Oct 9, 2012

Molly

I had some time at the week-end to do some more work on the chondrocyte report. My editorial comments are attached.

When working on this material the time I noted the following omissions:

1. There is no description (highlighted) of the chondrocyte:
   (a) size
   (b) capacitance
   (c) R_{ZN}
   (d) intracellular volume: (i) total (ii) minus nucleus, mitochondria and ER
2. There appears to be no formalism for cytosol Ca^{2+} buffering

3. No information was provided regarding the steady-state voltage dependence of the kinetics of the delayed rectifier. This was key because I believe the main ~~not~~ illustrations of the model paper will be showing alterations are a consequence of osmolarity, fixed charge, Zeta potential etc.

4. The 2 pore data may have been corrected for permeant ion concentration $[k^+]_o$ but the remaining unclear.

I will draft a speculative Results and Discussion section. We will need to speak by telephone this week. I suggest Wed. 9 a.m - 10th.

Oct 8, 2012 (1)

A Computational Approach for Studying Functional Role of K+ Currents in Human Articular Chondrocyte ~~Electrophysiology: a Computational Perspective~~

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ABSTRACT

~~**Abstract:**~~

We have developed a computational model for studying the electrophysiology of the human articular chondrocyte based on experimental data which has identified ~~novel~~ K+ current ~~expression~~. The utility of the model is illustrated by focusing on the role of a novel 2-pore K+ current in regulating the resting potential and therefore intracellular ion (e.g. Ca2+ homeostasis).

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

Our work is mainly this

modulating

ical principles which govern by functional families.

significant expression of a number of distinct and excitation-secretion coupling

PLAN FOR 2nd Draft:

Needs to be rewritten completely. For BioPhys J, the abstract takes the following form:

- One general intro sentence, setting up
- Problem statement
- What we are going to do about it (methods)
- What happened (results) (Most important)
- Conclusion

Introduction

Articular cartilage ~~is aneural, avascular, alymphatic~~, flexible connective tissue that covers the articulating ends of diarthroidal joints (2,1) and permits stability and movement of the skeleton. This connective tissue consists of an extracellular matrix (ECM, composed primarily of collagen, elastin and proteoglycans, as detailed below), ~~and~~ one type of cell--the *chondrocyte*--~~which~~ is responsible for synthesis and homeostasis of the matrix. Articular cartilage is regularly exposed to mechanical stresses, ~~and~~ this exposure is essential for the health of the tissue (3). Chondrocytes occupy only 1-10% of the total volume of articular cartilage in mammals (4,5) and play no direct mechanical role. Instead, mechanical support is provided by the ECM, which is composed of (a) collagen fibers, which gives the tissue the ability to resist tension, (b) negatively-charged gel-like proteoglycans (PGs) trapped within the collagen mesh, allowing the tissue to bear compression (6,1) and (c) synovial fluid within the articular capsule which acts as a lubricant, allowing for free movement of the bones (7). The chondrocyte thus resides in a physiologically atypical and dynamic environment. Its primary role is to maintain viable cartilage by balancing macromolecular synthesis and breakdown (see e.g. Wilkins et al. (8); Stockwell (3); Fassbender (9)).

Under abnormal conditions, chondrocyte damage may occur. As a result, the balance between matrix synthesis and degradation is lost, ~~causing~~ inflammation of the tissue and/or osteoarthritis: a thinning the cartilage layer which causes painful, bone-against-bone friction. It is ~~generally~~ known that the progression of osteoarthritis (Rush and Hall, 2003) and the ability of chondrocytes to respond to perturbations in the extracellular environment (Jones et al, 1999) is linked to ~~poorly~~ regulated volume changes (10); physical damage to cartilage is more frequent in the context of reduced osmolarity (Bushet et al, 2005). In turn, there is indication that these volume changes are linked to an abnormal resting membrane potential in these cells (10). In abnormal chondrocytes, the response to challenging external stimuli may be altered (e.g. much larger changes in resting membrane potential) as compared to healthy cells (Lewis et al, 2011; Wilson, et al 2004; Tsuga 2002; Tirabashi 2010a). It is likely that such changes in the regulation of the resting membrane potential are due to altered ion channel function (Lewis et al, 2011; WIlson, et al 2004; Tsuga 2002; Tirabashi 2010a). Direct experimental investigation of the links between chondrocyte electrophysiology and chondrotoxicity is complicated, however, by small cell size and the associated limitations of in vitro electrophysiological studies. For the purpose of integrating available data and attempting to understand its functional significance, we have developed a detailed biophysical model of chondrocyte electrophysiology. This detailed model, the first of its kind, will facilitate investigation of questions related to chondrocyte electrophysiology, intra- and intercellular signaling, and biomechanical sensitivity and transduction. The role of the chondrocyte in articular pathophysiology can also be considered.

Osteoarthritic changes may develop in even young patients following orthopedic surgery (cite) via chondrolysis, a condition in which accelerated loss of articular cartilage occurs over a short time period (Webb editorial, 2009). Several clinical studies have suggested that this significant chondrotoxicity can occur as a result of postoperative administration of bupivacaine, a local anesthetic (Busfield and Romero, 2009; Bailie and Ellenbecker, 2009; Rapley et al, 2009; Wiater, et al, 2011). Experimental work has confirmed that bupivacaine can cause profound chondrotoxic effects in both cell (11) and animal studies (Gomoll et al, 2006; Chu et al, 2010). However, the cellular and subcellular mechanisms leading to chondrotoxicity remain unclear. The family of two-pore K+ channels, recently identified in human articular chondrocytes (Clark, et al, manuscript) can be inhibited by bupivacaine application (Clark et al, manuscript; Punke et al 2003). We therefore hypothesized that the blockade of the two-pore K+ channel in human articular chondrocytes by the local anesthetic bupivacaine leads to compensated regulation of the resting membrane potential in these cells. The main goals of our work are (1) to develop the first detailed mathematical model of chondrocyte electrophysiology and, (2) to use this model to investigate the potential role of

bupivacaine in the homeostasis of the chondrocyte resting membrane potential.

~~Model and~~ Methods

Our model assumes a single chondrocyte is an isolated cell ~~residing~~ in deep regions of cartilage. This extracellular environment can be modeled by specifying external concentrations [Na+]o , [K+]o , [Ca2+]o, [H+]o and [Cl-]o within physiologically-relevant, slightly hyperosmotic ranges (see Table 1). The chondrocyte ~~cell~~ membrane is known to express a number of voltage- and ligand-gated ion channels as well as pumps and exchangers (12). The channels under consideration in this model are illustrated in Figure 1 and described in the following section.

Ionic current formulations

Note (W. Giles): State which family.

Needs some introductory text here. Point to the fact that the discussion contains other identified channels not explicitly modelled in this work.

