Role of Ion Channel Mechanosensitivity in the Gut: Mechano-Electrical Feedback Exemplified By Stretch-Dependence of Nav1.5

CHAPTER · JANUARY 2013

DOI: 10.1007/978-94-007-6561-0_2

3 AUTHORS, INCLUDING:

Arthur Beyder

Mayo Clinic - Rochester

46 PUBLICATIONS **394** CITATIONS

SEE PROFILE

ROLE OF ION CHANNEL MECHANOSENSITIVITY IN GUT MECHANO-ELECTRICAL FEEDBACK EXEMPLIIFIED BY STRETCH-DEPENDENCE OF $Na_V1.5$

Arthur Beyder[1], Rachel Lees-Green[2] & Gianrico Farrugia[1]

[1]Division of Gastroenterology & Hepatology, Enteric Neuroscience Program, Mayo Clinic, Rochester, MN

[2]Auckland Bioengineering Institute, The University of Auckland, Auckland, New Zealand

$Na_V1.5$ is a voltage-gated sodium channel found in the human gastrointestinal tract. In smooth muscle cells (SMC) and interstitial cells of Cajal (ICC), $Na_V1.5$ regulates the resting potential as well as slow wave upstroke and frequency. Mutations in SCN5A, the gene coding for $Na_V1.5$, are associated with gastrointestinal functional disorders. Some patients with irritable bowel syndrome (IBS) have SCN5A mutations that result in functionally abnormal channels. $Na_V1.5$ is mechanosensitive, and some of the mutations associated with gastrointestinal (GI) motility disorders have impaired mechanosensitivity. $Na_V1.5$ mechanosensitivity involves the actin cytoskeleton and associating proteins as well as the lipid bilayer. Mechanical stimulation of $Na_V1.5$ results in an increase in peak current, acceleration of the voltage-dependent activation & inactivation and slowed recovery from inactivation. Biophysical modeling is increasingly used as a tool for investigating the effect of $Na_V1.5$ and other mechanosensitive components in slow wave generation. We summarize the existing models of gastrointestinal cellular electrical activity, and specifically a model of $Na_V1.5$ mechanosensitivity that has been incorporated into one of the cell models. In agreement with experimental data, mechanical stimulation of $Na_V1.5$ results in increased excitability of the cell model in silico. In this chapter we discuss the current knowledge of the molecular mechanism of $Na_V1.5$ mechanosensitivity, mechano-electrical

consequences of $Na_V1.5$ stretch in cells and propose physiologic and pathophysiologic consequences.

All organisms, even unicellular organisms and plants, are sensitive to mechanical input. In the larger organisms, mechanosensitivity modulates a variety of physiological processes at the organ, cellular and molecular levels. Effective mechanical function is central for the gastrointestinal system, mechanosensitivity underlies several of fundamental processes involved in digestion, absorption and motility. Abnormalities in mechano-transduction have been implicated in a variety of gastrointestinal diseases such as achalasia, gastroparesis, irritable bowel syndrome, intestinal pseudo-obstruction and slow transit constipation. Mechanical force is usually transduced at the plasma membrane level by mechano-sensitive ion channels. While there have been significant advances in our understanding of bacterial mechanosensitive ion channels, much less is known about their mammalian counterparts. This chapter will focus on an example of a mechanically sensitive voltage-gated sodium selective ion channel $Na_V1.5$ found in the heart and in the human gastrointestinal tract. We will discuss the current knowledge of the molecular mechanism of $Na_V1.5$ mechanosensitivity, mechano-electrical consequences of $Na_V1.5$ stretch in cells and propose physiologic and pathophysiologic consequences.

2.1 Mechanosensitivity of voltage-gated ion channels may contribute to mechano-electrical feedback

Voltage-gated ion channels are essential for proper function of the electro-mechanical organs such as the heart and the gut. In the gut, cyclical electrical activity generated by the interstitial cells of Cajal (ICC), called slow waves, requires voltage-gated ion channels. Slow waves are transmitted to the smooth muscle cells (SMC), which use voltage gated ion channels to generate the cyclical contractile activity that underpins gastrointestinal motility. Voltage-gated sodium channels (Na_V), both TTX-sensitive [1-3] and TTX-resistant [4-7], are found in both the SMC and ICC of the gastrointestinal tract of many animals including humans. In the humans $Na_V1.5$ is expressed the jejunum circular layer [5,7], and this channel is also found in the dog [8] and rat small intestine. This channel is functionally relevant, as block by lidocaine and QX-314 slows the upstroke and shortens the duration of the slow wave, with both effects

contributing to the decrease in slow wave frequency [7]. Furthermore, abnormalities in $Na_V1.5$ are associated with pathology. The patients with mutations in SCN5A gene, which codes for $Na_V1.5$, have more abdominal symptoms [9] and increased IBS prevalence [10,11] compared to controls. Recent studies also suggest that $Na_V1.5$ channels from patients with IBS are functionally abnormal [10,12]. Abnormalities in the Na channel macromolecular complex are also associated with intestinal pseudo-obstruction [13].

The mechanical state of the electromechanical organs alters the electrical activity, a so-called mechano-electrical feedback. Multiple mechanisms are responsible for the mechano-electrical feedback in the gastrointestinal tract. To a large degree, stretch stimuli within the gut are processed by the mechanisms residing in the wall by enteroendocrine cells [14], by the enteric nervous system [15], the ICC [16] and the SMC [17]. Since voltage-gated channels participate in generation and coordinate the slow waves, the most direct mechano-electrical feedback mechanism may involve mechanosensitivity of voltage-gated ion channels. A subset of voltage-gated ion channels are mechanically sensitive, with mechanical input regulating the voltage-dependent activity. The voltage-dependent properties of these channels are of overriding importance to the electrical and electro-mechanical activity in the gut [18], mechanoelectric feedback on voltage gated channels may be an important physiologic and potentially pathophysiologic mechanism.

Pressure sensitivity of voltage-gated ion channels was realized even in the early squid axon experiments [19,20]. Since those early discoveries, and as the tools to probe mechano-electrical feedback at the molecular level have improved, representatives from all voltage-gated ion channel families (K_V [21-24], Ca_V [17,25,26], Na_V [27-29]) have been shown to be mechanically sensitive. In the sections below we will detail the current state of knowledge on the mechanism of $Na_V1.5$ mechanosensitivity.

2.1.1 Cellular mechanosensitivity is multifaceted

Multiple mechanisms are involved in cellular mechanosensation, including intracellular (e.g. cytoskeleton) [30] and extracellular (e.g. extracellular matrix) [31] response elements as well as the stretch-sensitive ion channels [32]. We propose that mechanosensation by voltage-gated ion channels in cells of electrically active tissues pro-

vides mechano-electrical feedback to the cell in order modify the underlying electrical behavior. To achieve this goal the stretch-sensitive ion channels need to be able to receive input on the mechanical state of the surrounding environment. It is known that the eukaryotic stretch sensitive channels receive a variable amount of input from each of the above components, depending on cell type, channel type, level of connection to the anchoring protein and location within the membrane. We present the evidence below that the $Na_V1.5$ mechanosensitivity mechanism involves both cytoskeletal connections and lipid membrane.

