **471,** 1–11.
Ledwell, J. L., and Aldrich, R. W. (1999). Mutations in the S4 region isolate the final voltage-dependent cooperative step in potassium channel activation. *J. Gen. Physiol.* **113,** 389–414.
Lee, S. Y., Lee, A., Chen, J., and MacKinnon, R. (2005). Structure of the KvAP voltage-dependent K+ channel and its dependence on the lipid membrane. *Proc. Natl. Acad. Sci. USA* **102,** 15441–15446.
Levitan, I., Helmke, B. P., and Davies, P. F. (2000). A chamber to permit invasive manipulation of adherent cells in laminar flow with minimal disturbance of the flow field. *Ann. Biomed. Eng.* **28,** 1184–1193.
Li, J., and Correa, A. M. (2002). Kinetic modulation of HERG potassium channels by the volatile anesthetic halothane. *Anesthesiology* **97,** 921–930.
Lin, W., Latiko, U., Juranka, P. F., and Morris, C. E. (2007). Dual stretch responses of mHCN2 pacemaker channels: Accelerated activation, accelerated deactivation. *Biophys. J.* [Epub ahead of print 2006 Dec 1].
Liu, L., Zhu, W., Zhang, Z. S., Yang, T., Grant, A., Oxford, G., and Simon, S. A. (2004). Nicotine inhibits voltage-dependent sodium channels and sensitizes vanilloid receptors. *J. Neurophysiol.* **91,** 1482–1491.
Long, S. B., Campbell, E. B., and Mackinnon, R. (2005a). Crystal structure of a mammalian voltage-dependent Shaker family K+ channel. *Science* **309,** 897–903.
Long, S. B., Campbell, E. B., and Mackinnon, R. (2005b). Voltage sensor of Kv1. 2: Structural basis of electromechanical coupling. *Science* **309,** 903–908.
Lundbaek, J. A., Birn, P., Hansen, A. J., Sogaard, R., Nielsen, C., Girshman, J., Bruno, M. J., Tape, S. E., Egebjerg, J., Greathouse, D. V., Mattice, G. L., Koeppe, R. E., II, *et al.* (2004). Regulation of sodium channel function by bilayer elasticity: The importance of hydrophobic coupling. Effects of micelle-forming amphiphiles and cholesterol. *J. Gen. Physiol.* **123,** 599–621.
Lundbaek, J. A., Birn, P., Tape, S. E., Toombes, G. E., Sogaard, R., Koeppe, R. E., II, Gruner, S. M., Hansen, A. J., and Andersen, O. S. (2005). Capsaicin regulates voltage-dependent sodium channels by altering lipid bilayer elasticity. *Mol. Pharmacol.* **68,** 680–689.
Ly, H. V., and Longo, M. L. (2004). The influence of short-chain alcohols on interfacial tension, mechanical properties, area/molecule, and permeability of fluid lipid bilayers. *Biophys. J.* **87,** 1013–1033.
Lyford, G. L., Strege, P. R., Shepard, A., Ou, Y., Ermilov, L., Miller, S. M., Gibbons, S. J., Rae, J. L., Szurszewski, J. H., and Farrugia, G. (2002). α(1C) (Ca(V)1. 2) L-type calcium channel mediates mechanosensitive calcium regulation. *Am. J. Physiol. Cell Physiol.* **283,** C1001–C1008.
Macdonald, A. G. (2002). Ion channels under high pressure. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **131,** 587–593.
Mannikko, R., Elinder, F., and Larsson, H. P. (2002). Voltage-sensing mechanism is conserved among ion channels gated by opposite voltages. *Nature* **419,** 837–841.
Maroto, R., and Hamill, O. P. (2001). Brefeldin A block of integrin-dependent mechanosensitive ATP release from Xenopus oocytes reveals a novel mechanism of mechanotransduction. *J. Biol. Chem.* **276,** 23867–23872.
Maroto, R., Raso, A., Wood, T. G., Kurosky, A., Martinac., B., and Hamill, O. P. (2005). TRPC1 forms the stretch-activated cation channel in vertebrate cells. *Nat. Cell Biol.* **7,** 179–185.
McNaughton, N. C., Green, P. J., and Randall, A. D. (2001). Inhibition of human alpha1E subunit-mediated Ca2+ channels by the antipsychotic agent chlorpromazine. *Acta Physiol. Scand.* **173,** 401–408.

