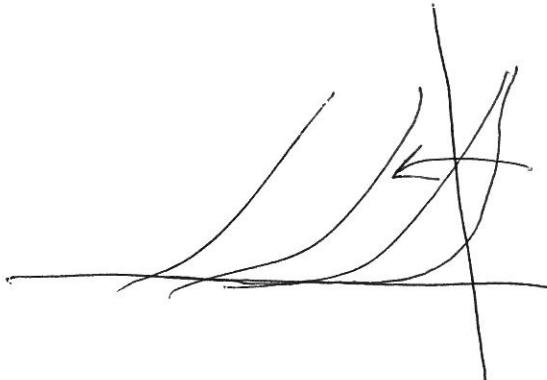


Chandrasekhar Paper 02/22/13

① Re-scale diagrams

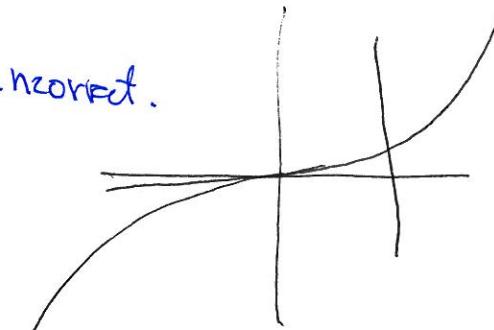
② Add raw data

- I_{K-DR}
- 2-pore
- TRP_{V4}



③ $I_{K-Ca} -$ is the insilce current 'noisy'
scale for $[Ca^{2+}]_i$: $5 \times 10^{-8} M$
 $5 \times 10^{-6} M \longrightarrow \times 5$

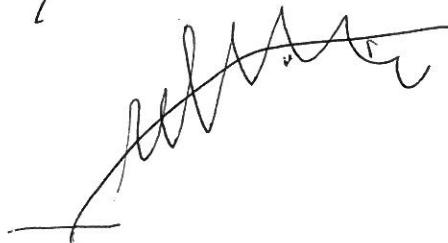
④ Na^+/Ca^{2+} exchange 2-V is incorrect.



⑤ Journal?

b. Jim Wilson.

1st review



⑦ Stretch $\propto \Delta [Ca^{2+}]_i$

⑧ $I_{K-Ca} -$

The Roles of K⁺ Currents in Human Articular Chondrocyte Electrophysiology: A Computational Perspective

Harish Narayanan*

Center for Biomedical Computing,
Simula Research Laboratory, Lysaker, Norway

Mary M. Maleckar,

Center for Biomedical Computing,
Simula Research Laboratory, Lysaker, Norway

Robert B. Clark,

Faculty of Kinesiology,

University of Calgary, Calgary, Canada

James R. Wilson

Wayne R. Giles,

Faculty of Kinesiology,

University of Calgary, Calgary, Canada

*Corresponding author. Address: Center for Biomedical Computing, Simula Research Laboratory, P.O. Box 134, 1325 Lysaker, Norway Tel.: +47 4003-4801, Fax: +47 6782-8201

Abstract:

A has been developed
We have developed a computational model for studying the electrophysiology of the human chondrocyte. This model is based on an initial experimental data set which identified the main K⁺ currents expressed in single cells isolated from knee joints of healthy adult human donors. The applicability and utility of this model are illustrated by focusing on the role of a novel 2-pore K⁺ current in regulating the chondrocyte resting potential and modulating intracellular Ca²⁺ homeostasis. This model is demonstrated to be useful for integrating available data from electrophysiological, PCR and gene array experiments. It is also an important adjunct for tool

rationalization of working hypotheses, design of new experiments and understanding the principles and limitations of patch clamp methods as applied to the isolated human chondrocyte.

Key words: chondrocyte; electrophysiology; potassium currents; computational model

INTRODUCTION

Articular cartilage is considered to be an aneural, avascular, alymphatic component of the flexible connective tissue that covers the articulating ends of diarthroidal joints (1-3). It is essential for the ~~required~~ stability and low friction movement of the associated long bones. This connective tissue consists of an extracellular matrix (ECM, composed primarily of collagen, elastin and proteoglycans, (as detailed below) and only one type of cell - the *chondrocyte*. Articular cartilage is exposed to cyclical mechanical forces; in fact, this dynamic environment is essential for the health

of the tissue (2). Chondrocytes occupy only approximately 1-10% of the total volume of articular cartilage in mammals (4,5) and play no direct mechanical role. ^{However} Instead, chondrocytes are responsible for synthesis and homeostasis of the extracellular matrix. Mechanical support is provided by the ECM, which is composed of (a) collagen fibers that give the tissue the ability to resist tension, (b) negatively-charged gel-like proteoglycans (PGs) that are trapped within the collagen mesh and allow the tissue to bear compression (1,8) and (c) synovial fluid within the articular capsule which acts as a lubricant, thus ensuring low friction movement of the bones (7).

The primary role of the human chondrocyte is to maintain viable cartilage ~~by balancing~~

macromolecular synthesis and breakdown (see e.g. 2,7,9).

In a variety of conditions (including healthy aging), ~~or~~ as a consequence of injury, progressive chondrocyte damage and dysfunction may occur (10-12). As a result, the balance between matrix synthesis and degradation is altered and the low friction environment of the joint may be reduced or lost (9). Under these conditions, there is often an inflammatory response within the articular joint. This is one factor that increases the early development of osteoarthritis: a thinning of the cartilage layer which causes painful, bone-against-bone friction (1,11). The progression of osteoarthritis (12,13) and the ability of chondrocytes to respond to perturbations in the extracellular environment (14) are associated with deficiencies in chondrocyte volume regulation (15). It is known that physical damage to cartilage is more frequent in the setting of altered osmolarity (16). In turn, there is evidence that these volume changes are linked to an abnormal resting potential in chondrocytes (17), which may be due to changes in K^+ and/or Cl^- channel activity. ~~However,~~ ^{In the case} direct experimental investigations that address possible functional relationships between chondrocyte electrophysiology and pathophysiology are challenging due to the small cell size of the mature chondrocyte and the associated limitations of *in vitro* electrophysiological studies. In fact, it is not at all certain that conventional patch pipette methods (18,19) can accurately determine the resting potential of isolated single chondrocytes (20). Accordingly, for the purpose of integrating available experimental data and attempting to understand its functional significance, we have developed a ^{view page.} ~~Mathematical~~ detailed biophysical model of human chondrocyte electrophysiology. This model ~~can~~ facilitate investigation of electrophysiological mechanisms, ^{which} regulate chondrocyte physiology and pathophysiology and are the targets of intra- and intercellular signaling pathways responsible for ~~biomechanical~~ roles, including excitation-secretion coupling.

The main goals of this work are (i) to develop the first detailed mathematical model of chondrocyte

electrophysiology and (ii) to illustrate the utility of the model in investigating the dependence of the chondrocyte resting membrane potential, and by extension the volume and signaling regimes of the chondrocyte, on key voltage- and ligand-gated membrane channels. currents.

MODEL DEVELOPMENT AND METHODS

The chondrocyte cell membrane is known to express a number of voltage- and ligand-gated ion channels as well as pumps and exchangers (30). Patch clamp experiments performed on enzymatically isolated individual human chondrocytes obtained (with permission) from a knee replacement surgery program (The Southern Alberta Transplant Service) provide the experimental basis for this model development. These chondrocytes evinced resting membrane potentials ranging from -30 to -60 mV when superfused with normal Tyrodes solution and studied using antibiotic-permeabilized (amphotericin) patch clamp methods (28). This significant range of resting membrane potential values may reflect the heterogeneous physiological state of these cells.

However, as we have illustrated previously, some of this variability is likely to result from the fact that in these very small, approximately spherical cells (diameter, 7 microns; capacitance 10-15 pF) the patch pipette recording method is being applied very near its technical limitations (20). That is,

This is because

The input resistance of the target cell (chondrocyte) is very large (1-10 gigohms), and the seal resistance between the surface membrane of the chondrocytes and the polished surface of the glass pipette has a typical value of 1-5 gigohms. Thus, the actual chondrocyte membrane potential may be underestimated due to the current flow through the seal resistance, which in most circumstances results in an apparent depolarization. The Appendix of this manuscript describes the known biophysical principles which, when combined, regulate the ways in which the combination of patch pipette seal resistance and intrinsic input resistance result in apparent values for the resting potential in small cells such as the human chondrocyte.