2.1.2 Actin cytoskeleton is critical for $Na_V1.5$ shear-stress mechanosensitivity

$Na_V1.5$ is known to connect to multiple intracellular accessory proteins [33], including some that have been implicated in mechanosensation. Some established mechano-relevant associating proteins include ankyrin B [34], actin [35] via dystrophin [36] via syntrophin γ2 [37] and titin via telethonin [13]. Disruption or alteration of these associations has been linked to $Na_V1.5$ dysfunction.

The initial studies demonstrating $Na_V1.5$ mechanosensitivity were done in voltage clamped whole freshly dissociated ICC and SMC from human jejunum. In one study, freshly isolated ICC were stimulated by 10 mL/min perfusion. This shear stress increased $Na_V1.5$ peak currents up to 40% (from 146±30 to 191±43 pA) [7]. Na_V current was also identified in the SMC [5], and was later found to be $Na_V1.5$ [6]. Similar to the ICC, in SMC $Na_V1.5$ current was also shear sensitive, with shear increasing peak currents by 27±3% (from 147±21 pA to 175±23 pA). Shear stress also accelerated activation, shortening time to peak for activating voltages (-50 to -2 mV). On the other hand, inactivation kinetics were not affected by shear [35]. The change in the activation kinetics without a change in the inactivation kinetics suggested that the increase in peak current seen with shear-stress is unlikely simply from insertion of channels into the membrane.

It is generally known that the mechanism of shear sensitivity involves the cytoskeleton [38], and that $Na_V1.5$ function in the cardiac myocyte is modified by cytoskeletal components, such as actin [39]. The Farrugia group proceeded to test the involvement of the actin, microtubule and intermediate filament cytoskeletal components in

$Na_V1.5$ shear mechanosensitivity. Pharmacological disruption of multiple cytoskeletal components did not affect $Na_V1.5$ function at rest, and disruption of the microtubule and intermediate filament networks did not affect $Na_V1.5$ shear sensitivity. However, actin cytoskeleton disruption by cytochalasin D and gelsolin effectively abolished shear sensitivity. On the other hand, actin network stabilization by phalloidin preserved $Na_v1.5$ mechanosensitivity [35]. Actin is not known to directly associate with ion channels. Instead, actin connection to ion channels, including $Na_V1.5$, involves adapter proteins. In the case of $Na_V1.5$, one of the adapter proteins is syntrophin γ2. In the human gastrointestinal SMCs syntrophin γ2 PDZ domain was found to directly interact with the last 10 amino acids of $Na_V1.5$ and this interaction altered $Na_V1.5$ function at rest [37]. In a series of subsequent experiments, the disruption of this interaction eliminated $Na_V1.5$ shear stress mechanosensitivity [37].

It appears that $Na_V1.5$ shear stress mechanosensitivity is dependent on the connection between the channel's cytoplasmic C-terminus and syntrophin γ2 connecting to the f-actin cytoskeleton. This mechanism of $Na_V1.5$ mechanosensitivity requires further exploration. Three broad outstanding questions remain regarding the mechanism of $Na_V1.5$ shear sensitivity. One, are other mechano-relevant associating proteins (e.g. ankyrin and telethonin) involved? Two, what is the rate-limiting mechanotransducer? Three, how does shear actually gate $Na_V1.5$?

2.1.3 The mechanism of stretch-dependent $Na_V1.5$ peak current increase

The details of mechanosensitivity by the ion-channel forming $Na_V1.5$ α-subunit can be determined in some detail using heterologous expression, such as HEK293 cells transfected with $Na_V1.5$. Shear stress mechanosensitivity was confirmed in this system, and effects were similar to those in native cells [10]. In such a system $Na_V1.5$ mechanosensitivity can be tested down to single channel level by using pressure- and voltage-clamped membrane patches, which provide an opportunity for well defined mechanical stimuli [40]. The mechanical stimulus applied to the patch is pressure, while the mechanical stimulus relevant to the ion channel is membrane tension [41]. Pressure is related to tension (T) in the simplest terms through Laplace law, $\Delta T=P\Delta r/2$, where pressure is P and the

radius of curvature is *r*. Thus for accurate estimation of tension, imaging of the patch is required [40]. In reality the membrane complex is non-linear, so caution is required when interpreting data, but to date patch pressure has been the most robust mechanical stimulus for ion channels. In the few patch studies reported to date, $Na_V1.5$ has been consistently mechanosensitive [28,29]. Similar to the shear stress findings, patch pressure increases peak current and accelerates kinetics. In the patch, both positive and negative pressure produced an increase in peak current, with a -30 mmHg stimuli resulting in a 33% peak current increase [29]. The increase in pressure-induced current appears even larger for voltages near activation, where a roughly 50% increase was noted in a different study [28]. Since total patch current $I=NPi$, where N is the number of channels, P is open probability and i is single channel current, one of these variables is responsible for the pressure-dependent current increase. Single channel experiments found that patch pressure 10-50 mmHg does not change single channel conductance, which remained fixed at ~17 pS. In this study pressure was found not to change the maximum probability of channel opening ($P_{o,max}$) and the increase in pressure-induced current was a result of an increase in the number of active channels by about 20% [29]. However, it is unclear what mechanism is responsible for the increase in the active channel number. Two distinct possibilities exist. The first possibility is pressure-induced recruitment of channels either from additional membrane or changes in membrane fluidity. The second possibility is an awaking of a subset of previously "sleepy channels," which are channels that have been previously shown to awake with changes in temperature [42]. It is also unclear whether the mechanism of shear-induced peak current increase is simi-

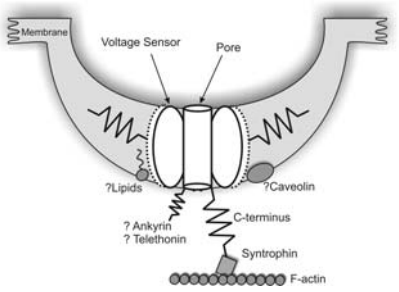

Figure 2.1. $Na_V1.5$ mechanosensitivity components. A $Na_V1.5$ protein complex is associated with multiple intracellular proteins. Syntrophin and actin have been directly linked to $Na_V1.5$ mechanosensitivity. Other targets include ankyrin and telethonin. Multiple potential targets exist in the membrane, including caveolin and bilayer lipids.

lar to that experienced in the patch.