Potassium ~~channels~~

Experimental results reported by Clark et al. (13) suggest that potassium ~~channels~~ play a dominant role in ~~controlling~~ the RMP of the human tibial joint articular chondrocyte. ~~Motivated~~ by this experimental work, our mathematical model incorporates the following three potassium channels.

Two-pore potassium channels. These are a set of widely-expressed K^+-selective channels. Their activation is largely independent of membrane potential. They play a vital role in determining the RMP of the human articular chondrocyte. Using the classic Hille-Goldman-Katz equation (ref) for voltage-gated, time-independent single-species ion channels, this current can be represented by:

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

Comment [MM1]: Reference for this is Hille's classic text, "Ion Channels of the Excitable Membrane"

and Figure 2a shows the current-voltage curve for this channel fit to experimental data (12)

Ca2+-activated K+ ~~channels~~. In chondrocytes from a number of different mammals, ~~the~~ so-called large-conductance Ca2+-activated K+ channels have been identified. Such channels are hypothesized to act as ``osmolytic channels'' and are responsible for decreasing intracellular osmotic strength by allowing efflux of potassium ions. This ~~effects~~ the ability of the chondrocyte to regulate its volume under rapid changes in the physiochemical environment (10). It has been suggested (12) that this type of K+ channel can be stretch-activated (stretch causes an increase in calcium influx, which results in markedly increased potassium current).

In the present formulation, we ignore the stretch dependence and model the (large) calcium-activated potassium channel using a functional form defined by Horrigan and Aldrich (15):

$$I_{K_{Ca-vf}} = N_{K_{Ca-vf}} P_0 G_{max} (V - E_K), \quad (2)$$

where,

$$kTe = 23.54\,(T/273),$$
$$L_v = L0 \exp((V Z_L)/kTe),$$
$$J_v = \exp(((V - Vh_j) Z_j)/Kate),$$
$$K = Ca_i/KDc,$$
$$P_0 = \frac{L_v(1 + KC - J_v D + J_v KC DE)^4}{L_v(1 + KC + J_v D + J_v KC DE)^4 + (1 \quad J_v + K + J_v KE)^4},$$
$$E_K = \frac{RT}{z_K F} \ln\left(\frac{[K^+]_o}{[K^-]_i}\right).$$

Figure 2b shows the current-voltage relationship for this channel fit to experimental data (13).

Delayed rectifier K+ Current. A markedly time- and voltage-dependent or delayed-rectifier has been identified in chondrocytes (17,18,16). These usually repolarize active cells following action potentials, but their role in chondrocytes are not known because chondrocytes are far more depolarised. Previous studies have identified Kv 1.4 and 1.6 (18,19). In this work, the mathematical expression for the delayed rectifier is motivated by the slow-rapidly rectifying potassium channel (20):

$$I_{K_{ur}} = g_{K_{ur}} a_{ur} i_{ur} (V - E_K), \quad (3)$$

where aur and iur are time-dependent channel activation and inactivation, and are defined in the model via the following expression:

$$E_K = \frac{RT}{z_K F} \ln\left(\frac{[K^+]_o}{[K^+]_i}\right),$$
$$a_{ur_\infty} = \frac{1}{1 + \exp(-(V_{ai} - 6.0)/8.6)},$$
$$i_{ur_\infty} = \frac{1}{1 + \exp(-(V_{ai} - 7.5)/10.0)} + 0.7,$$
$$\tau_{a_{ur}} = \frac{0.009}{1 + \exp((V + 5.0)/12.0)} + 0.0005,$$
$$\tau_{i_{ur}} = \frac{0.5}{1 + \exp((V + 60.0)/20.0)} + 6.$$

Figure 2c shows the voltage-current curve for this channel fit to experimental data (13) and Figure 2d shows the time-current curve over the same period.

Ion Pumps and Exchangers

Na+ / K+ Pump

~~Sodium-potassium pump~~

The ~~effective expulsion~~ of Na+ ~~ions~~ from the ~~cell~~ is achieved by the ~~activity~~ of ~~the~~ Na+-K+ -ATPase. ~~and 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 from Nygren et al. (22):

$$I_{NaK} = \bar{I}_{NaK}\left(\frac{[K^+]_o}{[K^+]_o + k_{NaK_K}}\right)\left(\frac{[Na^-]_i^{1.5}}{[Na^+]_i^{1.5} + k_{NaK_{Na}}^{1.5}}\right)\left(\frac{V+150}{V+200}\right) \quad (4)$$

Figure X shows a representative current-voltage curve for this electrogenic oump.

Na+ / Ca2+

~~Sodium-calcium~~ exchanger

As in many other cell types, the sodium-calcium exchanger plays a key role in Ca2+ homeostasis in articular chondrocytes (ref). Here, we model this ~~channel~~ using the following mathematical expression (22):

$$I_{NaCa} = k_{NaCa}\frac{[Na^+]_i^3[Ca^{2+}]_o \exp(\frac{\gamma VF}{RT}) - [Na^+]_o^3[Ca^{2+}]_i \exp(\frac{(\gamma-1.0)VF}{RT})}{1.0 - d_{NaCa}([Na^+]_o^3[Ca^{2+}]_i + [Na^+]_i^3[Ca^{2+}]_o)} \quad (5)$$

Na+ / H+

~~Sodium-hydrogen~~ exchanger

Mammalian Na+ / H+

Chondrocytes ~~possess~~ a ~~sodium-hydrogen~~ **antiporter** (8,5) which ~~allows~~ the cell to sense extracellular pH. In order to model this channel, we use the following functional form described in Cha et al. (23):

$$I_{NaH} = N_{NaH} I_{NaH_{mod}} I_{NaH_{exch}} \quad (6)$$

where,

$$I_{NaH_{mod}} = \frac{1}{1 + (K_i^{nH}/[H^-]_i^{nH})},$$

$$t_1 = \frac{k_1^+[Na^+]_o/K_{Na}^o}{(1 + [Na^-]_o/K_{Na}^o + [H^+]_o/K_H^o)}$$

$$t_2 = \frac{k_2^+[H^+]_i/K_H^i}{(1 + [Na^-]_i/K_{Na}^i + [H^+]_i/K_H^i)}$$

$$t_3 = \frac{k_1^-[Na^+]_i/K_{Na}^i}{(1 + [Na^-]_i/K_{Na}^i + [H^+]_i/K_H^i)}$$

$$t_4 = \frac{k_2^-[H^+]_o/K_H^o}{(1 + [Na^-]_o/K_{Na}^o + [H^+]_o/K_H^o)},$$

$$I_{NaH_{exch}} = \frac{(t_1 t_2 - t_3 t_4)}{(t_1 + t_2 + t_3 + t_4)}. \quad (7)$$

3x larger

Background Leakage Currents

~~The~~ model accounts for background leakage of Na+ and K+ ~~through the~~ use of time-independent channels whose mathematical expressions are based on ~~Hodgkin-Huxley~~ formalism:

$$I_{Na_b} = \bar{g}_{Na_b}(V_m - E_{Na}),$$
$$I_{K_b} = \bar{g}_{K_b}(V_m - E_K). \quad (8)$$

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

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

~~This model can account~~ for chloride leakage using a similar mathematical formulation:

$$I_{Cl_b} = \bar{g}_{Cl_b}(V_m - E_{Cl}), \quad (9)$$

where

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

is the Nernst potential set up by the difference in Cl- concentration inside and outside the cell.