The main objective of this ~~first~~ model is to obtain insights into the ionic mechanisms that underlie the resting potential ~~in the human chondrocyte~~ *in situ*. The raw data that forms the basis of this ~~was~~ model ~~is~~ derived mainly from our previously published recordings of the K^+ currents in human (28), mouse (31) and canine chondrocytes (32). Other elements summarized diagrammatically in Figure 1 below ~~are~~ obtained from published literature on isolated chondrocytes from rabbit, canine and equine tissue.

[Figure 1 near here]

The Atypical Environment of the Chondrocyte

~~trans membrane~~
The chondrocyte is situated in a physiological environment that differs significantly from that of most other mammalian cells (as summarized in Table 1). Despite ~~this unique extracellular environs,~~ ~~the~~ ~~one~~, the large electrochemical gradients for Na^+ , K^+ and Cl^- ~~across the cell membrane~~ are very similar to those in other mammalian tissue types. These gradients lead to the requirement for an ATP-dependent Na^+/K^+ pump mechanism, (assumed to be electrogenic,) in order to maintain ionic homeostasis. Note, however, that the extracellular fluid within the articular joint is hypertonic (approximately 320 mOsm vs. blood plasma which is approximately 280 mOsm). In addition, the extracellular pH of the synovial fluid that bathes the chondrocyte is somewhat acidic, pH 7.2 (8,24) although apparently an intra- to extracellular pH gradient also typical for other cell types can be identified (references?). [x x x] ~~that are~~

The substantial number of fixed negative charges on proteoglycans immediately adjacent to the chondrocyte can attract free cations (e.g. Na^+) and exclude free anions from the matrix. As a result of this cation accumulation (Table 1), there is an osmotically driven water influx ~~to the cell~~ (10,17).

The articular joint receives little or no vascular supply. As a result, the synovial fluid must supply adult articular cartilage with the required small amounts of nutrients as well as oxygen to e.g., maintain Na^+/K^+ pump activity (33). Metabolic byproducts are removed mainly by diffusion (34,35). A second consequence of the avascular nature of this tissue is that chondrocytes generate ATP by substrate-level phosphorylation during anaerobic respiration. This generates H^+ ions as a byproduct, which further lowers the pH in this micro environment. The dynamic changes in mechanical loading within the knee joint during activity also exposes chondrocytes to very significant fluctuations in vector forces and shear. The resulting mechanical changes would be expected to activate mechano- or shear-sensitive ion channels (36-40).

Experimentally-reported values for the external cation concentrations differ markedly from those of extracellular fluid in other mammalian tissues. We have also incorporated these atypical values for extracellular ion concentrations (Table 1).

in the Articular Joint

Table 1: Consensus Values of Ion Concentrations for Chondrocyte Electrophysiology, given in millimolars (mM) (8)

	Cytoplasm	Matrix	Serum/Synovium
$[\text{Na}^+]_o$ (mM)	?	240-350	140
$[\text{K}^+]_o$ (mM)	40	7-12	5

6

ref 8

[Ca²⁺]_o (mM) 8.e-5 6-15 1.5

[Cl]_o (mM) 60-90 60-100 140

[HCO₃⁻]_o (mM) 20 15 23

[SO₄²⁻]_o (mM) 0.17 0.30 0.81

pH (mM) 7.1 6.6-6.9 7.4

Osmolarity
(mOsm) -- 350-
450 300

Membrane Currents

Background Currents

Three different

Background or leakage conductances, including resting Na^+ , K^+ , and Cl^- fluxes, are included in the present model.

The model accounts for leakage of Na^+ and K^+ through time-independent, non-specific cation channels using standard Hodgkin-Huxley formalism:

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Comment: Please note that all equations will be typeset properly in submitted version.

$$I_{\text{Na}_b} = \bar{g}_{\text{Na}_b}(V_m - E_{\text{Na}}), \quad (1)$$

$$I_{\text{K}_b} = \bar{g}_{\text{K}_b}(V_m - E_{\text{K}}), \quad (2)$$

To

Where XX is XX...

Mary M Maleckar 13-1-31 4:48 PM

Comment: Please note that these definitions for preceding equations will also be set in when typesetting.

And the Nernst potentials for Na^+ and K^+ are computed based on their respective intra- and extracellular concentrations:

$$E_{\text{Na}} = \frac{RT}{z_{\text{Na}}F} \ln \left(\frac{[\text{Na}^+]_o}{[\text{Na}^+]_i} \right), \quad (3)$$

$$E_{\text{K}} = \frac{RT}{z_{\text{K}}F} \ln \left(\frac{[\text{K}^+]_o}{[\text{K}^+]_i} \right). \quad (4)$$

Where XX is XX...

In a variety of mammalian chondrocyte preparations, one or more types of Cl^- conductance have been identified (59,c.f. 30). This quasi-linear Cl^- conductance has also been identified in human

chondrocyte cell lines (61), and in experiments in which selected transmitters or pharmacological agonists (e.g., histamine) have been applied (62). We have incorporated this type of current into our model by treating it as a background current, specified by the equations below.

$$I_{\text{Cl}_b} = g_{\text{Cl}_b}(V_m - E_{\text{Cl}}) \quad (5)$$

Where XX is XX...

and

$$E_{\text{Cl}} = \frac{RT}{z_{\text{Cl}}F} \ln \left(\frac{[\text{Cl}^-]_o}{[\text{Cl}^-]_i} \right) \quad (6)$$

is the Nernst potential due to the difference in Cl⁻ concentration inside and outside the cell. Where XX is XX...

The current-voltage (IV) relationships for these sodium, potassium, and chloride background currents (INab, IKb, and IClb, respectively) are shown in Figure 2A.

Ion Pumps and Exchangers

(a) Na⁺/K⁺ Pump

The active extrusion of Na⁺ ions from the chondrocyte is assumed to be achieved by the turnover of an electrogenic Na⁺/K⁺-ATPase. Mobasher et al (33) have characterized some of the functional properties of an electrogenic Na⁺/K⁺ pump in bovine articular chondrocytes. Cell volume is maintained by altered balance of leaks and pumps to hold cell water constant. In this model, we employ the following sodium-potassium pump formulation (42):

$$I_{NaK} = J_{NaK} \left(\frac{[K^+]_o}{[K^{+}]_o + k_{NaK_K}} \right) \left(\frac{[Na^{+}]_i^{1.5}}{[Na^{+}]_i^{1.5} + k_{NaK_Na}^{1.5}} \right) \left(\frac{V + 150}{V + 200} \right) \quad (7)$$

Where XX is XX...

This conductance has been scaled to achieve a steady-state intracellular Na^+ concentration of 10-12 mM.

(b) Na^+/Ca^{2+} Exchanger

As in many other cell types, the Na^+/Ca^{2+} exchanger plays a key role in Ca^{2+} homeostasis in articular chondrocytes (41). We model this electrogenic exchange process using the following mathematical expression (42).

$$I_{NaCa} = k_{NaCa} \frac{[Na^+]_i^3 [Ca^{2+}]_o \exp(\frac{VF}{RT}) - [Na^+]_o^3 [Ca^{2+}]_i \exp(\frac{(V-1.0)VF}{RT})}{1.0 + d_{NaCa}([Na^+]_o^3 [Ca^{2+}]_i + [Na^+]_i^3 [Ca^{2+}]_o)} \quad (8)$$

Note

L-V curve
looks 'odd'

Where XX is XX...

This conductance has been scaled to achieve an intracellular Na^+ concentration of 12 mM, and assumed free intracellular Ca^{2+} concentration of 3×10^{-8} M.