2.1.4 Pressure-induced acceleration in kinetics and shifts in voltage-sensitivity

Patch pressure increased peak current, accelerated kinetics and hyperpolarized voltage-sensitivity [28,29]. Morris & Juranka found a reversible acceleration of both activation and inactivation rate constants by about 40% for $Na_V1.5$ channels expressed in Xenopus oo-

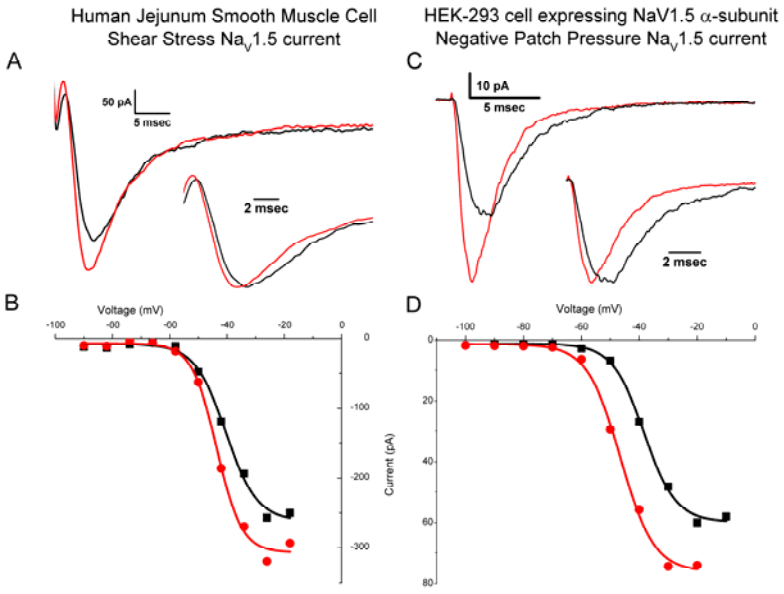

Figure 2.2. Mechanosensitivity of $Na_V1.5$ to shear and patch pressure. A) In a whole-cell voltage-clamped human jejunum smooth muscle cell, time-dependence of $Na_V1.5$ current is shown at -20 mV (black trace). Flow at 10 mL/min increases peak current (red trace). When normalized to peak current, the acceleration in activation kinetics is notable (inset). B) Current-voltage (IV) curves at rest (black) and during flow (red) demonstrate an increase in peak current. C) In a cell-attached patch from HEK293 cell, time-dependent $Na_V1.5$ current is shown at rest (0 mmHg, black) and an increase in peak current with negative pressure (-30 mmHg, red). The kinetics of activation and inactivation are noticeably accelerated as shown in the traces normalized to peak current (inset). D) IV curves for this patch at rest (black) and with -30 mmHg pressure (red) showing a hyperpolarizing shift in the voltage-dependence of activation.

cytes [28]. Beyder and colleagues used $Na_V1.5$ expressed in HEK293 cells and found similar acceleration in the kinetics for all activating voltage steps, but this was only partially reversible [29]. In both studies, a single scaling constant was used to normalize kinetics, suggesting that pressure did not introduce novel gating states. The acceleration of the kinetics can be due to a shift in voltage-sensitivity. Indeed, when the current-voltage (IV) relationship was fit to a two-state Boltzmann function, pressure (10-50 mmHg) shifted the voltage dependence of activation and inactivation by a remarkable 0.7 mV per mmHg [29]. These shifts in voltage sensitivity markedly hyperpolarize the voltage range of the window current with likely physiological consequences. The shift in voltage-dependence can be due to mechanical sensitivity of the voltage-sensitive activation steps or the relatively voltage-insensitive but rate-limiting gate opening, or a combination of both. Kinetics can be normalized using a single constant, suggesting that pressure did not introduce novel kinetic states and that likely a single kinetic step was affected. Since closed-state inactivation follows voltage-activation but precedes gate opening, this transition can be used to assess the likely transition affected by pressure. It was found that closed-state inactivation appeared enhanced, suggesting that a voltage-sensing step is accelerated by pressure. The movement of $Na_V1.5$ voltage-sensors is responsible for the voltage-sensitive transitions, and so it appears that the voltage-sensors are pressure sensitive.

Voltage sensor mechanosensitivity awaits confirmation by a more direct approach, but there are several supporting pieces of evidence for their involvement. Mechanosensitivity of an ion channel requires that at least one of the conformational transitions undergoes an area change, such that undergoing a mechanosensitive transition involves a change in free energy $\Delta G = T\Delta A$, where T is tension and A is in-plane area [32]. Since ion channels are residents of the lipid bilayer, expansion within the bilayer is very likely to be sensitive to the protein-lipid interface. Indeed, previous studies have demonstrated that Na_V function is sensitive to the surrounding lipids [43] and amphiphiles [44]. In the recently published crystal structure of Na_V, the voltage-sensors have significant exposure to lipid membrane and based on that structure (discussed below), the voltage sensors would be expected to have a significant impact from a stretched

bilayer. Previous studies also show that even the isolated voltage sensor domains have a dimpling effect on the lipid bilayer [45-47], adding a third dimension to the mechanical sensitivity of bilayer-voltage sensor interface.

In addition to the in-plane area expansion, the curvature of the lipid bilayer may add a third dimension in the consideration of $Na_V1.5$ mechanosensitivity. For example, highly curved areas called caveoli are known to be mechanosensitive [48], and thus molecules found within these domains would be exposed to mechanical input. In fact, it is known that in the cardiac myocytes $Na_V1.5$ are targeted to specific membrane domains with intrinsic curvature, including caveoli [49,50] and T-tubules [51].

While it is likely that the voltage sensor domains are the mechanically sensitive parts of $Na_V1.5$, it is unclear whether 1) all four of the voltage sensors are equally involved, 2) there is a connection to the cytoskeletal mechanism described above.

2.1.5 Pressure stabilizes $Na_V1.5$ entry into inactivation and slows recovery

In cell-attached patches pressure consistently accelerates inactivation, which is not seen in whole cell bath perfusion experiments. The kinetic acceleration of fast inactivation and the hyperpolarizing shift in the voltage-dependence of inactivation mirrors those of kinetic acceleration of activation, and the shift in the voltage-dependence of activation, respectively. It is most likely that the changes in fast inactivation are secondary to the changes seen in the activation. In single channel studies the process of activation appears to be rate limiting, so the appearance of kinetic link and voltage-dependence of the much more rapid inactivation reflect those measures in activation [52]. This explanation is also consistent with the results of the pressure-induced changes in $Na_V1.5$. Namely, pressure accelerated the kinetics of activation and inactivation by the same linear constant, and the shift in voltage dependence was also equivalent in direction and magnitude over a range of pressures [29,28]. Thus, the appearance of the change in the kinetics and voltage-dependence of inactivation with pressure is likely linked to the activation.

An additional finding was that the recovery from inactivation of $Na_V1.5$ was significantly slowed with pressure. At –30 mmHg for

all but one holding potential the recovery from fast inactivation was slowed. While the left-shift in the voltage-dependence of inactivation explains some of the slowing of the recovery from inactivation, the experiments were done at the voltages that were sufficiently hyperpolarized to provide full channel availability.

2.1.6 Na_V crystal structure provides some answers

The data presented above detail the currently known functional aspects of $Na_V1.5$ mechanosensitivity. Our understanding is incomplete and more data are needed to understand the mechanisms of $Na_V1.5$ mechanosensitivity. A major recent advance is the publication of the Na_V crystal structure [53]. Although this is the first Na_V structure available, a significant progress has been made over the last decade as K_V crystal structures of multiple channel species and in several conformations have been worked out [54-58]. None of these crystals were grown in a well-controlled mechanical environment. Nevertheless, several structural aspects are of interest given the body of the above functional data. First, crystal structures of all voltage-sensitive channels show that voltage sensor domains are loosely associated with the pore domain and have significant exposure to the lipid bilayer. Since the bilayer is known to be important for mechanotransduction, it is likely that this extensive interaction between the lipid bilayer and the voltage-sensor mediates at least some mechanical input. Second, water filled vestibules were found within the voltage-sensor domains, and these likely change during the activation process of the channels, and are also potential sites for mechanosensitivity. Third, the attachment of the voltage-sensor domains to the intracellular gate is via a string-like linker. This linker is another potential area of mechanosensitivity [59,24].