Meyer, G. R., Gullingsrud, J., Schulten, K., and Martinac, B. (2006). Molecular dynamics study of MscL interactions with a curved lipid bilayer. *Biophys. J.* **91,** 1630–1637.

Meyer, R., and Heinemann, S. H. (1997). Temperature and pressure dependence of Shaker K+ channel N- and C-type inactivation. *Eur. Biophys. J.* **26,** 433–445.

McEwen, D. P., and Isom, L. L. (2004). Heterophilic interactions of sodium channel beta1 subunits with axonal and glial cell adhesion molecules. *J. Biol. Chem.* **279,** 52744–52752.

Mitchell, D. C., and Litman, B. J. (2000). Effect of ethanol and osmotic stress on receptor conformation. Reduced water activity amplifies the effect of ethanol on metarhodopsin II formation. *J. Biol. Chem.* **275,** 5355–5360.

Mitchell, D. C., Lawrence, J. T., and Litman, B. J. (1996). Primary alcohols modulate the activation of the G protein-coupled receptor rhodopsin by a lipid-mediated mechanism. *J. Biol. Chem.* **271,** 19033–19036.

Moran, O., Tammaro, P., Nizzari, M., and Conti, F. (2000). Functional properties of sodium channels do not depend on the cytoskeleton integrity. *Biochem. Biophys. Res. Commun.* **276,** 204–209.

Morris, C. E. (2001). Mechanoprotection of the plasma membrane in neurons and other non-erythroid cells by the spectrin-based membrane skeleton. *Cell Mol. Biol. Lett.* **6,** 703–720.

Morris, C. E., and Sigurdson, W. J. (1989). Stretch-inactivated ion channels coexist with stretch-activated ion channels. *Science* **243,** 807–809.

Morris, C. E., and Laitko, U. (2005). The mechanosensitivity of voltage-gated channels may contribute to cardiac M.E.F. *In* "Cardiac Mechano-Electric Feedback and Arrhythmias: From Pipette to Patient" (P. Kohl, M. Franz, and F. Sachs, eds.), pp. 33–41. Saunders Philadelphia, Pennsylvania.

Morris, C. E., Juranka, P. F., Lin, W., Morris, T. J., and Laitko, U. (2006). Studying the mechanosensitivity of voltage-gated channels using oocyte patches. *In* "Xenopus Protocols: Cell Biology and Signal Transduction" (X. J. Liu, ed.). *Methods Mol. Biol.* **322,** 315–329.

Mozrzymas, J. W., Teisseyre, A., and Vittur, F. (1996). Propofol blocks voltage-gated potassium channels in human T lymphocytes. *Biochem. Pharmacol.* **52,** 843–849.

Murata, Y., Iwasaki, H., Sasaki, M., Inaba, K., and Okamura, Y. (2005). Phosphoinositide phosphatase activity coupled to an intrinsic voltage sensor. *Nature* **435,** 1239–1243.

Namba, T., Ishii, T. M., Ikeda, M., Hisano, T., Itoh, T., Hirota, K., Adelman, J. P., and Fukuda, K. (2000). Inhibition of the human intermediate conductance Ca(2+)-activated K(+) channel, hIK1, by volatile anesthetics. *Eur. J. Pharmacol.* **395,** 95–101.

Nicholson, R. A., Liao, C., Zheng, J., David, L. S., Coyne, L., Errington, A. C., Singh, G., and Lees, G. (2003). Sodium channel inhibition by anandamide and synthetic cannabimimetics in brain. *Brain Res.* **978,** 194–204.