The atypical environment of the chondrocyte

As a result of being deep within cartilaginous tissue, the ~~chondrocyte's~~ extracellular environment is ~~unique in comparison with~~ other cell types. The high number of fixed negative charges on immediately adjacent proteoglycans attracts free cations (e.g. Na+) and excludes free anions from ~~the~~ matrix. As a results of this cation accumulation, water ~~osmotically~~ enters. The ~~resulting~~ ionic strength is in the range of XX osmoles. It is also known that the pH in this microenvironment is somewhat acidic in comparison with other extracellular environments (24,8).

As the tissue is avascular, synovial fluid supplies adult articular cartilage with small amounts of nutrients as well as oxygen, and byproducts are removed by diffusion (24,25). Due to the avascular nature of the resident tissue, chondrocytes generate ATP by substrate-level phosphorylation during

anaerobic respiration. This generates H+ ions as a byproduct, which further lowers surrounding pH (24). Mechanical loading during activity also exposes chondrocytes to profound fluctuations in their physiochemical environment (27,26).

This atypical environment is reflected in measurements taken in tissue samples (see Table 1). Experimentally-reported values for the external concentrations of different species reveal some marked extremity in cation concentrations compared to e.g. cardiac tissue. We have utilized these extracellular concentrations in our model in concert with measured values from (13) to characterize the extracellular environment of the chondrocyte in our model.

Table 1: Consensus Values of Ion Concentrations for Chondrocyte Electrophysiology (8).

	Cytoplasm	Matrix	Serum/Synovium
$[Na^+]$ (mM)	40	240-350	140
$[K^+]$ (mM)	120-140	7-12	5
$[Ca^{2-}]$ (mM)	8.e-5	6-15	1.5
$[Cl^-]$ (mM)	60-90	60-100	140
$[HCO_3]$ (mM)	20	15	23
$[SO_4^2]$ (mM)	0.17	0.30	0.81
pH (mM)	7.1	6.6-6.9	7.4
Osmolarity (mOsm)	--	350-450	300

Comment [MM2]: Table will be remade; bad formatting a result of translation from LaTex format.

~~Note from Wayne: Need a transition to next section.~~

Elements of the

Mathematical Model of Chondrocyte Electrophysiology

Comment [MM3]: Comment from Wayne: This section is key and needs an appropriate introduction.

The individual currents above are defined by Equations 2-9, and the ODE system (10) is solved for the primary vector of unknowns: Vm, [Na+]i , [K+]i , [Ca2+]i , [H+]i , [Cl-]i , aur , and iur . ~~In the time period of the numerical experiment~~. The initial conditions are chosen from previous calculations run to steady state. ~~The equation~~ system is solved using LSODE (28),. ~~and~~ the corresponding GNU Octave code is available free and open source ~~for anyone~~ to use and ~~extend~~ (12).

modify / extend

This & equation

We assume that there are no spatial variations in quantities of interest, allowing us to model the chondrocyte as the following set of ordinary differential equations (ODEs) in time.

$$\frac{d}{dt}\begin{pmatrix} V_m \\ [Na^+]_i \\ [K^+]_i \\ [Ca^{2+}]_i \\ [H^+]_i \\ [Cl^-]_i \\ a_{ur} \\ i_{ur} \end{pmatrix} = \begin{pmatrix} -I_i/C_m \\ -(I_{Na_b} + 3I_{NaK} + 3I_{NaCa} - I_{NaH})/(v_i F) \\ -(I_{K_b} - 2I_{NaK} + I_{K_{ur}} + I_{K_{2pore}} + I_{K_{Ca-act}})/(v_i F) \\ I_{NaCa}/(v_i F) \\ -I_{NaH}/(v_i F) \\ I_{Cl_b}/(v_i F) \\ (a_{ur_\infty} - a_{ur})/\tau_{a_{ur}} \\ (i_{ur_\infty} - i_{ur})/\tau_{i_{ur}} \end{pmatrix} \quad (10)$$

Significant spatial variation (intra- or extra cellular subcompart

3x larger.

where,

$$I_i = \underbrace{I_{K_{ur}} + I_{K_{2pore}} + I_{K_{Ca-act}}}_{\text{Potassium currents}} + \underbrace{I_{NaK} + I_{NaCa} + I_{NaH}}_{\text{Pumps and exchangers}} + \underbrace{I_{Na_b} - I_{K_b} - I_{Cl_b}}_{\text{Background currents}}$$

But need to note & exclude
- mitochondrial volume
- volume of nucleus

Figure Legends

Figure 1.

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

Figure 2.

Potassium current-voltage relationships which have been fit to experimental values (in red) from Clark et al. (13). The external concentrations correspond to the experimental conditions: [K+]o = 5 mM, [Na+]o = 140 mM, [Ca2+]o = 2 mM, pH = 7.4, except for IK2pore , where [K+]o = 145 mM, pH = 8.5.

Comment [MM4]: This was to match experimental conditions – the visualization shows correspondence to experiment, but the modeled channel certainly does not have a reversal potential around 0mV.

Figure 3.

I-V relations for the other currents. These are not fit to experimental data, but used to tune simulation results.

Figure 4.

Overall behaviour of the model when voltage is ramped from -130 mV to +90 mV in 1 s. The model output closely matches experimental data (red) from Clark et al. (13).

Figure 5.

Comment [MM5]: Will move to Appendix.

Time-evolution of the concentrations over 1800 s to show that the initial conditions we have chosen for the model were at steady state. The initial conditions for the concentrations used in the computations were [Na+]i = 2.814 mM, [K+]i = 121.59 mM, [Ca2+]i = 2.371e-06 mM, [H+]i = 6.188e-10 mM, [Cl-]i = 13.209 mM.