2.1.7 $Na_V1.5$ mechanosensitivity abnormalities may be pathologic

It is already known that for some mutations of SCN5A, the $Na_V1.5$ channels have disrupted mechanosensitivity [60,10]. $Na_V1.5$ is expressed in the cardiac muscle and in the human gastrointestinal tract. $Na_V1.5$ mutations in both of these tissues are linked to disease. In the heart, $Na_V1.5$ dysfunction leads to LQT3 if gain-of-function [61] and Brugada syndrome if loss-of-function [62]. In the gut, $Na_V1.5$ mutations have been linked to abdominal pain syndromes [9]

and specifically IBS [11]. $Na_V1.5$ mutants with impaired mechanosensitivity have been described in LQT3 [60] and IBS [11]. A G298S mutation discovered in a cohort of IBS patients resulted in $Na_V1.5$ channels with decreased peak currents in four known common backgrounds. In addition, in one of the backgrounds G298S led to a significant decrease in mechanosensitivity to shear stress [10]. Multiple other $Na_V1.5$ mutations have been described in a larger cohort of patients IBS [11], and several of these have also been found to be functionally abnormal [12], but their mechanosensitive behavior awaits further exploration. In the heart, two classic mutations linked to LQT3 were found to have mechanosensitive behavior different from the controls [60]. More specifically, 1623 and 1626 were found to be mechanosensitive, but the typical coupling in the acceleration of activation and inactivation with stretch was disrupted. Thus, while in the control channels stretch accelerates both activation and inactivation to the same degree, the 1623 mutant has a typical acceleration of the activation, but the acceleration in inactivation is sped up to a smaller degree. The result is a larger and prolonged Na^+ current with stretch in 1623, which would predispose to early after-depolarizations and therefore arrhythmias.

2.2 Modeling the effects of ion channel mechanosensitivity *in silico*

Mechanosensitivity and mechanoelectrical feedback is difficult to study. Experimental tools necessary to obtain high fidelity data in both mechanical and electrical domains continue to be developed, but major limitations still exist. Biophysically-based computational models are an ideal tool for integrating diverse findings from a wide variety of experimental scales and systems. This approach uses experimental results and knowledge about the electrophysiology of the cells being modeled, but also allows the experimental conditions to be simulated by changing appropriate parameter values. *In silico* approach enables models to be used to test theories about cell electrophysiology and to predict the results for difficult experiments.

2.2.1 Introduction to biophysically-based cell models

Biophysically-based cell models simulate the electrical activity of excitable cells like ICC and SMC by modeling the components and

processes that are believed to underlie the electrical activity of the cell [63]. Different types of ion channels, pumps and other ion transport mechanisms can be integrated into a global model of the homogenized membrane potential and ion concentrations in the cell. Ion fluxes between different subcellular compartments in the cell may also be included, such as between endoplasmic reticulum and cytoplasm.

These biophysical cell models are modular and multi-scale, as each of the components in a model can be independently replaced or updated when new data become available. In this way, mechanosensitivity can be introduced into multiple components of a cell model. In addition, the influence of individual components on whole cell behavior can be predicted by altering the relative contribution of the component to the cell model.

The equations for the voltage-gated ion currents in biophysical cell models are typically developed using the classical Hodgkin-Huxley type approach. The whole cell ion currents are simulated by modeling the voltage dependence of activation and inactivation using voltage clamp data. The ion current through a particular type of ion channel is modeled as $I_{\text{ion}}=g_{ion}\cdot d\cdot f\cdot(V_m - E_{rev})$, where g_{ion} is the maximum whole cell conductance, V_m is the cell membrane potential, E_{rev} is the equilibrium (or Nernst) potential of the ion, and d and f are the activation and inactivation gating variables respectively [63,64]. The equilibrium potential for a particular ion, E_{rev}, is the membrane potential at which the concentration gradient and the electrical gradient across the cell membrane are balanced such that there is no net ionic current across the membrane. Thus, the equilibrium potential is determined by the intracellular and extracellular ion concentrations.

The gating variables, d and f, used to model the sensitivity of the ion channel open state to various stimuli, including voltage, mechanical stimuli, and ligand concentrations, are calculated using:

$$\frac{dx}{dt} = \frac{x_\infty - x(t)}{\tau_x},$$

where x_∞ is the steady-state value for gating variable x, and τ_x is the time constant. The steady-state of the gating variables, d_∞ and f_∞, are often described using a Boltzmann function of the form:

$$\frac{I_{ion}}{I_{\max}} = \frac{1}{1 + e^{(V_{1/2} - V_m)/dV}},$$

where I_{max} is the maximum current recorded through the ion channel, V_m is the membrane potential, $V_{1/2}$ is the voltage at which I_{ion} is half-maximal, and dV represents the slope of a sigmoidal curve describing the voltage dependence of the current [65].

These simple ion current models, while elegant and effective for describing the activation and inactivation of whole cell or macroscopic currents within a cell model, can be insufficient to capture the full complexity of ion currents, especially at the level of single ion channels or small clusters of channels.

An alternative way to model ion currents is as a Markov process, in which the kinetics of the ion channel at any point in time depend only on the current state of the channel, and not on any of the previous channel transitions. This enables simulation of the stochastic opening and closing of individual ion channels [66,67].

The Hodgkin-Huxley formulation starts from the assumption that a channel has two states: open and closed. A Markov model can incorporate multiple open, closed and inactive states. The transition rates between each of the different states can be dependent on factors such as membrane potential, ion and ligand concentrations, and mechanical stretch. Unlike the Hodgkin-Huxley model, Markov models are able to capture the varied responses of ion channels in different states. For example, sodium channel activation and inactivation kinetics are known to be linked [52,66,68], and Beyder et al. observed that stretch caused acceleration of $Na_V1.5$ inactivation secondary to accelerated activation kinetics [29]. Markov models can incorporate the direct kinetic link between activation and inactivation of $Na_V1.5$ [69], whereas in a Hodgkin-Huxley type model activation and inactivation kinetics are independent [68].

Hodgkin-Huxley models are a subclass of Markov models, and therefore can indirectly include the behavior of channels with multiple closed, open and inactivated states, as well as recovery from inactivation, and multiple inactivation time constants, as evidenced by Markov adaptations of Hodgkin-Huxley models. Nonetheless, Markov models are essential for simulating the stochastic nature of individual or small groups of ion channels, and for describing more

complex behaviors including the relationship between the state of an ion channel and its response to a stimulus [68].

Due to the extra states in Markov models, they are more difficult to develop and more computationally expensive to solve than Hodgkin-Huxley ion channel models. For modeling studies that aim to investigate the behavior of ion currents, Markov models provide a more accurate and detailed representation, but Hodgkin-Huxley type models are often sufficient for studies that aim to simply reproduce dynamic membrane potential activity like slow waves [68].

2.2.2 Established models of slow waves

Four biophysically-based models of cellular level gastrointestinal slow wave activity have been published: a small intestine ICC slow wave model by Youm et al. [70], Corrias and Buist gastric SMC [71] and ICC [72] models, and a small intestine mouse ICC slow wave model by Faville et al. [73], which was based on a preceding model of ICC unitary potentials [74]. Each model will be briefly discussed below; for more detail see the recent review by Lees-Green et al. [75].