(c) Na^+/H^+ Exchanger

contributes (importantly) to regulation

Chondrocytes express a Na^+/H^+ antiporter (43,44), which ~~allows the cell to regulate pH and~~ establish an intra- to extracellular pH gradient. This pH gradient is essential for maintaining intracellular Na^+ levels and the intracellular pH indirectly regulates a number of essential enzymatic processes in both physiological and pathophysiological settings.

In order to model this channel, we use the following functional form (45):

electroneutral antiporter.

$$I_{\text{NaH}} = N_{\text{NaH}} I_{\text{NaH}_{\text{med}}} I_{\text{NaH}_{\text{exch}}}$$

(9)

where,

$$\begin{aligned} I_{\text{NaH}_{\text{med}}} &= \frac{1}{1 + (K_i^{\text{pH}} / [\text{H}^+]_i^{\text{pH}})}, \\ t_1 &= \frac{k_1^+ [\text{Na}^+]_o / K_{\text{Na}}^o}{(1 + [\text{Na}^+]_o / K_{\text{Na}}^o + [\text{H}^+]_o / K_{\text{H}}^o)} \\ t_2 &= \frac{k_2^+ [\text{H}^+]_i / K_{\text{H}}^i}{(1 + [\text{Na}^+]_i / K_{\text{Na}}^i + [\text{H}^+]_i / K_{\text{H}}^i)} \\ t_3 &= \frac{k_1^- [\text{Na}^-]_i / K_{\text{Na}}^i}{(1 + [\text{Na}^+]_i / K_{\text{Na}}^i + [\text{H}^+]_i / K_{\text{H}}^i)} \\ t_4 &= \frac{k_2^- [\text{H}^+]_o / K_{\text{H}}^o}{(1 + [\text{Na}^+]_o / K_{\text{Na}}^o + [\text{H}^+]_o / K_{\text{H}}^o)}, \\ I_{\text{NaH}_{\text{exch}}} &= \frac{(t_1 t_2 - t_3 t_4)}{(t_1 + t_2 + t_3 + t_4)}. \end{aligned}$$

Figure 2B shows I-V curve for these electrogenic pump and exchangers INaK, INaCa, and INaH.

Where XX is XX...

time- and voltage dependent

Potassium Currents

This model also incorporates mathematical expressions for a total of four distinct K^+ currents identified from electrophysiological studies performed on mammalian chondrocytes. Two of these, a delayed rectifier K^+ current, which we denote $I_{\text{K-DR}}$, and a K^+ current due to a 2-pore K^+ channel, which we denote $I_{\text{K-2P}}$, have been studied in detail in our laboratory (28,32) and by others (45-57). The remaining two K^+ currents, a Ca^{2+} -activated K^+ current ($I_{\text{K-Ca}}$) and an ATP-dependent K^+ current ($I_{\text{K-ATP}}$) have been identified in other patch clamp studies. These are also included.

*, each of which
has been*

} from other investigations (REF)

(a) Delayed Rectifier K⁺ Current: I_{K-DR}

A time- and voltage-sensitive K⁺ current has been identified in the human articular chondrocyte (28-32). The biophysical properties of this current and the details of its pharmacological blockade suggest that it is generated by the family of alpha subunit K⁺ conductances denoted Kv1.4 or Kv1.6. Accordingly, IK-DR is modeled by the following:

$$I_{K-DR} = g_{K_dr} \cdot a_{dr} \cdot (V - E_K) \quad (10)$$

where a_{dr} is computed as part of the system using:

$$a_{dr_inf} = 1.0 / (1.0 + \exp(-(V + 26.7)/4.1))$$

$$\tau_{a_dr} = 0.005 / (1.0 + \exp((V + 5.0)/12.0))$$

in our model.

Where XX is XX...

The two trees in
(i) and the (ii)
Panel A of Figure 3 shows an experimental I-V relationship generated under physiological conditions, that is, a normal electrochemical gradient for K⁺, with the corresponding model results. 2-V curve.
Figure 3B shows steady-state activation, and Figure 3C illustrates the kinetics of activation and inactivation.

[Figure 3 near here]

(b) 2-Pore K⁺ Current: I_{K₂pore}

Our recent work has identified recording conditions under which an additional K⁺ current, generated by what is believed to be the TASK family of 2-pore K⁺ channels (28), is measured (I_{K2pore}). These ~~exhibit~~ ^{K+} channels show no detectable time dependence. TASK channels additionally show a significant increase in conductance in response to an increase in pH or alkalinization of the extracellular medium. A peak I-V curve for this current is shown in Panel A of Figure 4, while Figure 4B illustrates the enhancement of this current when extracellular pH was changed from ~~xx~~ ^{its} ~~xx~~ ^{test} ~~xx~~.

These recordings were performed under conditions of elevated extracellular K⁺ so channel currents were of a detectable magnitude such that biophysical properties could be resolved. Before this data could be appropriately represented and incorporated into the mathematical model, it needed to be corrected to physiological conditions. This was achieved via the Eisenman principle (54): the conductance of an ion-selective channel scales according to the square root of the extracellular concentration of that permeant ion. The I-V curve in Figure 4C shows the original data recorded in isotonic K⁺ (~145 mM), with the expected reversal potential of 0 mV, together with the corrected data (assuming external K⁺ to be 5.4 mM with a corresponding reversal potential of approximately -85 mV).

(normal [K⁺]_o levels).

[Figure 4 near here]

Mary M Malek 13-1-31 4:33 PM

Comment: We suggested that this Figure (4) be entirely experimental, and we leave the mathematical descriptions for Figure 5.

Using the classic Goldman-Hodgkin-Katz equation (54) for voltage-gated, time-independent single-species ion channels, this current can be represented by:

$$I_{K_2 \text{part}} = P_K \frac{z_K^2 V F^2}{RT} \frac{([K^+]_i - [K^+]_o) \exp(-z_K V F / RT)}{(1 - \exp(-z_K V F / (RT)))}, \quad (11)$$

Where XX is XX...

A I-V

Figure 5 shows the current-voltage curve fitted to experimental data (28) obtained in isotonic conditions (panel A), and when corrected for an assumed normal extracellular potassium concentration of 5.4 mM (panel B). Experimental work also demonstrated that this particular K^+ current was strongly inhibited by bupivacaine; an effective concentration of bupivacaine also resulted in a significant depolarization of the resting potential (see Discussion).

[Figure 5 near here]

(c) Ca^{2+} -Activated K^+ Current: $I_{K,Ca\text{-act}}$

Virtually all recordings of global K^+ currents in human chondrocytes include a significant and very noisy outward current at depolarized potentials (55,56). The spontaneous fluctuations of this current suggest that it is generated by the so-called large conductance (often called BK) variant of the Ca^{2+} activated K^+ current family (57). ~~Our experiments work here~~ We have not explored the biophysical properties of this current or its pharmacological profile in any detail. However, it is consistently present in our recordings and a Ca^{2+} -activated K^+ current ($I_{K,Ca\text{-act}}$) has also been described in isolated chondrocytes from rabbits, dogs and horses (47), and is thus included in the present model.

For the given data set (34), the (large) Ca^{2+} -activated K^+ channel can be represented by (57,58):

$$\underline{I_{\text{KCa-net}}} = \underline{N_{\text{KCa-net}} P_0 G_{\max} (V - E_K)}, \quad (12)$$

where,

$$kTe = 23.54 (T/273),$$

$$L_v \approx L_0 \exp((V Z_L)/kTe),$$

$$J_v = \exp(((V - V h_j) Z_j)/kTe),$$

$$K = Ca_i/KDc,$$

$$P_0 = \frac{L_v (1 + K C + J_v D + J_v K C D E)^4}{L_v (1 + K C + J_v D + J_v K C D E)^4 + (1 + J_v + K + J_v K E)^4},$$

$$E_K = \frac{RT}{z_K F} \ln \left(\frac{[K^+]_o}{[K^+]_i} \right).$$

Where XX is XX...

Figure 6 shows the current-voltage relationship for this current as fit to recent experimental data.