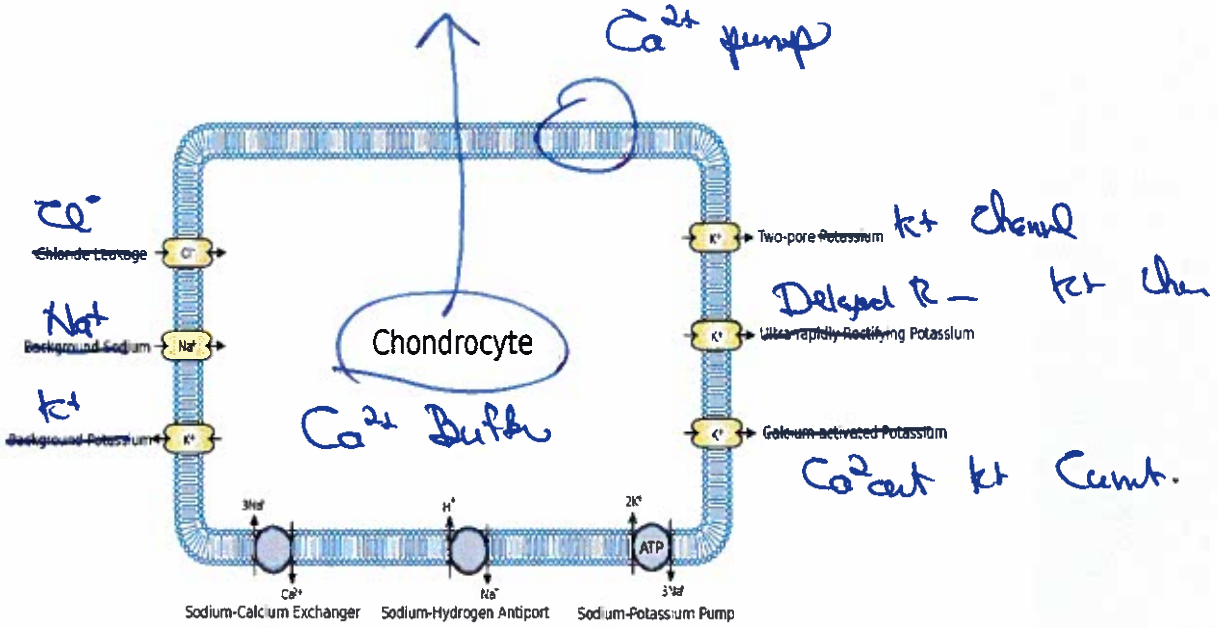
Figure 6

Evolution of the resting membrane potential with varying external potassium concentration. Note that while it is slightly more positive than experiment, it matches the qualitative behaviour quite closely (13).

Figure 7.

When the amount of IK2pore is varied from 100% to 0% (by blocking with increasing amounts of BUP), the RMP increases. These simulations were carried out at two different values of external concentrations [K+]o = 5 mM and [K+]o = 25 mM and results compare favorably with experimental data (13, Fig. 8B).

Figure 1:



Cl-

Na+

K+

Ca^{2+} pump

Ca^{2+} Buffer

Two-pore Potassium K+ channel

Delayed R— K+ Chan

Ultra-rapidly Rectifying Potassium

Calcium-activated Potassium

Ca^{2+} act K+ Count.

Sodium-Calcium Exchanger Na+/Ca2

Sodium-Hydrogen Antiport Na+/H+

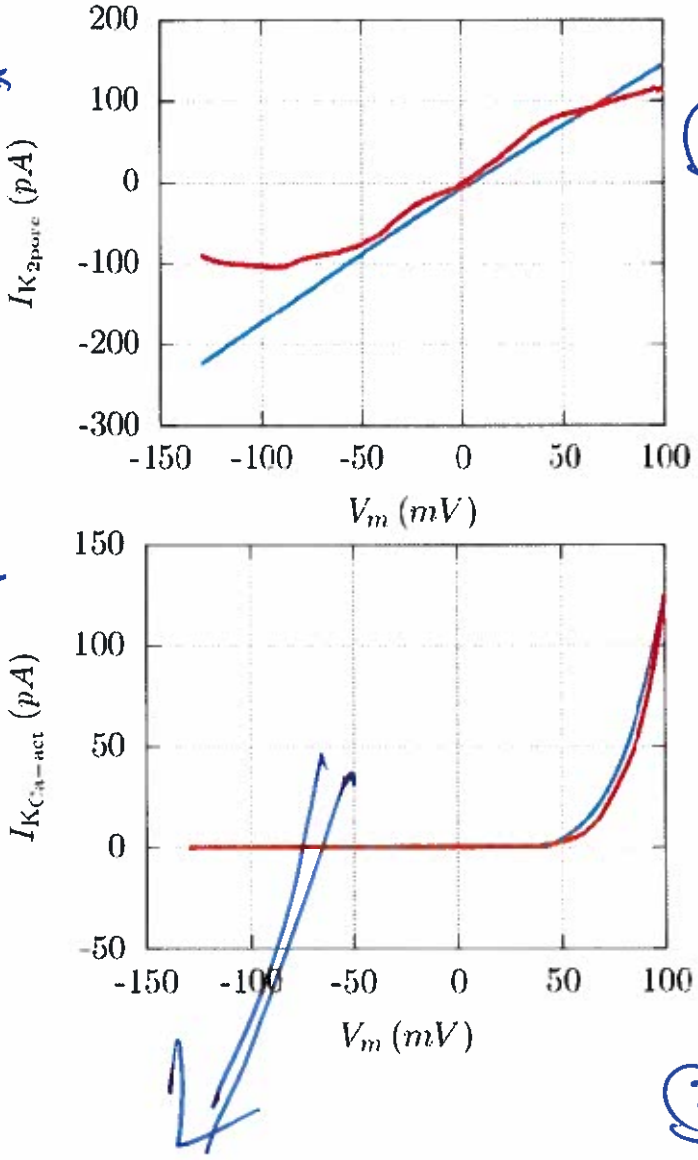
Sodium-Potassium Pump Na+/K+

Figure 1.

repeat Figure title and define terms.

(b)

Figure 2:



(2) The delayed Rectifier and 2-P current need to be separated

A. Delayed Rect
A I-V
B activ
inact
C kinetics.

(3) B 2-Pore

A. High $(Ca^{2+})_o$

B. Normal $(Ca^{2+})_o$

(4) 2_{K-Ca}
at what $[Ca^{2+}]_i$
How is $[Ca^{2+}]_i$
buffered.