The ICC model developed by Youm et al. [70] was based on cardiac cell models, so the ion channels and calcium signaling pathways implemented in the model do not match well with the current understanding of ICC electrophysiology. This model contained a complex description of inositol trisphosphate (IP_3) production and degradation, as well as calcium ion (Ca^{2+}) transport within the sarcoendoplasmic reticulum (ER or SR). However, it did not incorporate mitochondria, which have been implicated in slow wave generation [76]; nor did it incorporate $Na_V1.5$ [7] or Cl^- channels [77,78], both of which are now believed to play an important role in slow wave generation [75].

Both the Faville et al. [73,74] and the Corrias and Buist [72] ICC models were based on a hypothesis of pacemaking mechanisms that has since been called into question [78,79,75]. Nevertheless, these models provide a good system for testing various aspects of ICC function, as the majority of the ion channels and other components in the models are still consistent with the present understanding of ICC electrophysiology. The Corrias and Buist model incorporated $Na_V1.5$ and a calcium-activated chloride current [72].

Faville et al. developed a highly detailed model in which the slow waves are produced by the summation of unitary potentials, which are believed to be the underlying electrical activity responsible for generating slow waves [73]. In contrast, Corrias and Buist developed a model that reproduces the aggregate electrical activity of an ICC [72]. It is less demanding computationally; therefore it is much more suitable than the Faville model for inclusion in tissue- and organ-level multi-scale models of the gastrointestinal tract. For example, two modeling studies have used the Corrias and Buist ICC model to test the viability of different entrainment mechanisms in networks of ICC – a voltage-dependent Ca^{2+} influx [80] and a voltage-dependent intracellular Ca^{2+} release mechanism [81].

Only one model of gastrointestinal SMC electrical activity from the stomach is available [71]. Unlike the ICC models, the SMC model is not self-excitable; slow waves are generated by the SMC model in response to a pacing stimulus current, and the signal generated by the model depends in large part on the shape of the input stimulus current.

The SMC model has been used to test the effects of different gastric electrical stimulation protocols on gastric slow waves in order to evaluate the motility outcomes and energy efficiency of each protocol [82]. Experiments on isolated rodent gastric SMC were used to validate the simulation results for a handful of selected stimulation protocols. This study demonstrated the utility of cell models for investigating a comprehensive range of protocols while reducing reliance on animal experiments [82].

The SMC model [71] has also been used in several multi-scale modeling studies in conjunction with the Corrias and Buist ICC model [72], to investigate the electrical activity of the whole stomach. Buist et al. developed a model of slow wave propagation along the stomach [80]. Du et al. used a multiscale model to predict a body surface EGG recording corresponding to a known slow wave activity on the stomach surface [83].

2.2.3 Introducing stretch sensitivity to existing cell models

The existing cell models of gastrointestinal slow wave activity do not contain mechanosensing mechanisms, while only two models of

ICC [72] and SMC [71] included a description of voltage-dependent $Na_V1.5$ currents. However, mechanosensitive ion channels can be easily incorporated into existing slow wave models by substituting a stretch-dependent $Na_V1.5$ model in place of an existing model of $Na_V1.5$ [71,72] or another appropriate cation current, such as the phenomenological cation leak current [73], as demonstrated recently [84].

A highly detailed $Na_V1.5$ model is required to incorporate all of the experimentally observed $Na_V1.5$ mechanosensitive parameters. However, this may not be necessary for simulating the main effects of stretch on slow waves at a cell or tissue level. We integrated several aspects of $Na_V1.5$ pressure-sensitivity [29] using a Hodgkin-Huxley formulation into the available small intestine ICC slow wave model [73]. Specifically, the pressure-dependent hyperpolarizing shift in the voltage half-points of activation and inactivation were incorporated into calculations of the activation and inactivation steady-state parameters and time constants.

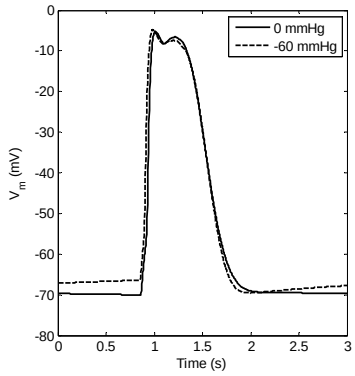

Fig. 2.3 Simulated slow waves from the modified Faville ICC model with no pressure (0 mmHg) and with –60 mmHg pressure applied to $Na_V1.5$.

To simulate the effects of stretch with this model, negative pressures of –40 to –60 mmHg were applied to $Na_V1.5$ channels. The largest pressure-induced changes in the simulated slow wave were observed at the highest pressure of –60 mmHg, including a 5.3% depolarization of resting membrane potential (from –70.2 mV at 0 mmHg to –66.4 mV at –60 mmHg), an 11.4% increase in upstroke rate (from 623 mV s^{-1} to 693 mV s^{-1}), a 4.9% increase in slow wave duration (from 615 ms to 645 ms) and a 0.85% increase frequency (from 17.0 cpm to 17.1 cpm). Although the increase in frequency was small, a similar increase has also been observed in a study on strips of human small intestine tissue, where stretch caused slow wave frequency to increase from 7 cpm to 7.3 cpm [7].

These changes all suggest an excitatory role for $Na_V1.5$ during stretch, which is in agreement with the effects of stretch on sections of human small intestine [7], confirming that $Na_V1.5$ mechanosensitivity is likely to contribute to the effects of stretch on slow waves in GI tissue.

2.2.4 The future of modeling $Na_V1.5$ mechanosensitivity

The initial results from modeling $Na_V1.5$ mechanosensitivity in small intestine ICC are promising. Further modeling and experimental studies can provide additional insight into the role of $Na_V1.5$ in mediating stretch-dependent changes of slow waves in the gastrointestinal tract.

The stretch-dependent shift in voltage half-points implemented in the Hodgkin-Huxley formulation of $Na_V1.5$ in small intestine ICC altered the slow wave by causing a hyperpolarizing shift in the window current towards the resting membrane potential, thus increasing the baseline Na^+ current during the resting phase. It is likely that the resultant depolarization of the resting membrane potential contributed to the increased frequency and upstroke rate of the slow waves [84].

Some aspect of $Na_V1.5$ mechanosensitivity were not included in the Hodgkin-Huxley formulation [84]. These include the stabilization of inactivated states and slowed recovery from inactivation [29], which may affect the repolarization phase of slow waves and further contribute to the resting potential and excitability. A Markov formulation of $Na_V1.5$ [69] could be adopted to directly model the effect of stretch on the inactivated states and the kinetic link between activation and inactivation.

An important goal of modeling stretch is to expand this work to investigate mechanosensitivity on the tissue scale. The dynamic effects of mechanoelectrical and electromechanical feedback cycles on the tissue and organ level can be further explored by modeling, using both ICC and SMC cell models with an integrated model of $Na_V1.5$ mechanosensitivity.

It is likely that most channels in the GI tract have some mechanosensitivity. In general, any channel that has changes in its in-plane area during gating should be mechanically sensitive. Many channels found in the GI tract have already been shown to be mechanosesitive, including L-type Ca^{2+} channels [26] as well as $Na_V1.5$,

but the level of available data, especially from primary cells, is limited. Tissue level models will be enhanced by incorporating each of these ion channel models into both ICC and SMC, where appropriate.