Nilius, B., Talavera, K., Owsianik, G., Prenen, J., Droogmans, G., and Voets, T. (2005). Gating of TRP channels: A voltage connection? *J. Physiol.* **567,** 35–44.

Niu, S. L., Mitchell, D. C., and Litman, B. (2002). Manipulation of cholesterol levels in rod disk membranes by methyl-beta-cyclodextrin: Effects on receptor activation. *J. Biol. Chem.* **277,** 20139–20145.

Niu, S. L., and Mitchell, D. C. (2005). Effect of packing density on rhodopsin stability and function in polyunsaturated membranes. *Biophys. J.* **89,** 1833–1840.

Ogata, N., and Narahashi, T. (1989). Block of sodium channels by psychotropic drugs in single guinea-pig cardiac myocytes. *Br. J. Pharmacol.* **97,** 905–913.

Olcese, R., Usai, C., Maestrone, E., and Nobile, M. (1994). The general anesthetic propofol inhibits transmembrane calcium current in chick sensory neurons. *Anesth. Analg.* **78,** 955–960.

Okamoto, T., Harnett, M. T., and Morikawa, H. (2006). Hyperpolarization-activated cation current (Ih) is an ethanol target in midbrain dopamine neurons of mice. *J. Neurophysiol.* **95,** 619–626.

Oliver, D., Lien, C. C., Soom, M., Baukrowitz, T., Jonas, P., and Fakler, B. (2004). Functional conversion between A-type and delayed rectifier K+ channels by membrane lipids. *Science* **304,** 265–270.

Ou, X., Blount, P., Hoffman, R. J., and Kung, C. (1998). One face of a transmembrane helix is crucial in mechanosensitive channel gating. *Proc. Natl. Acad. Sci. USA* **95,** 11471–11475.

Ouyang, W., Wang, G., and Hemmings, H. C., Jr. (2003). Isoflurane and propofol inhibit voltage-gated sodium channels in isolated rat neurohypophysial nerve terminals. *Mol. Pharmacol.* **64,** 373–381.

Park, J. B., Kim, H. J., Ryu, P. D., and Moczydlowski, E. (2003). Effect of phosphatidylserine on unitary conductance and Ba2+ block of the BK Ca2+-activated K+ channel: Re-examination of the surface charge hypothesis. *J. Gen. Physiol.* **121,** 375–397.

Pascarel, C., Brette, F., and Le Guennec, J. Y. (2001). Enhancement of the T-type calcium current by hyposmotic shock in isolated guinea-pig ventricular myocytes. *J. Mol. Cell. Cardiol.* **33,** 1363–1369.

Pathak, M., Agarwal, G., Kurtz, L., Gandhi, C. S., and Isacoff, E. Y. (2006). A fluorescence scan to investigate the structural dynamics of the Shaker K+ channel. Abstract #2268 Biophysical Society.

Pawson, L., and Bolanowski, S. J. (2002). Voltage-gated sodium channels are present on both the neural and capsular structures of Pacinian corpuscles. *Somatosens. Mot. Res.* **19,** 231–237.

Pencer, J., Nieh, M. P., Harroun, T. A., Krueger, S., Adams, C., and Katsaras, J. (2005). Bilayer thickness and thermal response of dimyristoylphosphatidylcholine unilamellar vesicles containing cholesterol, ergosterol and lanosterol: A small-angle neutron scattering study. *Biochim. Biophys. Acta* **1720,** 84–91.

Piao, L., Li, Y., Li, L., and Xu, W. X. (2001). Increment of calcium-activated and delayed rectifier potassium current by hyposmotic swelling in gastric antral circular myocytes of guinea pig. *Acta Pharmacol. Sin.* **22,** 566–572.

Piao, L., Ying Li, H., Park, C. K., Cho, I. H., Piao, Z. G., Jung, S. J., Choi, S. Y., Lee, S. J., Park, K., Kim, J. S., and Oh, S. B. (2006). Mechanosensitivity of voltage-gated K(+) currents in rat trigeminal ganglion neurons. *J. Neurosci. Res.* **83,** 1373–1380.