(c)

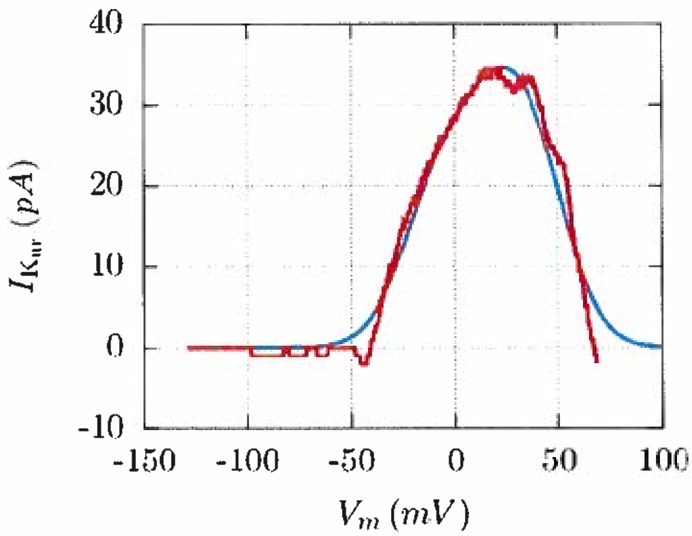


Fig 5

What 'other currents' need

I_{ex} at which $(Ca^{2+})_c$

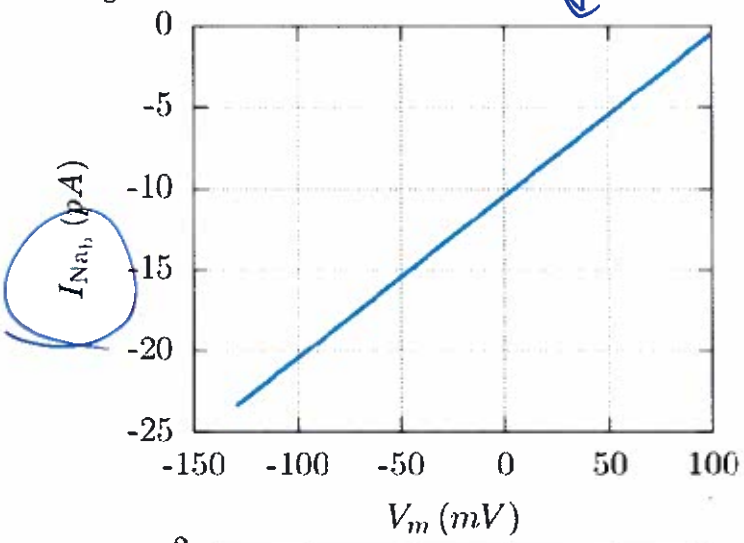
I_{pump} at which $(Na^+)_i$

(d)

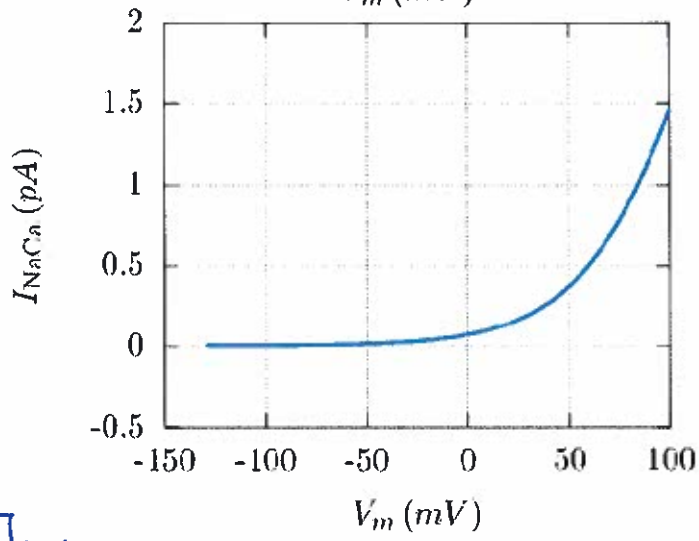
Expose as Current Density

b.

Figure 4.

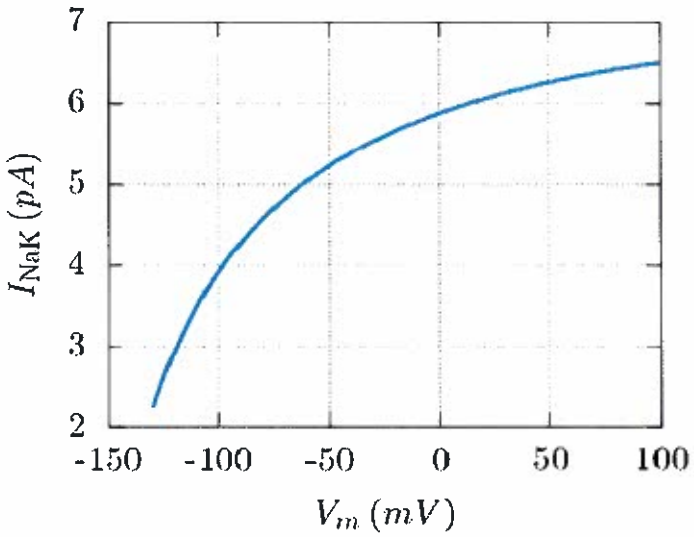


This should have a E_{rev} about +30 mV



= Separate Figure

Jkl.



Show at

$(Na^+)_i$ = 10 mM

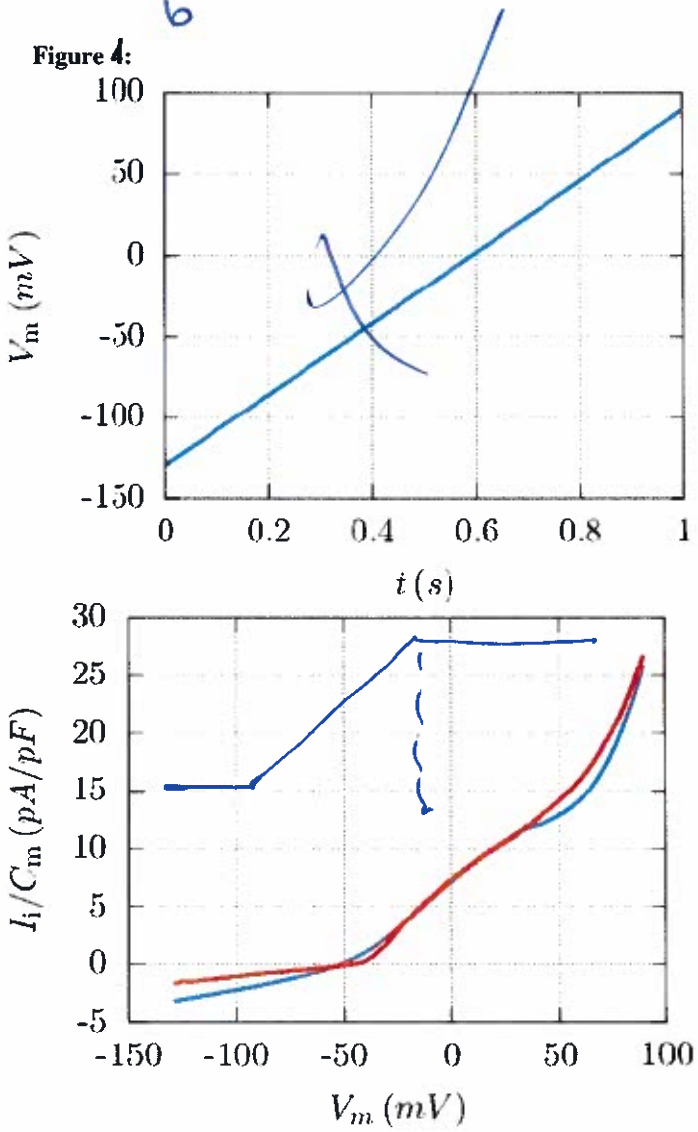
20 mM

40 mM

(f.)