The future of mechano-electrical feedback experimentation and modeling

In this chapter we summarized the experimental and modeling data available for gut mechano-electrical feedback at the cellular and molecular scale using $Na_V1.5$ as an example. It is clear that much more remains to be done in both the experimental and modeling arenas. The experimentalists will need to continue to develop the experimental tools for study of molecular electro-mechanics, to expand the focus to other ion channels and to assess mechano-sensitive states of ion channels comprehensively. The modelers will need to refine the existing cellular and molecular models to include a more comprehensive repertoire of ion channels, to have the flexibility to integrate multiple kinetic states into the molecular models and to couple mechano-electrical feedback with electro-mechanical output [85]. This combined work will allow the exciting opportunity to examine mechano-electrical feedback at enlarging anatomic scales, to predict the impact of the individual molecular components on tissue physiology, to understand the pathophysiology relating to ion channel mechanosensitivity, and to most importantly discover therapeutic options for our patients.

Work supported by NIH DK52766.

References

1. Muraki K, Imaizumi Y, Watanabe M (1991) Sodium currents in smooth muscle cells freshly isolated from stomach fundus of the rat and ureter of the guinea-pig. J Physiol 442:351-375
2. Smirnov SV, Zholos AV, Shuba MF (1992) Potential-dependent inward currents in single isolated smooth muscle cells of the rat ileum. J Physiol 454:549-571
3. Xiong Z, Sperelakis N, Noffsinger A, Fenoglio-Preiser C (1993) Fast Na+ current in circular smooth muscle cells of the large intestine. Pflugers Arch 423 (5-6):485-491
4. Fernandez-Tenorio M, Gonzalez-Rodriguez P, Porras C, Castellano A, Moosmang S, Hofmann F, Urena J, Lopez-Barneo J Short communication: genetic ablation of L-type Ca2+ channels abolishes depolarization-induced Ca2+ release in arterial smooth muscle. Circulation research 106 (7):1285-1289
5. Holm AN, Rich A, Miller SM, Strege P, Ou Y, Gibbons S, Sarr MG, Szurszewski JH, Rae JL, Farrugia G (2002) Sodium current in human jejunal circular smooth muscle cells. Gastroenterology 122 (1):178-187
6. Ou Y, Gibbons SJ, Miller SM, Strege PR, Rich A, Distad MA, Ackerman MJ, Rae JL, Szurszewski JH, Farrugia G (2002) SCN5A is expressed in human jejunal circular smooth muscle cells. Neurogastroenterol Motil 14 (5):477-486
7. Strege PR, Ou Y, Sha L, Rich A, Gibbons SJ, Szurszewski JH, Sarr MG, Farrugia G (2003) Sodium current in human intestinal interstitial cells of Cajal. Am J Physiol Gastrointest Liver Physiol 285 (6):G1111-1121
8. Strege PR, Mazzone A, Kraichely RE, Sha L, Holm AN, Ou Y, Lim I, Gibbons SJ, Sarr MG, Farrugia G (2007) Species dependent expression of intestinal smooth muscle mechanosensitive sodium channels. Neurogastroenterol Motil 19 (2):135-143
9. Locke GR, 3rd, Ackerman MJ, Zinsmeister AR, Thapa P, Farrugia G (2006) Gastrointestinal symptoms in families of patients with an SCN5A-encoded cardiac channelopathy: evidence of an intestinal channelopathy. Am J Gastroenterol 101 (6):1299-1304
10. Saito YA, Strege PR, Tester DJ, Locke GR, 3rd, Talley NJ, Bernard CE, Rae JL, Makielski JC, Ackerman MJ, Farrugia G (2009) Sodium channel mutation in irritable bowel syndrome: evidence for an ion channelopathy. Am J Physiol Gastrointest Liver Physiol 296 (2):G211-218
11. Saito YA, Tester DJ, Mazzone A, Beyder A, Locke GR, 3rd, Talley NJ, Ackerman M, Farrugia G Sodium channel mutations in irritable bowel syndrome. In: Neurogastroenterology & Motility, Chicago, IL, 2009.
12. Beyder A, Strege P, Mazzone A, Bernard C, Tester DJ, Saito YA, Ackerman M, Farrugia G Mutations in SCN5A from patients with IBS result in abnormal Nav1.5 function. In: Digestive Diseases Week, Chicago, IL, 2011.
13. Mazzone A, Strege PR, Tester DJ, Bernard CE, Faulkner G, De Giorgio R, Makielski JC, Stanghellini V, Gibbons SJ, Ackerman MJ, Farrugia G (2008) A mutation in telethonin alters Nav1.5 function. J Biol Chem 283 (24):16537-16544

14. Nozawa K, Kawabata-Shoda E, Doihara H, Kojima R, Okada H, Mochizuki S, Sano Y, Inamura K, Matsushime H, Koizumi T, Yokoyama T, Ito H (2009) TRPA1 regulates gastrointestinal motility through serotonin release from enterochromaffin cells. Proc Natl Acad Sci U S A 106 (9):3408-3413. doi:0805323106 [pii]
10.1073/pnas.0805323106
15. Kunze WA, Clerc N, Bertrand PP, Furness JB (1999) Contractile activity in intestinal muscle evokes action potential discharge in guinea-pig myenteric neurons. J Physiol 517 (Pt 2):547-561. doi:PHY_8802 [pii]
16. Won KJ, Sanders KM, Ward SM (2005) Interstitial cells of Cajal mediate mechanosensitive responses in the stomach. Proc Natl Acad Sci U S A 102 (41):14913-14918
17. Farrugia G, Holm AN, Rich A, Sarr MG, Szurszewski JH, Rae JL (1999) A mechanosensitive calcium channel in human intestinal smooth muscle cells. Gastroenterology 117 (4):900-905
18. Beyder A, Farrugia G (2011) Targeting ion channels for the treatment of gastrointestinal motility disorders. Therap Adv Gastroenterol in review
19. Conti F, Fioravanti R, Segal JR, Stuhmer W (1982) Pressure dependence of the sodium currents of squid giant axon. J Membr Biol 69 (1):23-34
20. Conti F, Inoue I, Kukita F, Stuhmer W (1984) Pressure dependence of sodium gating currents in the squid giant axon. Eur Biophys J 11 (2):137-147
21. Gu CX, Juranka PF, Morris CE (2001) Stretch-activation and stretch-inactivation of Shaker-IR, a voltage-gated K+ channel. Biophys J 80 (6):2678-2693
22. Tabarean IV, Morris CE (2002) Membrane stretch accelerates activation and slow inactivation in Shaker channels with S3-S4 linker deletions. Biophys J 82 (6):2982-2994
23. Laitko U, Morris CE (2004) Membrane tension accelerates rate-limiting voltage-dependent activation and slow inactivation steps in a Shaker channel. J Gen Physiol 123:135-154
24. Laitko U, Juranka PF, Morris CE (2006) Membrane stretch slows the concerted step prior to opening in a Kv channel. J Gen Physiol 127 (6):687-701
25. Calabrese B, Tabarean IV, Juranka P, Morris CE (2002) Mechanosensitivity of N-type calcium channel currents. Biophys J 83 (5):2560-2574
26. Lyford GL, Strege PR, Shepard A, Ou Y, Ermilov L, Miller SM, Gibbons SJ, Rae JL, Szurszewski JH, Farrugia G (2002) alpha(1C) (Ca(V)1.2) L-type calcium channel mediates mechanosensitive calcium regulation. Am J Physiol Cell Physiol 283 (3):C1001-1008
27. Tabarean IV, Juranka P, Morris CE (1999) Membrane stretch affects gating modes of a skeletal muscle sodium channel. Biophys J 77 (2):758-774
28. Morris CE, Juranka PF (2007) Nav channel mechanosensitivity: activation and inactivation accelerate reversibly with stretch. Biophys J 93 (3):822-833
29. Beyder A, Rae JL, Bernard C, Strege PR, Sachs F, Farrugia G (2010) Mechanosensitivity of Nav1.5, a voltage-sensitive sodium channel. J Physiol 588 (Pt 24):4969-4985. doi:jphysiol.2010.199034 [pii]