[Figure 6 near here]

What $[Ca^{2+}]$: level

d) ATP-sensitive K⁺ Current: I_{K,ATP}

An ATP-sensitive K⁺ current has been reported in chondrocytes from a variety of mammalian species (59). Previous experimental work in human articular chondrocytes (34) did not address this possibility and there are no reports of this current being activated in human chondrocytes ~~in the literature~~. However, the hypoxic environment of the articular chondrocyte makes the presence and the activation of this current during normal biomechanical activity plausible, and thus this current (I_{K,ATP}) is included in the present model, as shown in Figure 7:

$$I_{K,ATP} = \sigma g_0 p_0 f_{ATP} (V - E_K) \quad (15)$$

Where XX is XX...

[Figure 7 near here]

Other Sarcolemmal Currents

Transient Receptor Potential or TRP Current: I_{TRPV4}

Ligand-gated channels that exhibit properties similar to ~~TRP~~ ion channels are expressed in mammalian chondrocytes (63). ~~and the~~ the TRPV4 family is prominently expressed in mouse chondrocytes (64,65). It is likely that this type of conductance ~~is needed to explain~~ chondrocyte electrophysiological responses to naturally occurring paracrine substances (e.g., ATP) or to cytokines that are liberated in the setting of acute or chronic inflammation (e.g., XX). A further reason for incorporating this type of conductance into this early model is that ~~this type of ion channel~~ exhibits significant permeability to both Na⁺ and Ca²⁺ (68). Either or both of these cation

species are likely important in regulating excitation-secretion coupling and could modulate cell volume. It is also known that this type of ion channel can couple to purinergic receptors and/or to specific connexin proteins which function in the context of intercellular electrotonic communication, or as hemi-channels. *For these reasons*, on these bases, the TRPV4 ligand-gated ion channel, thought to be involved in the regulation of systemic osmotic pressure, has been incorporated into this initial model of the human chondrocyte as I_{TRPV4} (see Figure 8).

(REF)

An I-V curve is shown
in Figure 8

[Figure 8 near here]

$$\underline{I_{TRPV4} = \bar{g}_{TRPV4} (V_m - E_g)}$$

(13)

Where XX is XX...

Intracellular Ca^{2+} Homeostasis

The chondrocyte's intracellular Ca^{2+} , $[\text{Ca}^{2+}]_i$, is maintained by the sodium calcium exchanger (eq. 8), a sarcolemmal electroneutral Ca^{2+} ATP-ase (eq. 12) and via intracellular buffering (eq. 13 and 14). In the absence of more concrete data, the intracellular Ca^{2+} buffering in the present model is attributed mainly to calmodulin within the cytosol and is represented as in our previous models of the atrial myocyte (42,48).

$$I_{\text{Ca_ATP}} = I_{\text{Ca_ATP_bar}} * (\text{Ca}_i / (\text{Ca}_i + k_{\text{Ca_ATP}})) \quad (12)$$

$$dO_c/dt = 200,000 * \text{Ca}_i * (1.0 - O_c) - 476.0 * O_c \quad (13)$$

$$d\text{Ca}_i/dt = (I_{\text{NaCa}} - I_{\text{Ca_ATP}}) / (\text{vol}_i * F) - 0.045 * dO_c/dt \quad (14)$$

Where XX is XX...

Model Implementation

Resting $[\text{Ca}^{2+}]_0$

In this first-order model, spatial variations in quantities of interest are not accounted for. As a result, the human articular chondrocyte can be modeled by the following set of ordinary differential equations (ODEs) in time:

$$\frac{d}{dt} \begin{pmatrix} V_m \\ [Na^+]_i \\ [K^+]_i \\ [Ca^{2+}]_i \\ [H^+]_i \\ [Cl^-]_i \\ a_{ur} \\ i_{ur} \end{pmatrix} = \begin{pmatrix} -I_r/C_m \\ -(I_{Na_i} + 3I_{NaK} + 3I_{NaCa} - I_{NaH})/(v_i F) \\ -(I_{K_p} - 2I_{NaK} + I_{K_m} + I_{K_{x,pote}} + I_{K_{Ca-act}})/(v_i F) \\ I_{NaCa}/(v_i F) \\ -I_{NaH}/(v_i F) \\ I_{Cl_b}/(v_i F) \\ (a_{ur\infty} - a_{ur})/\tau_{aur} \\ (i_{ur\infty} - i_{ur})/\tau_{i_{ur}} \end{pmatrix}$$

that is restricted
diffusion condition
extracellular in
accumulation

$$I_t = \underbrace{I_{K_{tr}} + I_{K_2 \text{ pore}} + I_{K_{Ca \cdot act}}}_{\text{Potassium currents}} \\ + \underbrace{I_{NaK} - I_{NaCa} + I_{NaH}}_{\text{Pumps and exchangers}} \\ + \underbrace{I_{Na_b} + I_{K_b} + I_{Cl_b}}_{\text{Background currents}}$$

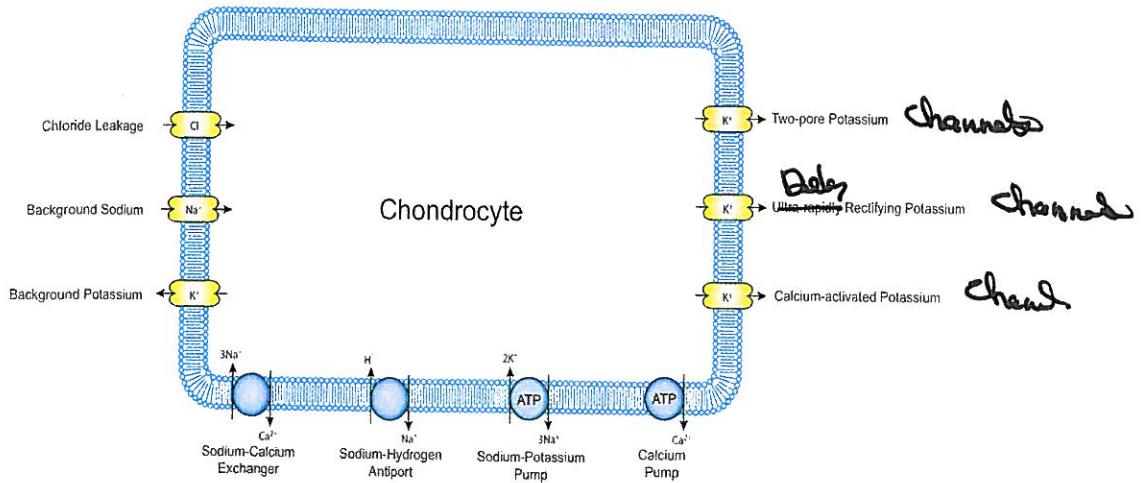
Where XX is XX...

The system of ordinary differential equations defined above was solved using LSODE (68). All initial conditions were chosen from previous calculations run to steady state. The corresponding

GNU Octave code is available freely and is open source.