6

Figure 4:



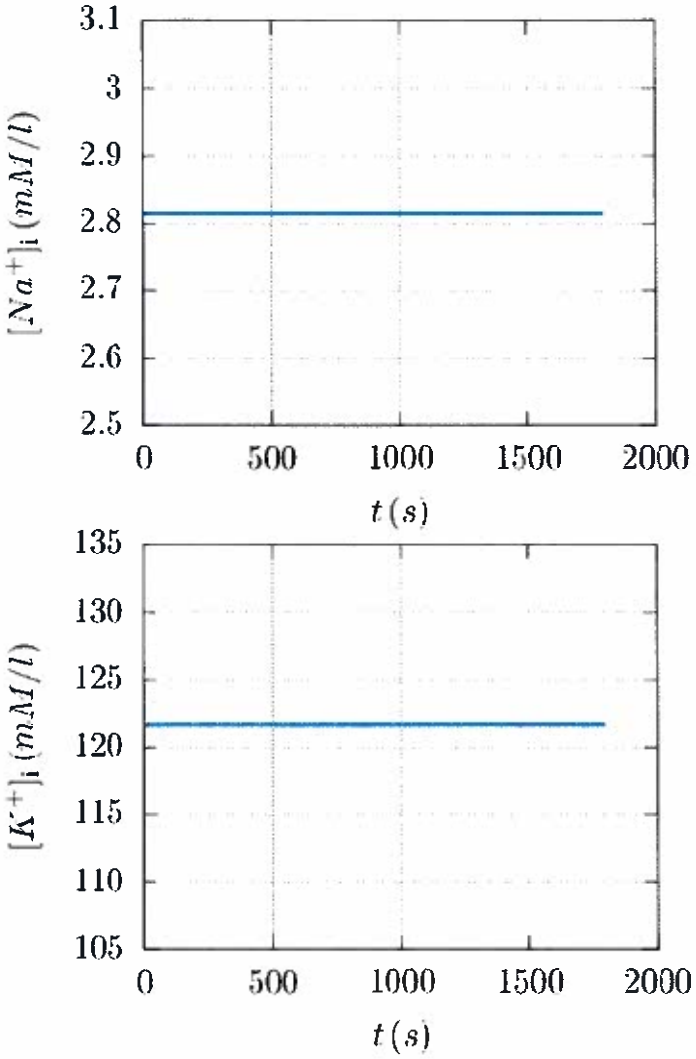
should show voltage to E_R as well

Figure 1

Supplem. / Appendix

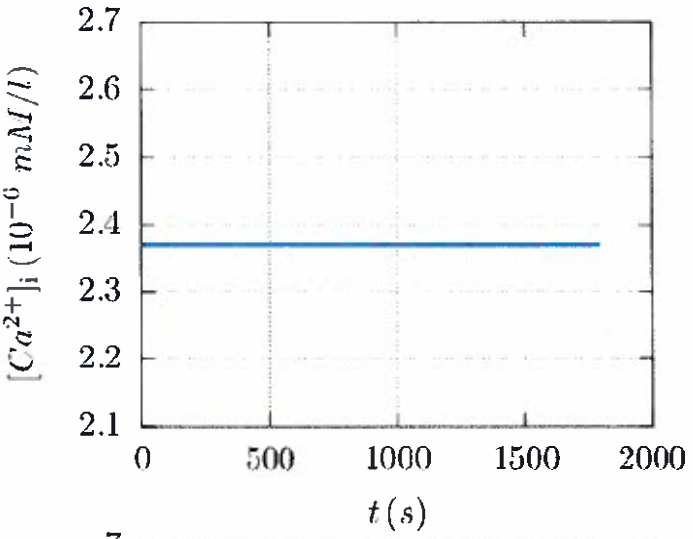
Figure 5:

Comment [MM6]: Will be moved to an appendix.



(h)

Figure 1 Appendix | Sample



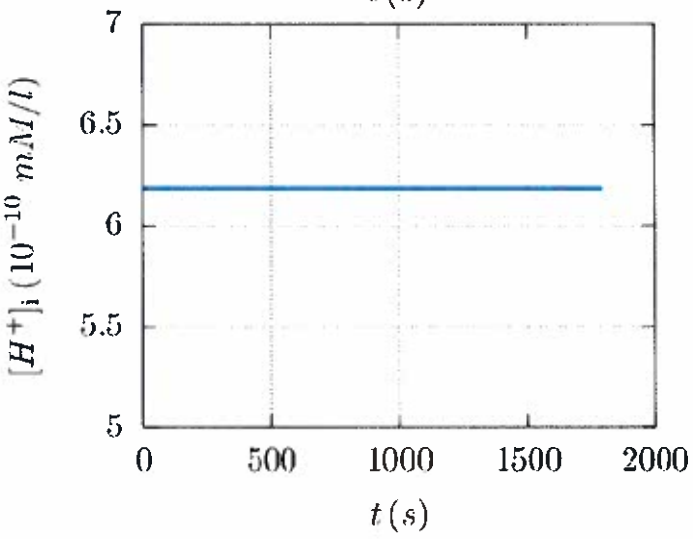
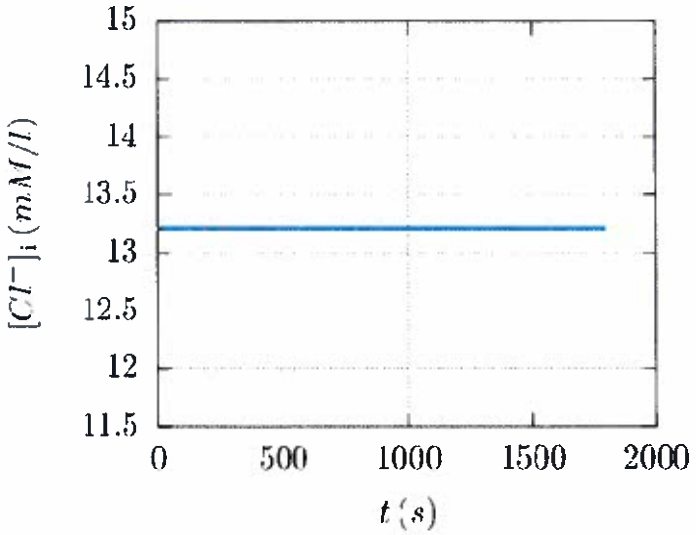