10.1113/jphysiol.2010.199034
30. Trepat X, Deng L, An SS, Navajas D, Tschumperlin DJ, Gerthoffer WT, Butler JP, Fredberg JJ (2007) Universal physical responses to stretch in the living cell. Nature 447 (7144):592-595
31. Shi ZD, Abraham G, Tarbell JM (2010) Shear stress modulation of smooth muscle cell marker genes in 2-D and 3-D depends on mechanotransduction by heparan sulfate proteoglycans and ERK1/2. PLoS One 5 (8):e12196
32. Sachs F (2010) Stretch-activated ion channels: what are they? Physiology (Bethesda) 25 (1):50-56. doi:25/1/50 [pii]
10.1152/physiol.00042.2009
33. Abriel H (2010) Cardiac sodium channel Na(v)1.5 and interacting proteins: Physiology and pathophysiology. J Mol Cell Cardiol 48 (1):2-11
34. Bennett V, Healy J (2008) Organizing the fluid membrane bilayer: diseases linked to spectrin and ankyrin. Trends Mol Med 14 (1):28-36
35. Strege PR, Holm AN, Rich A, Miller SM, Ou Y, Sarr MG, Farrugia G (2003) Cytoskeletal modulation of sodium current in human jejunal circular smooth muscle cells. Am J Physiol Cell Physiol 284 (1):C60-66
36. Petitprez S, Zmoos AF, Ogrodnik J, Balse E, Raad N, El-Haou S, Albesa M, Bittihn P, Luther S, Lehnart SE, Hatem SN, Coulombe A, Abriel H (2011) SAP97 and dystrophin macromolecular complexes determine two pools of cardiac sodium channels Nav1.5 in cardiomyocytes. Circulation research 108 (3):294-304. doi:CIRCRESAHA.110.228312 [pii]
10.1161/CIRCRESAHA.110.228312
37. Ou Y, Strege P, Miller SM, Makielski J, Ackerman M, Gibbons SJ, Farrugia G (2003) Syntrophin gamma 2 regulates SCN5A gating by a PDZ domain-mediated interaction. J Biol Chem 278 (3):1915-1923
38. Geiger B, Bershadsky A (2002) Exploring the neighborhood: adhesion-coupled cell mechanosensors. Cell 110 (2):139-142. doi:S0092867402008310 [pii]
39. Undrovinas AI, Shander GS, Makielski JC (1995) Cytoskeleton modulates gating of voltage-dependent sodium channel in heart. Am J Physiol 269 (1 Pt 2):H203-214
40. Suchyna TM, Markin VS, Sachs F (2009) Biophysics and structure of the patch and the gigaseal. Biophys J 97 (3):738-747
41. Markin VS, Sachs F (2004) Thermodynamics of mechanosensitivity. Physical biology 1 (1-2):110-124
42. Matteson DR, Armstrong CM (1982) Evidence for a population of sleepy sodium channels in squid axon at low temperature. J Gen Physiol 79 (5):739-758
43. Milescu M, Bosmans F, Lee S, Alabi AA, Kim JI, Swartz KJ (2009) Interactions between lipids and voltage sensor paddles detected with tarantula toxins. Nat Struct Mol Biol 16 (10):1080-1085
44. Lundbaek JA, Birn P, Hansen AJ, Sogaard R, Nielsen C, Girshman J, Bruno MJ, Tape SE, Egebjerg J, Greathouse DV, Mattice GL, Koeppe RE, 2nd, Andersen OS (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 (5):599-621
45. Freites JA, Tobias DJ, von Heijne G, White SH (2005) Interface connections of a transmembrane voltage sensor. Proc Natl Acad Sci U S A 102 (42):15059-15064
46. Bjelkmar P, Niemela PS, Vattulainen I, Lindahl E (2009) Conformational changes and slow dynamics through microsecond polarized atomistic molecular simulation of an integral Kv1.2 ion channel. PLoS computational biology 5 (2):e1000289
47. Krepkiy D, Mihailescu M, Freites JA, Schow EV, Worcester DL, Gawrisch K, Tobias DJ, White SH, Swartz KJ (2009) Structure and hydration of membranes embedded with voltage-sensing domains. Nature 462 (7272):473-479. doi:nature08542 [pii]
10.1038/nature08542
48. Sinha B, Koster D, Ruez R, Gonnord P, Bastiani M, Abankwa D, Stan RV, Butler-Browne G, Vedie B, Johannes L, Morone N, Parton RG, Raposo G, Sens P, Lamaze C, Nassoy P (2011) Cells respond to mechanical stress by rapid disassembly of caveolae. Cell 144 (3):402-413. doi:S0092-8674(10)01523-0 [pii]
10.1016/j.cell.2010.12.031
49. Yarbrough TL, Lu T, Lee HC, Shibata EF (2002) Localization of cardiac sodium channels in caveolin-rich membrane domains: regulation of sodium current amplitude. Circulation research 90 (4):443-449
50. Vatta M, Ackerman MJ, Ye B, Makielski JC, Ughanze EE, Taylor EW, Tester DJ, Balijepalli RC, Foell JD, Li Z, Kamp TJ, Towbin JA (2006) Mutant caveolin-3 induces persistent late sodium current and is associated with long-QT syndrome. Circulation 114 (20):2104-2112. doi:CIRCULATIONAHA.106.635268 [pii]
10.1161/CIRCULATIONAHA.106.635268
51. Scriven DR, Dan P, Moore ED (2000) Distribution of proteins implicated in excitation-contraction coupling in rat ventricular myocytes. Biophys J 79 (5):2682-2691. doi:S0006-3495(00)76506-4 [pii]
10.1016/S0006-3495(00)76506-4
52. Aldrich RW, Corey DP, Stevens CF (1983) A reinterpretation of mammalian sodium channel gating based on single channel recording. Nature 306 (5942):436-441
53. Payandeh J, Scheuer T, Zheng N, Catterall WA (2011) The crystal structure of a voltage-gated sodium channel. Nature. doi:10.1038/nature10238
nature10238 [pii]
54. Jiang Y, Ruta V, Chen J, Lee A, MacKinnon R (2003) The principle of gating charge movement in a voltage-dependent K+ channel. Nature 423:42-48
55. Jiang Y, Lee A, Chen J, Ruta V, Cadene M, Chait BT, MacKinnon R (2003) X-ray structure of a voltage-dependent K+ channel. Nature 423:33-41
56. Long SB, Cambell EB, MacKinnon R (2005) Voltage sensor of kv1.2: Structural basis of electromechanical coupling. Science 309 (5736):903-908