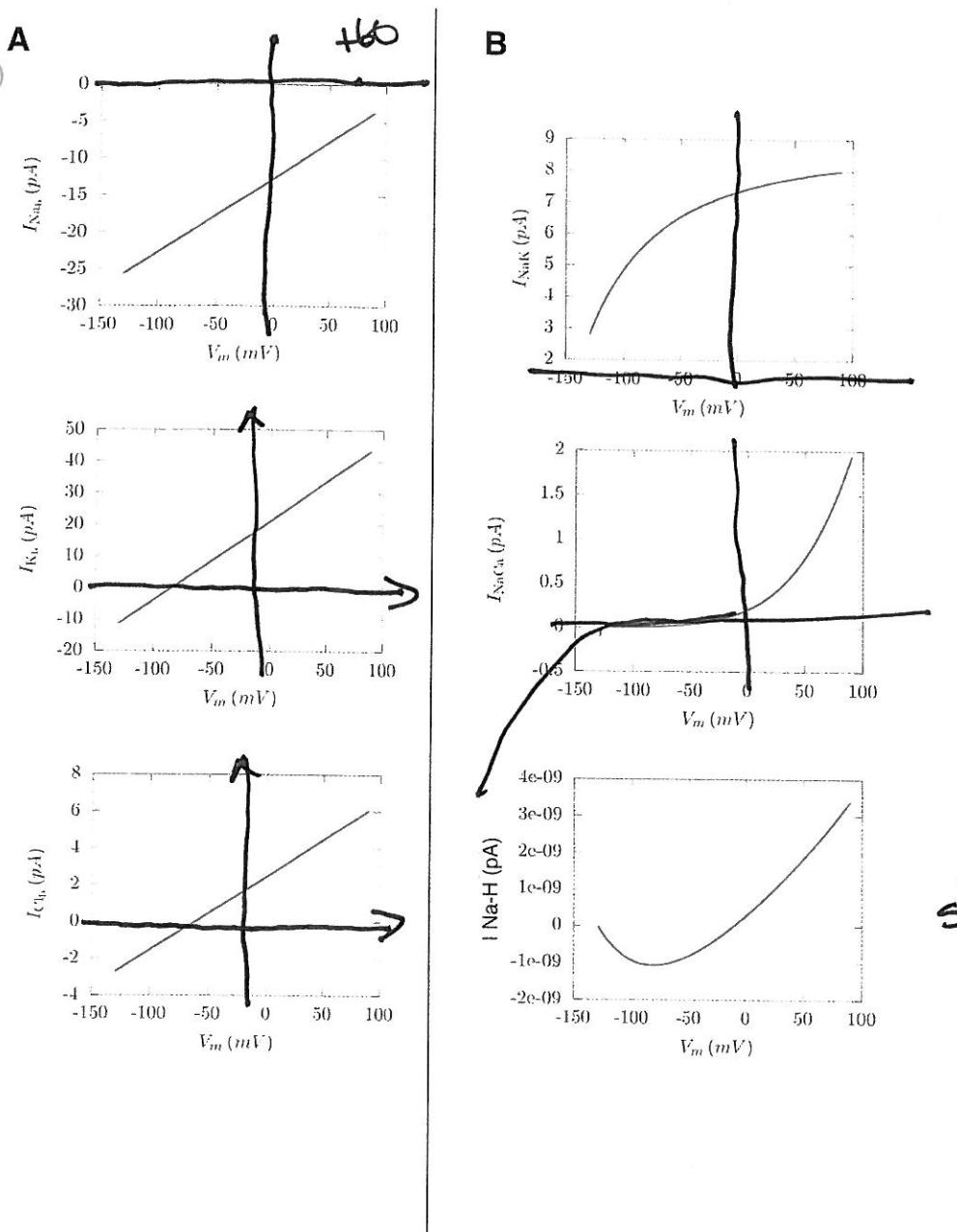
*that we'll
use*

Figure 1



All labels
30% larger.

Figure 2



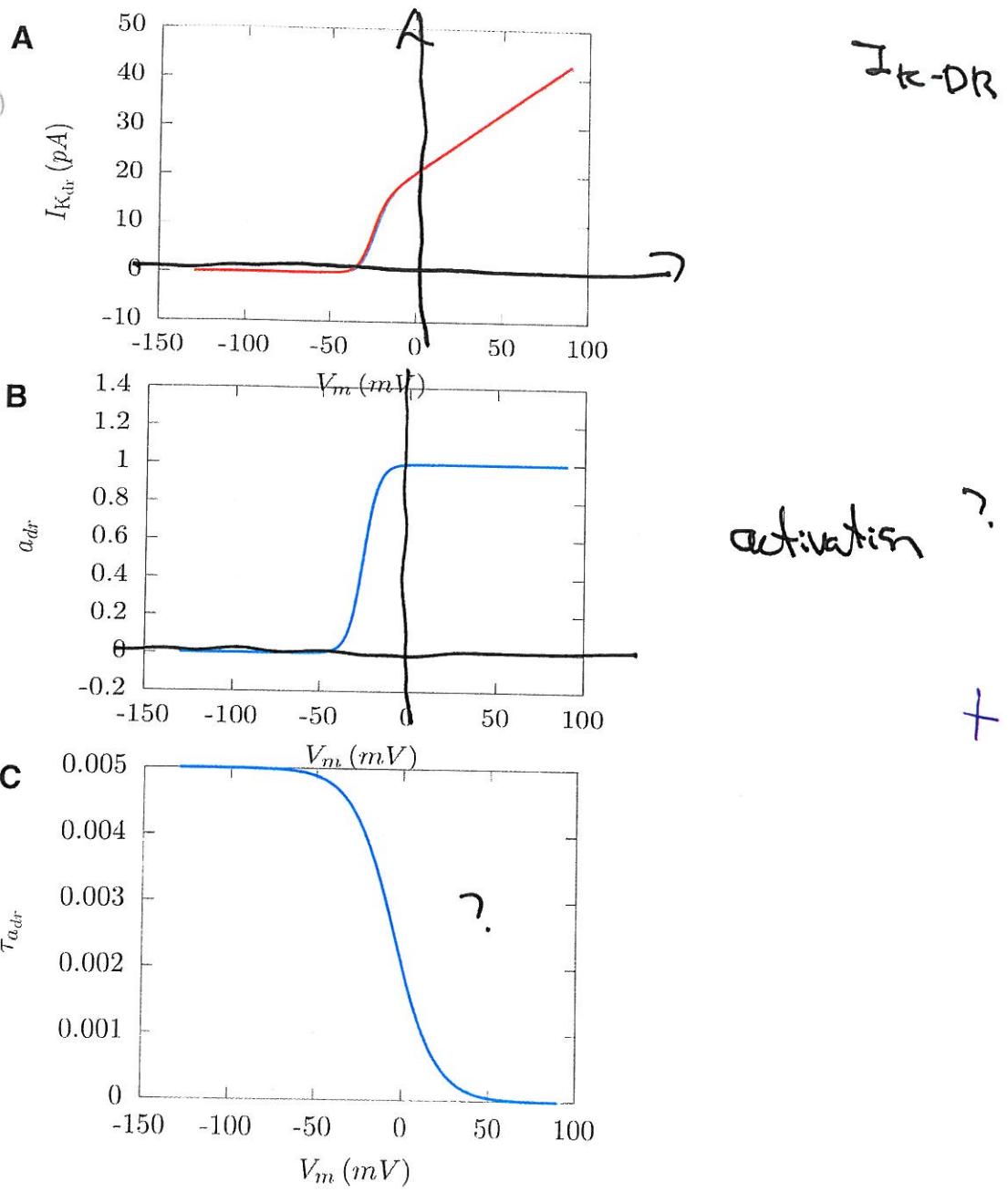
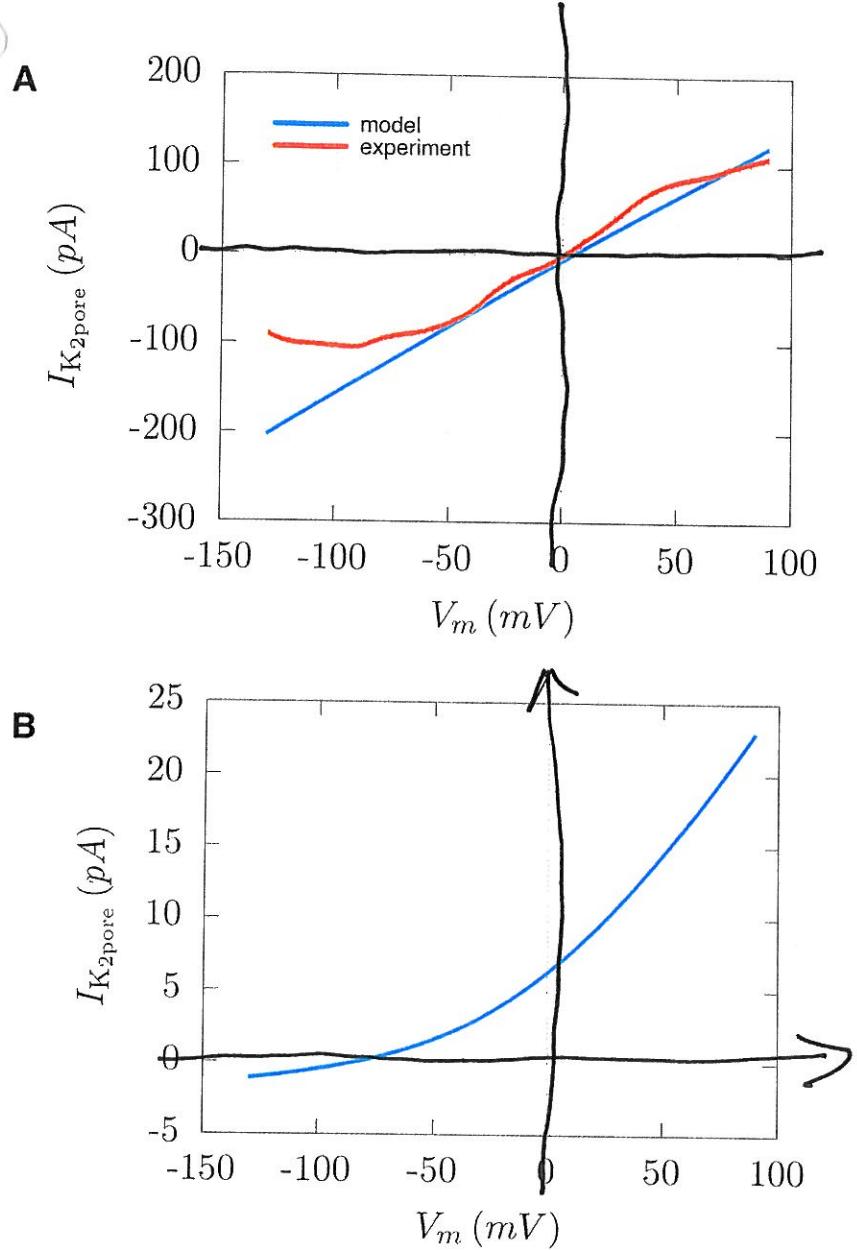