Figure 1

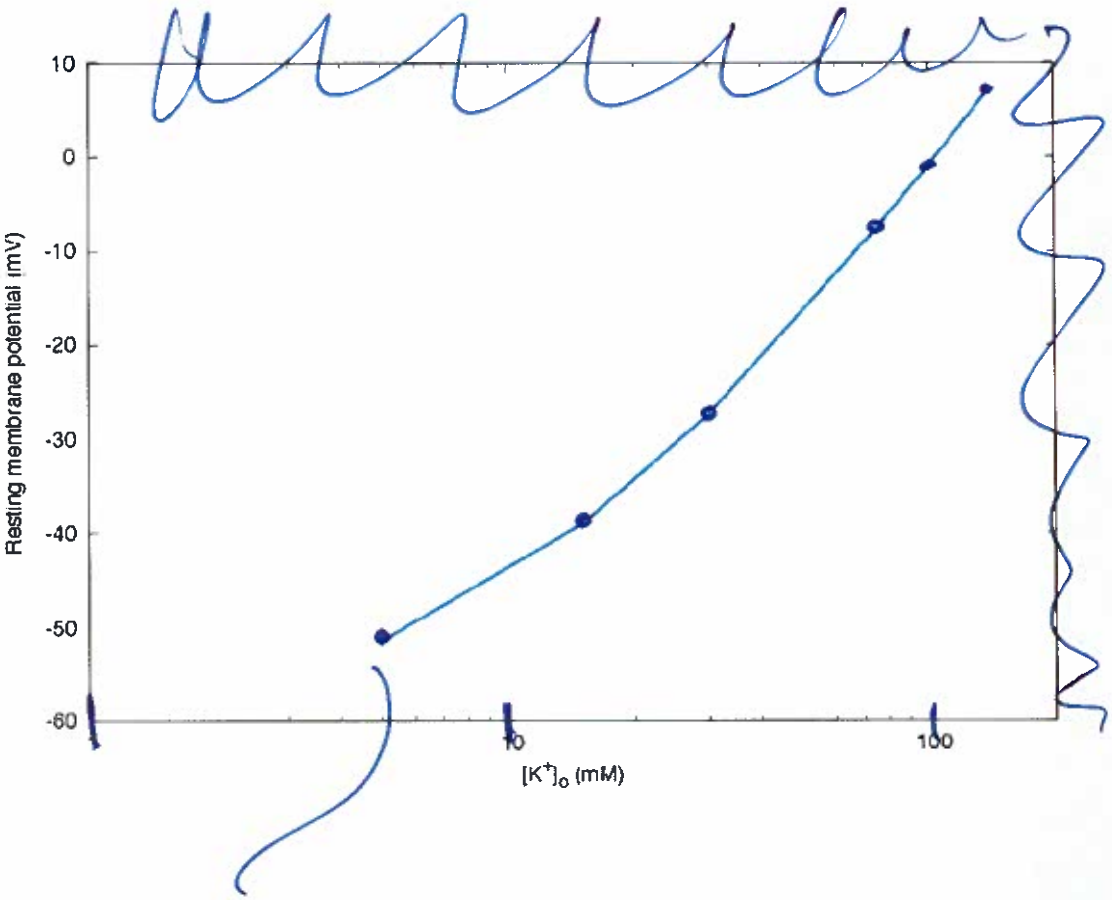
Screenshot



(j)

7

Figure 6



Are these data points. Either or the computed values shown.

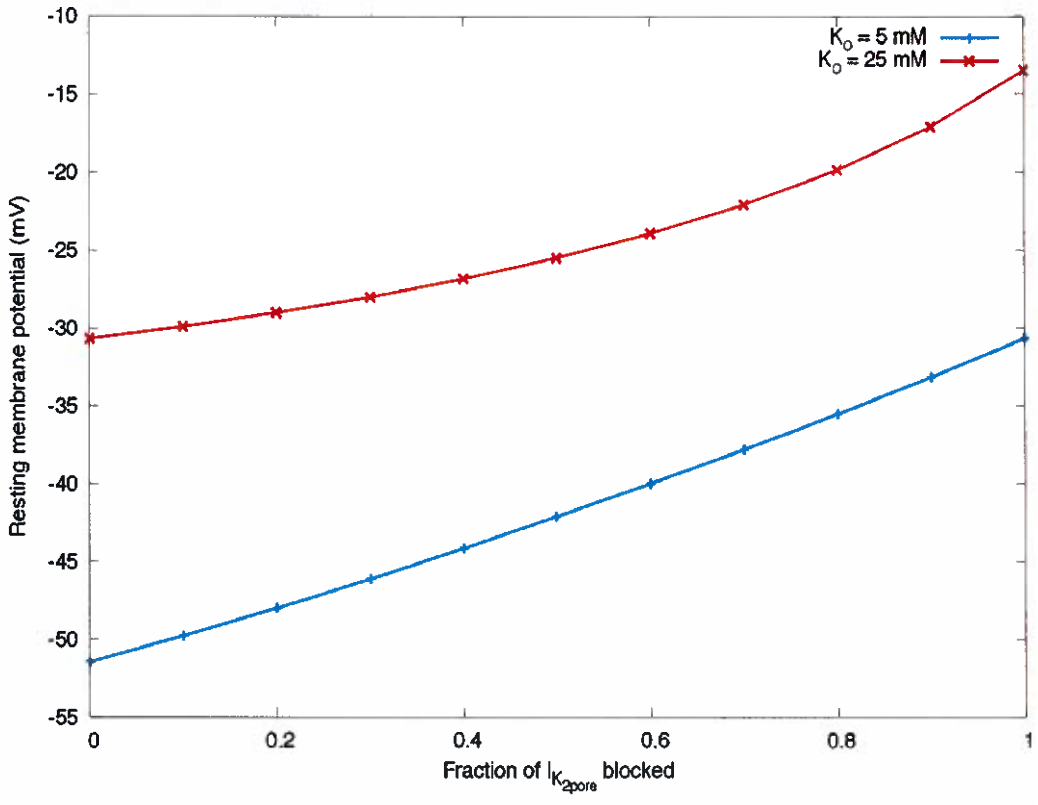
Is the due to 2-P tx or Delayed Rect or Both

Either as the saturation of the electrogenic pump

I_{pump} R_{24} $= \Delta V$.

8.

Figure 4:



Bupivocaine. Blkd.

2 don't see the point of the 25mM $[K^+]_o$ data.

What are the consequences for $[Ca^{2+}]_i$ and/or $[Na^+]_i$?

Footnotes

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Harish Narayanan 2012-10-05

REFERENCES:

~~Bibliography~~

1

Poole, C. A., 1997.
Articular cartilage chondrons: form, function and failure.
J. Anat. 191:1-13.