Poling, J. S., Rogawski, M. A., Salem, N., Jr., and Vicini, S. (1996). Anandamide, an endogenous cannabinoid, inhibits Shaker-related voltage-gated K+ channels. *Neuropharmacology* **35,** 983–991.

Ramsey, I. S., Moran, M. M., Chong, J. A., and Clapham, D. E. (2006). A voltage-gated proton-selective channel lacking the pore domain. *Nature* **440,** 1213–1216.

Riechmann, L., Lavenir, I., de Bono, S., and Winter, G. (2005). Folding and stability of a primitive protein. *J. Mol. Biol.* **348,** 1261–1272.

Rodriguez, B. M., Sigg, D., and Bezanilla, F. (1998). Voltage gating of Shaker K+ channels. The effect of temperature on ionic and gating currents. *J. Gen. Physiol.* **112,** 223–242.

Sako, Y., Nomura, N., Uchida, A., Ishida, Y., Morii, H., Koga, Y., Hoaki, T., and Maruyama, T. (1996). Aeropyrum pernix gen. nov., sp. nov., a novel aerobic hyperthermophilic archaeon growing at temperatures up to 100 degrees C. *Int. J. Syst. Bacteriol.* **46,** 1070–1077.

Scarlata, S., McBath, H., and Haspel, H. C. (1995). Effect of lipid packing on the conformational states of purified GLUT-1 hexose transporter. *Biochemistry* **34,** 7703–7711.

Shahidullah, M., Harris, T., Germann, M. W., and Covarrubias, M. (2003). Molecular features of an alcohol binding site in a neuronal potassium channel. *Biochemistry* **42,** 11243–11252.

Shcherbatko, A., Ono, F., Mandel, G., and Brehm, P. (1999). Voltage-dependent sodium channel function is regulated through membrane mechanics. *Biophys. J.* **77,** 1945–1959.

Shiraishi, M., and Harris, R. A. (2004). Effects of alcohols and anesthetics on recombinant voltage-gated Na+ channels. *J. Pharmacol. Exp. Ther.* **309,** 987–994.

Strege, P. R., Holm, A. N., Rich, A., Miller, S. M., Ou, Y., Sarr, M. G., and Farrugia, G. (2003). Cytoskeletal modulation of sodium current in human jejunal circular smooth muscle cells. *Am. J. Physiol. Cell Physiol.* **284,** C60–C66.

Su, Z., Martin, R., Cox, B. F., and Gintant, G. (2004). Mesoridazine: An open-channel blocker of human ether-a-go-go-related gene K+ channel. *J. Mol. Cell. Cardiol.* **36,** 151–160.

Tabarean, I. V., and Morris, C. E. (2002). Membrane stretch accelerates activation and slow inactivation in Shaker channels with S3-S4 linker deletions. *Biophys. J.* **82,** 2982–2994.

Tabarean, I. V., Juranka, P., and Morris, C. E. (1999). Membrane stretch affects gating modes of a skeletal muscle sodium channel. *Biophys. J.* **77,** 758–774.

Tanaka, T., Tamba, Y., Masum, S. M., Yamashita, Y., and Yamazaki, M. (2002). La(3+) and Gd(3+) induce shape change of giant unilamellar vesicles of phosphatidylcholine. *Biochim. Biophys. Acta* **1564,** 173–182.

Teisseyre, A., and Michalak, K. (2003). The voltage- and time-dependent blocking effect of trifluoperazine on T lymphocyte Kv1.3 channels. *Biochem. Pharmacol.* **65,** 551–561.

Terakawa, S., and Nakayama, T. (1985). Are axoplasmic microtubules necessary for membrane excitation? *J. Membr. Biol.* **85,** 65–77.

Tillman, T. S., and Cascio, M. (2003). Effects of membrane lipids on ion channel structure and function. *Cell Biochem. Biophys.* **38,** 161–190.