Figure 4

Experimental figure to be included.

Consolidated

Figure 5



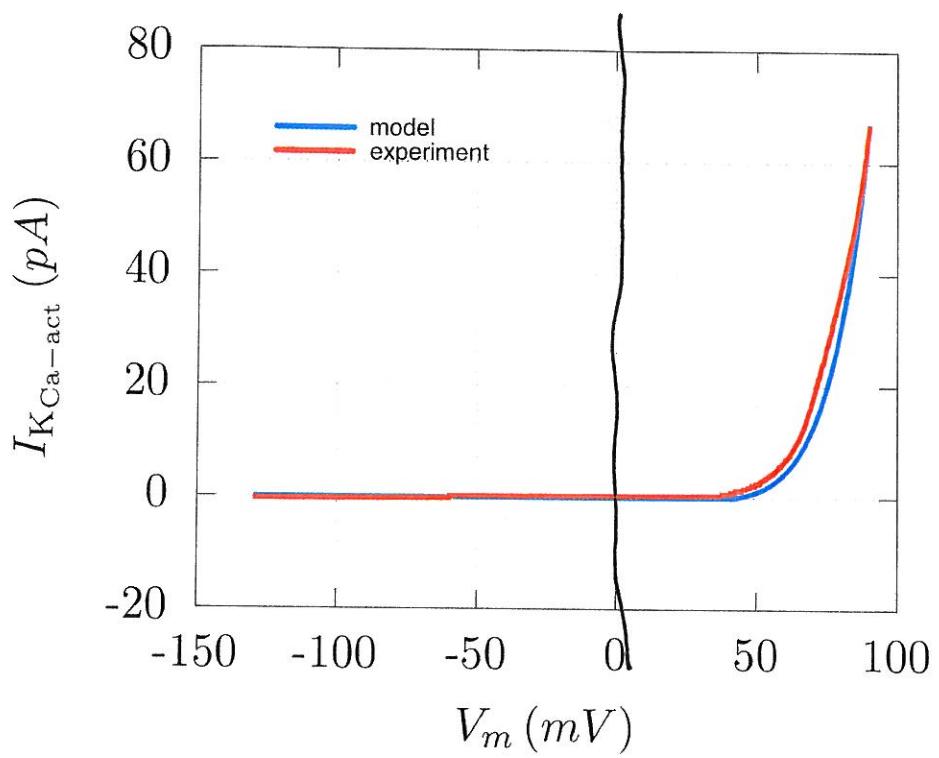
(2)

New Egg

A ERST

Lord Angel

Figure 6



at what $[Ca^{2+}]_i$

Figure 1. An illustration of the ion-selective channels included in the mathematical model of the chondrocyte.

Figure 2. Background & pump currents

- A. Background currents Na⁺, Ca²⁺, K⁺
- B. pump/exchanger currents
- C. Net current, A+B, measured slope resistance in range of 1-2 Gohms

Figure 3. I_K-DR

- A. IV curve, peak
- B. Activation curve
- C. Kinetics

Figure 4. I_K2pore (A, B, C, all experimental)

- A. peak IV
- B. peak IV with increased pH
- C. isotonic measurement data and "corrected" data

Figure 5. I_K2pore in computation and corrected experiment

Figure 6. Ca²⁺-activated K⁺ current, experimental and computed

Figure 7. ATP-sensitive K⁺ current, computed only

Figure 8. TRP4V channel, computed only

Figure 9. Whole-cell current-voltage relationship as compared to model results.

Figure 10. In Discussion; activation of TRPV4 influence on RMP

- A. Cell IV-relation including computational TRPV4 and without + experimental
-). Stretch increases the conductance 3-fold