2

Mankin, H. J., 1982.
The response of articular cartilage to mechanical injury.
J. Bone Joint Surg. Am. 64:460-466.

3

Stockwell, R. A., 1991.
Cartilage failure in osteoarthritis: Relevance of normal structure and function. A review.
Clin. Anat. 4:161-191.

4

Carney, S. L., and H. Muir, 1988.
The structure and function of cartilage proteoglycans.
Physiol. Rev. 68:858-910.

5

Hall, A. C., E. R. Horwitz, and R. J. Wilkins, 1996.
The cellular physiology of articular cartilage.
Exp. Physiol. 81:535-545.

6

Buckwalter, J. A., and H. J. Mankin, 1998.
Articular cartilage: Tissue design and chondrocyte-matrix interactions.
Instr. Course Lect. 47:477-486.

7

Edwards, J. C., L. S. Wilkinson, H. M. Jones, P. Soothill, K. J. Henderson, J. G. Worrall, and A. A. Pitsillides, 1994.
The formation of human synovial joint cavities: A possible role for hyaluronan and CD44 in altered interzone cohesion.
J. Anat. 185:355-367.

8

Wilkins, R. J., J. A. Browning, and J. C. Ellory, 2000.
Surviving in a Matrix: Membrane Transport in Articular Chondrocytes.
J. Membrane Biol. 177:95-108.

9

Fassbender, H. G., 1987.
Role of chondrocytes in the development of osteoarthritis.
Am. J. Med. 83:17-24.

10

Lewis, R., K. E. Asplin, G. Bruce, C. Dart, A. Mobasheri, and R. Barrett-Jolley, 2011.

remove caps.

The role of the membrane potential in chondrocyte volume regulation.
J. Cell. Physiol. xx:mm-nn.

11

Chu, C. R., N. J. Izzo, N. E. Papas, and F. H. Fu, 2006.
In Vitro Exposure to 0.5% Bupivacaine Is Cytotoxic to Bovine Articular Chondrocytes.
J. Arthroscopy 22:693-699.

remove copy.

12

Unknown, A., yyyy.
This is an unknown article.
J. Unknown xx:mm-nn.

13

Clark, R. B., C. Kondo, and W. R. Giles, 2011.
Two-pore K^+ channels contribute to membrane potential of isolated human articular chondrocytes.
J. Physiol. xx:mm-nn.

14

Barrett-Jolley, R., R. Lewis, R. Fallman, and A. Mobasheri, 2010.
The emerging chondrocyte channelome.
Front. Physiol. 1:mm-nn.

15

remove copy.

Horrigan, F. T., and R. W. Aldrich, 2002.
Coupling between Voltage Sensor Activation, Ca^{2+} Binding and Channel Opening in Large Conductance (BK) Potassium Channels.
J. Gen. Physiol. 120:267-305.

16

Walsh, K. B., S. D. Cannon, and R. E. Wuthier, 1992.
Characterization of a delayed rectifier potassium current in chicken growth plate chondrocytes.
Am. J. Physiol. 262:C1335-C1340.

17

Sugimoto, T., M. Yoshino, M. Nagao, S. Ishii, and H. Yabu, 1996.
Voltage-Gated Ionic Channels in Cultured Rabbit Articular Chondrocytes.
Comp. Biochem. Physiol. 115C:223-232.

remove copy.

18

Mobasheri, A., T. C. Gent, M. D. Womack, S. D. Carter, P. D. Clegg, and R. Barrett-Jolley, 2005.
Quantitative analysis of voltage-gated potassium currents from primary equine (Equus caballus) and elephant (Loxodonta africana) articular chondrocytes.
Am. J. Physiol. Regul. Integr. Comp. Physiol. 289:R172-R180.

19

Clark, R. B., N. Hatano, C. Kondo, D. D. Belke, B. S. Brown, S. Kumar, B. J. Votta, and W. R. Giles, 2010.
Voltage-gated K+ currents in mouse articular chondrocytes regulate membrane potential.

Channels 4:179-191.

20

Maleckar, M. M., J. L. Greenstein, W. R. Giles, and N. A. Trayanova, 2009.
K^+ current changes account for the rate dependence of the action potential in the human atrial myocyte.
Am. J. Physiol Heart Circ. Physiol. 297:1398-1410.

21

Mobasheri, A., R. J. Errington, S. Golding, A. C. Hall, and J. P. Urban, 1997.
Characterization of the Na+, K+-ATPase in isolated bovine articular chondrocytes; molecular evidence for multiple alpha and beta isoforms.
Cell Biol. Int. 21:201-212.

22

Nygren, A., C. Fiset, L. Firek, J. W. Clark, D. S. Lindblad, R. B. Clark, and W. R. Giles, 1998.
Mathematical Model of an Adult Human Atrial Cell: The Role of K^+ Currents in Repolarization.
Circ. Res. 82:63-81.

23

Cha, C. Y., C. Oka, Y. E. Earm, S. Wakabayashi, and A. Noma, 2009.
A Model of Na^+ /H^+ Exchanger and Its Central Role in Regulation of pH and Na^+ in Cardiac Myocytes.
Biophys. J. 97:2674-2683.

24

Lee, R. B., and J. P. Urban, 1997.
Evidence for a negative Pasteur effect in articular cartilage.
Biochem. J. 321:95-102.

25

Otte, P., 1991.
Basic cell metabolism of articular cartilage. Manometric studies.
Z. Rheumatol. 50:304-312.

26

Mow, V. C., C. C. Wang, and C. T. Hung, 1999.
The extracellular matrix, interstitial fluid and ions as a mechanical signal transducer in articular cartilage.
Osteoarthr. Cartil. 7:41-58.

27

Urban, J. P., 1994.
The chondrocyte: A cell under pressure.
Br. J. Rheumatol. 33:901-908.

28

Radhakrishnan, K., and A. C. Hindmarsh, 1993.
Description and use of LSODE, the Livermore Solver for Ordinary Differential Equations.
Technical Report UCRL-ID-113855, Lawrence Livermore National Laboratory.

(iv)

29

Dart, C., and N. B. Standen, 1994.
Hypoxia induces a potassium current in smooth muscle cells isolated from the porcine coronary artery.
J. Physiol. 477:P85-P86.

?

remove or replace w recent review.

30

Mobasheri, A., T. C. Gent, A. I. Nash, M. D. Womack, C. A. Moskaluk, and R. Barrett-Jolley, 2007.
Evidence for functional ATP-sensitive (K(ATP)) potassium channels in human and equine articular chondrocytes.
Osteoarthr. Cartil. 15:1-8.

Add

2-Pore Review

Kv 1.5 Review
1.6 including O_2 sensitive

Literature on Goss-Chapman Effects
- surface shape.

Review on Na^+/K^+ pump

cite - array findings.

cite - Wilson - measure of E_m