57. Long SB, Cambell EB, MacKinnon R (2005) Crystal structure of a mammalian voltage-dependent Shaker family K+ channel. Science 309 (5736):897-903
58. Long SB, Tao X, Campbell EB, MacKinnon R (2007) Atomic structure of a voltage-dependent K+ channel in a lipid membrane-like environment. Nature 450 (7168):376-382
59. Beyder A, Sachs F (2009) Electromechanical coupling in the membranes of Shaker-transfected HEK cells. Proc Natl Acad Sci U S A 106 (16):6626-6631
60. Banderali U, Juranka PF, Clark RB, Giles WR, Morris CE (2010) Impaired stretch modulation in potentially lethal cardiac sodium channel mutants. Channels (Austin) 4 (1):12-21. doi:10260 [pii]
61. Bennett PB, Yazawa K, Makita N, George AL, Jr. (1995) Molecular mechanism for an inherited cardiac arrhythmia. Nature 376 (6542):683-685
62. Tfelt-Hansen J, Winkel BG, Grunnet M, Jespersen T Inherited cardiac diseases caused by mutations in the Nav1.5 sodium channel. J Cardiovasc Electrophysiol 21 (1):107-115
63. Du P, O'Grady G, Davidson JB, Cheng LK, Pullan AJ (2010) Multiscale modeling of gastrointestinal electrophysiology and experimental validation. Crit Rev Biomed Eng 38 (3):225-254. doi:4b26e27c6af6b2f3,25b8e9a24ec08a0c [pii]
64. Hodgkin AL, Huxley AF (1952) A quantitative description of membrane current and its application to conduction and excitation in nerve. J Physiol 117 (4):500-544
65. Dubois JM, Ouanounou G, Rouzaire-Dubois B (2009) The Boltzmann equation in molecular biology. Prog Biophys Mol Biol 99 (2-3):87-93. doi:S0079-6107(09)00052-2 [pii]
10.1016/j.pbiomolbio.2009.07.001
66. Hille B (2001) Ion channels of excitable membranes. Third edn. Sinauer Associates, Inc., Sunderland, MA
67. Keener JP, Sneyd J (2009) Mathematical physiology: Cellular physiology. Springer,
68. Destexhe A, Huguenard JR (2009) Modeling voltage-dependent channels. In: Schutter ED (ed) Computational modeling methods for neuroscientists. MIT Press, Cambridge, MA, pp 107-137
69. Poh YC, Beyder A, Strege PR, Farrugia G, Buist ML (2011) Quantification of gastrointestinal sodium channelopathy. J Theor Biol 293C:41-48. doi:S0022-5193(11)00470-X [pii]
10.1016/j.jtbi.2011.09.014
70. Youm JB, Kim N, Han J, Kim E, Joo H, Leem CH, Goto G, Noma A, Earm YE (2006) A mathematical model of pacemaker activity recorded from mouse small intestine. Philos Transact A Math Phys Eng Sci 364 (1842):1135-1154. doi:X622288MN8G33RL0 [pii]
10.1098/rsta.2006.1759
71. Corrias A, Buist ML (2007) A quantitative model of gastric smooth muscle cellular activation. Ann Biomed Eng 35 (9):1595-1607

72. Corrias A, Buist ML (2008) Quantitative cellular description of gastric slow wave activity. Am J Physiol Gastrointest Liver Physiol 294 (4):G989-995
73. Faville RA, Pullan AJ, Sanders KM, Koh SD, Lloyd CM, Smith NP (2009) Biophysically based mathematical modeling of interstitial cells of Cajal slow wave activity generated from a discrete unitary potential basis. Biophys J 96 (12):4834-4852. doi:S0006-3495(09)00794-2 [pii]
10.1016/j.bpj.2009.03.058
74. Faville RA, Pullan AJ, Sanders KM, Smith NP (2008) A biophysically based mathematical model of unitary potential activity in interstitial cells of Cajal. Biophys J 95 (1):88-104. doi:S0006-3495(08)70286-8 [pii]
10.1529/biophysj.107.122507
75. Lees-Green R, Du P, O'grady G, Beyder A, Farrugia G, Pullan AJ (2011) Biophysically-based modelling of the interstitial cells of Cajal: Current status and future perspectives. Frontiers in Computational Physiology and Medicine 2:29
76. Ward SM, Baker SA, de Faoite A, Sanders KM (2003) Propagation of slow waves requires IP3 receptors and mitochondrial Ca2+ uptake in canine colonic muscles. J Physiol 549 (Pt 1):207-218. doi:10.1113/jphysiol.2003.040097
2003.040097 [pii]
77. Hwang SJ, Blair PJ, Britton FC, Odriscoll KE, Hennig G, Bayguinov JR, Rock JR, Harfe BD, Sanders KM, Ward SM (2009) Expression of anoctamin 1Tmem16a by inrerstitial cells of cajal is fundamental for slow wave activity in gastointestinal muscles. J Physiol
78. Zhu MH, Kim TW, Ro S, Yan W, Ward SM, Koh SD, Sanders KM (2009) A Ca2+-activated Cl- conductance in interstitial cells of Cajal linked to slow wave currents and pacemaker activity. J Physiol
79. Huang F, Rock JR, Harfe BD, Cheng T, Huang X, Jan YN, Jan LY (2009) Studies on expression and function of the TMEM16A calcium-activated chloride channel. Proc Natl Acad Sci U S A 106 (50):21413-21418. doi:0911935106 [pii]
10.1073/pnas.0911935106
80. Buist ML, Corrias A, Poh YC (2010) A model of slow wave propagation and entrainment along the stomach. Ann Biomed Eng 38 (9):3022-3030. doi:10.1007/s10439-010-0051-1
81. Du P, O'Grady G, Gibbons SJ, Yassi R, Lees-Green R, Farrugia G, Cheng LK, Pullan AJ (2009) Tissue-specific mathematical models of slow wave entrainment in wild-type and 5-HT(2B) knockout mice with altered interstitial cells of Cajal networks. Biophys J 98 (9):1772-1781
82. Du P, Li S, O'Grady G, Cheng LK, Pullan AJ, Chen JD (2009) Effects of electrical stimulation on isolated rodent gastric smooth muscle cells evaluated via a joint computational simulation and experimental approach. Am J Physiol Gastrointest Liver Physiol 297 (4):G672-680. doi:00149.2009 [pii]
10.1152/ajpgi.00149.2009
83. Du P, O'Grady G, Cheng LK, Pullan AJ (2010) A multiscale model of the electrophysiological basis of the human electrogastrogram. Biophys J 99 (9):2784-2792

84. Lees-Green R, Beyder A, Farrugia G, O'grady G, Poh YC, Buist ML, Pullan AJ Computational modeling of the sodium channel mechanical stretch effects on the electrical function of human interstitial cells of Cajal and smooth muscle cells. In: Digestive Diseases Week, Chicago, IL, May, 2011 2011.
85. Gajendiran V, Buist ML (2011) A quantitative description of active force generation in gastrointestinal smooth muscle. International Journal for Numerical Methods in Biomedical Engineering 27 (3):450-460