Figure 7

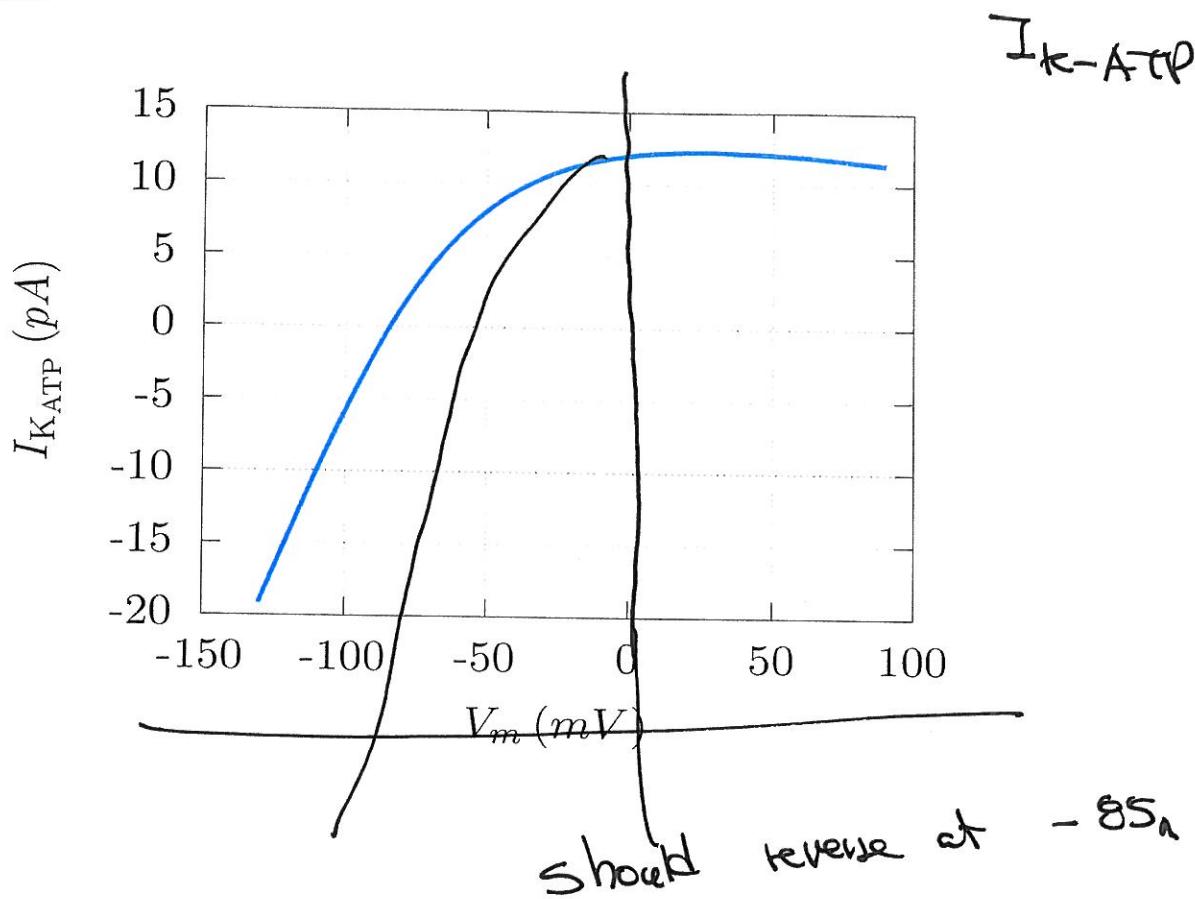
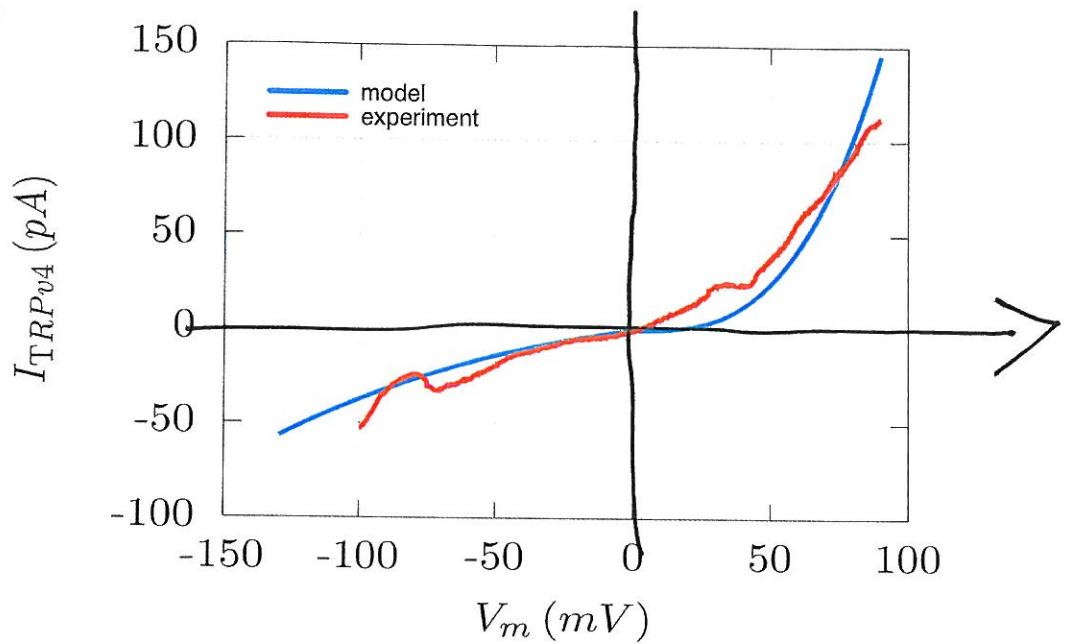


Figure 8



TRP V₄

Figure 9

Whole-cell current-voltage relationship as compared to model results.

?

A

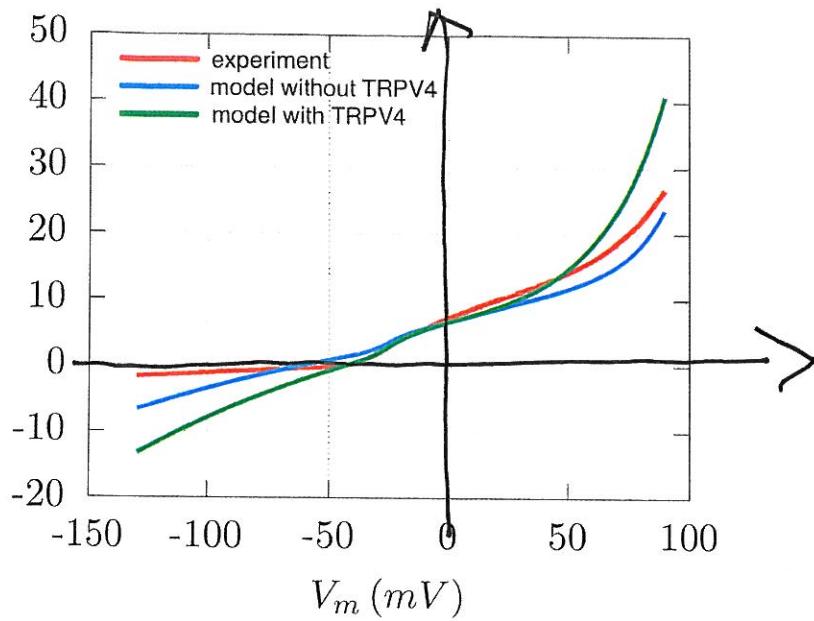
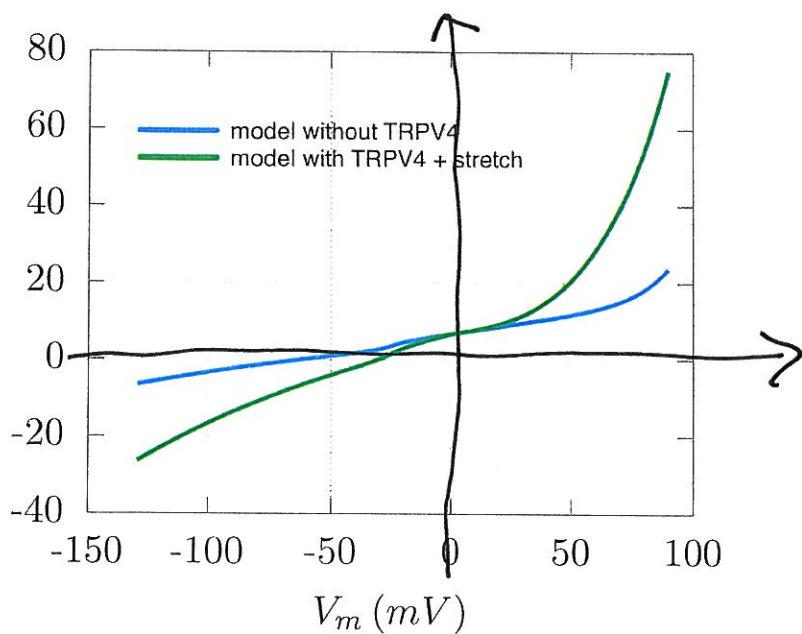


Fig. 16

B



APPENDIX:

Model of seal resistance re: membrane potential

Jim Wilson

The Role of K⁺ Currents in Human Articular Chondrocyte Electrophysiology:

A Computational Perspective

Results Section

Model Development / Methods

Figure 1 in the previous section includes a diagram of the major ion channel and ion transporter pathways that are incorporated into the initial version of this mathematical model of the resting membrane potential in the human chondrocyte. It is important to recall that the human chondrocyte functions as an isolated small cell. Significant biophysical parameters include the average whole cell capacitance, approximately 22 pF, and input resistance in normal physiological saline at room temperature of 2-5 Gohms.

Focuses on time-independent or electrogenic mechanisms. As indicated in the model development section, adult chondrocytes from a number of mammalian species show small but measurable time-independent background Cl⁻, Na⁺, and K⁺ conductances. Other than making measurements of membrane potential, and of input resistance, none of these ion transfer relationships are well characterized.