Thomas, D., Wu, K., Kathofer, S., Katus, H. A., Schoels, W., Kiehn, J., and Karle, C. A. (2003). The antipsychotic drug chlorpromazine inhibits HERG potassium channels. *Br. J. Pharmacol.* **139,** 567–574.

Tombola, F., Pathak, M. M., and Isacoff, E. Y. (2005). How far will you go to sense voltage? *Neuron* **48,** 719–725.

Tombola, F., Pathak, M. M., and Isacoff, E. Y. (2006). How does voltage open an ion channel? *Annu. Rev. Cell Dev. Biol.* May 5 [Epub ahead of print].

Toselli, M., Biella, G., Taglietti, V., Cazzaniga, E., and Parenti, M. (2005). Caveolin-1 expression and membrane cholesterol content modulate N-type calcium channel activity in NG108–15 cells. *Biophys. J.* **89,** 2443–2457.

Traub, O., Ishida, T., Ishida, M., Tupper, J. C., and Berk, B. C. (1999). Shear stress-mediated extracellular signal-regulated kinase activation is regulated by sodium in endothelial cells. Potential role for a voltage-dependent sodium channel. *J. Biol. Chem.* **274,** 20144–20150.

Treistman, S. N., and Wilson, A. (1987). Alkanol effects on early potassium currents in Aplysia neurons depend on chain length. *Proc. Natl. Acad. Sci. USA* **84,** 9299–9303.

Van den Brink-van der Laan, E., Chupin, V., Killian, J. A., and de Kruijff, B. (2004). Small alcohols destabilize the KcsA tetramer via their effect on the membrane lateral pressure. *Biochemistry* **43,** 5937–5942.

Wallace, C. H., Baczko, I., Jones, L., Fercho, M., and Light, P. E. (2006). Inhibition of cardiac voltage-gated sodium channels by grape polyphenols. *Br. J. Pharmacol.* **149,** 657–665.

Wang, J., Wang, H., Han, H., Zhang, Y., Yang, B., Nattel, S., and Wang, Z. (2001). Phospholipid metabolite 1-palmitoyl-lysophosphatidylcholine enhances human ether-a-go-go-related gene (HERG) K(+) channel function. *Circulation* **104,** 2645–2648.

Wiggins, P., and Phillips, R. (2005). Membrane-protein interactions in mechanosensitive channels. *Biophys. J.* **88,** 880–902.

Wong, W., and Schlichter, L. C. (2004). Differential recruitment of Kv1. 4 and Kv4. 2 to lipid rafts by PSD-95. *J. Biol. Chem.* **279,** 444–452.

Xiao, Y. F., Ma, L., Wang, S. Y., Josephson, M. E., Wang, G. K., Morgan, J. P., and Leaf, A. (2006). Potent block of inactivation-deficient Na+ channels by n-3 polyunsaturated fatty acids. *Am. J. Physiol. Cell Physiol.* **290,** C362–C370.

Xu, W. X., Li, Y., Wu, L. R., and Li, Z. L. (2000). Effects of different kinds of stretch on voltage-dependent calcium current in antrial circular smooth muscle cells of the guinea-pig. *Sheng Li Xue Bao* **52,** 69–74.

Yang, X. C., and Sachs, F. (1989). Block of stretch-activated ion channels in Xenopus oocytes by gadolinium and calcium ions. *Science* **243,** 1068–1071.

Yao, X., McIntyre, M. S., Lang, D. G., Song, I. H., Becherer, J. D., and Hashim, M. A. (2005). Propranolol inhibits the human ether-a-go-go-related gene potassium channels. *Eur. J. Pharmacol.* **519,** 208–211.

Ying, S. W., Abbas, S. Y., Harrison, N. L., and Goldstein, P. A. (2006). Propofol block of I(h) contributes to the suppression of neuronal excitability and rhythmic burst firing in thalamocortical neurons. *Eur. J. Neurosci.* **23,** 465–480.