Accordingly, in Panel A, they are represented as ohmic or linear current-voltage (I-V) relationships, crossing the axis of the current-voltage relationship at their respective Nernst or electrochemical equilibrium potentials for Na⁺ (top), K⁺ (middle), and Cl⁻ (bottom). The three current voltage relationships in Panel B are scaled to the measured membrane capacitance while being taken from our previous models of atrial myocytes. In Panel B, the top current voltage I-V

and have been normalized to the human chondrocyte using the

relationship is ~~that~~ for the ~~the~~ electrogenic Na^+/K^+ pump, the middle ~~current~~- $I-V$ voltage relationship is ~~for~~ for the $\text{Na}^+/\text{Ca}^{2+}$ exchanger (at an intercellular $[\text{Ca}^{2+}]$ 10^{-8} M), and the bottom panel depicts the current-voltage relationship for the Na^+/H^+ ion exchanger. We note that the Na^+/H^+ ion exchanger is known to be electroneutral. In this diagram, the very small net current, which is generated in the physiological range of membrane potentials, is due to ~~xxxxx~~.

[Figure 2 near here]

$I_{\text{K-DR}}$

The results in Figure 3 illustrate the important functional parameters, which describe the ~~current-voltage~~ $I-V$ relationship or ion transfer mechanism (Panel A) for the delayed rectifier K^+ current, ~~which has been recorded in human and canine chondrocytes. The steady state voltage dependent activation relationship for this current is shown in Panel B and the corresponding steady state inactivation~~

~~kinetic parameters for it are summarized~~

~~and is illustrated in Panel C. Note that although based on this information, this~~

~~K^+ current is very small or negligible~~

~~at membrane potentials negative to -50 mV ; nevertheless, it is a prominent outward current at~~

~~membrane potentials positive to about -40 mV . As a consequence, it could contribute to or stabilize a relatively depolarized resting membrane potential or~~

$I_{\text{K-DR}}$

in principle

~~and does provide a significant outward repolarizing current following transient or~~

~~maintained depolarizing stimuli to the human articular chondrocyte. However, the~~

~~ability of this current to provide outward flux in the physiological range of~~

~~membrane potentials is regulated by very slow inactivation. Nevertheless, as~~

~~shown in Panel C, the current should be fully available (that is, inactivation is~~

~~almost completely removed) at any membrane potential negative to -50 mV .~~

Figure 3 near I_{DR}

a family of current traces

Figure 4 shows the corresponding raw data for this current. These recordings, as outlined in detail in the methods section, were obtained using conventional patch clamp recording methods with intercellular $[Ca^{2+}]$ buffered to approximately $10^{-8} M$ molar levels using EGTA. Note that this current, which we denote I-K_{DR} shows prominent time and voltage dependant activation with little if any sign of inactivation, even in response to prolonged and substantial depolarization.

[Figure 4 near I_{DR}]

and novel

An interesting new feature of the human chondrocyte (apparently in distinction to chondrocytes of other mammalian species) is the expression of a two-pore K⁺ channel mediated current. In our experiments, this current was identified under specialized physiological recording conditions, which were tailored to enhance the probabilities of identifying this small outward current. The data in Figure 5 shows a quasi-linear current voltage relationship recorded obtained in isotonic [K⁺] concentration. Note that this conductance is substantial in the entire physiological range of membrane potentials in the human chondrocyte. Its identity has a two-pore K⁺ conductance, in fact likely of the TASK-3 variety, was made based upon its enhancement with acidification of extracellular pH and its sensitivity to volatile local anaesthetics. These key identifiers have been described previously in our original publication on this current.

Fig SA do

Although the data in Panel A provided convincing evidence for the functional expression for a two-pore K⁺ current, they do not, however, provide any field.

significant information concerning its physiological role. This is because, as mentioned, these recordings were obtained under ~~conditions~~^{superficial with} of isotonic K^+ . The ~~comparative~~ data shown in ~~Figure 2~~^{Panel B} depict the same ion channel activation pattern, this time recorded ~~in~~^{in silico}. The current remains quasi-linear with some indication of a tendency for rectification, ~~that reverses at approximately -80 mV~~^{As expected the reversal potential is} very close to the predicted electrochemical equilibrium potential of K^+ under these recording conditions, ~~and that indeed does generate a small but significant outward current under physiological recording conditions.~~^{When adjusted or converted the 2-pore K^+ conductance can indeed}

The large input resistance of the human chondrocyte (see discussion section) ~~has the consequence that creates conditions under which even a very small net current can significantly alter resting membrane potential. This was demonstrated in our original study of this current and a corresponding simulation or plot of the two-pore K^+ current under physiological conditions is shown under Figure 6, together with the insight, which is previously published raw data. In summary, this novel two-pore K^+ channel appears to have properties such that it can be a major contributor to the resting potential and can also explain, at least in part, the known sensitivity of the chondrocyte membrane potential and intercellular Ca^{2+} homeostasis to alterations in extracellular pH and to responsiveness to volatile anaesthetic compounds.~~

[Figure 6 near here]

Perhaps the most consistent electrophysiological feature of the electrophysiological recordings that have been made from mammalian

chondrocytes is that these cells exhibit a significant Ca^{2+} activated K^+ current. It is now well known that the molecular correlates of Ca^{2+} activated K^+ channels can be divided into three sub-groups based on a number of different properties, characteristics, including the specific biophysical properties of the current under consideration: (1) (e.g. its voltage dependence), their pharmacological properties (e.g. sensitivity to blank by apamin or tetraethylammonium), or their single channel conductance. In the case of human chondrocytes, as shown in the inset of Figure 6, the pronounced current fluctuations or noise ~~are~~ strongly suggestive of the presence of the variant of Ca^{2+} activated K^+ channels known as the large conductance subtype.

The properties of this current and detailed formalisms for mathematical

simulation of it have been developed by Aldrich and Colleagues. We have used

this mathematical formalism to simulate the current-voltage relationship, which is

to have been recorded from isolated human chondrocytes under conditions in which the

composition of the pipette solution includes buffered intercellular $[\text{Ca}^{2+}]$ values

from approximately 10^{-8} M. In this setting Under this condition, this current is activated at only

very positive potentials. However, as intercellular $[\text{Ca}^{2+}]$ rises, the current is

activated at more negative potentials as shown reflected in Panel C of Figure 6. From

the data at hand, it is not possible to state in a critical way what the physiological

role of this Ca^{2+} activated K^+ current in the human chondrocyte is likely to be.

However, the properties, which we have identified and been able to accurately

model, would strongly suggest that it is a major repolarizing current but not one,

that contributes substantially to the resting potential. Perhaps the best evidence

for this can be put forward deduced by again recalling that the input resistance of that cell is

the zero potential)

(6)

resting membrane potential

very high. Under this circumstance, activation of a small number of these large Ca^{2+} activated K^+ channels would give rise to a resting potential that would be characterized by significant fluctuations in membrane voltage as opposed to what is found - a stable value in the range of -40 mV .

~~observed writing~~

Figure 7 now see

TRP

Results Cont'd

It is acknowledged that a major physiological role of the rathe chondrocyte is to synthesize and secrete basic collagen and ~~other~~ peptide substances (e.g. ATP and growth factors) by both constitutive and regulated mechanisms. This gives rise to important questions regarding the major cellular mechanisms for transmembrane Ca^{2+} entry, intracellular (Ca^{2+} release and buffering), and Ca^{2+} extrusion.

In this initial model we have addressed one of the important functional questions as follows: We note that in most isolated mammalian chondrocyte preparation a net current mediated by ligand or

(2)

Voltage-activated TRP channels can be identified. In the case of both mouse and human channels the predominant isoform appears to be ~~TRPV4~~ TRPV₄. This pH, stretch, and temperature sensitive ~~channel~~ current is carried by both Na⁺ and Ca²⁺ under physiological conditions. When activated the small net Ca²⁺ influx is ~~sufficient~~ sufficient to trigger a much larger release of ~~intracellular~~ Ca²⁺ from intracellular stores (endoplasmic reticulum). We have preliminary ~~metaphysiological~~ and ~~open~~ ~~current~~ data concerning TRP channel expression/function in the human channels.

(3)

(2-V zone)

This experimental data and a superimposed curve fit based on the mathematical model described by equation (x1) is shown in Figure 8

[Figure 8 now lost]

