October 19 - Second Draft

METHODS:

Model Description

Patch clamp experiments done on enzymatically isolated individual human chondrocytes obtained with permission from a knee replacement surgery program (XXXXX) provide the basis for this model development. These chondrocytes have resting membrane potentials which range from -30 to -60 mV when superfused with normal Tyrodes solution and studied using antibiotic-permeablized (amphotericin) patch clamp methods. 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. That is, 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 is underestimated due to the current flow through the seal resistance which in most circumstances results in a depolarization.

The main objective of this first order model is to obtain insights into the ionic mechanisms which underlie the resting potential in the human chondrocyte *in situ*. The raw data which forms the basis of this model is derived mainly from our previously published recordings of the K+ currents in these chondrocytes. 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]

As noted in the Introduction, the chondrocyte is situated in a physiological environment which differs significantly from that of most other mammalian cells. This information is summarized in Table 1. The large electrochemical gradients for Na+, K+ and Cl- are very similar to those in other mammalian systems. These gradients lead to the requirement for an ATP-dependent Na+/K+ pump mechanism that is assumed to be electrogenic. 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 which bathes the chondrocyte is somewhat acidic, pH 7.2, although apparently the normal intra- to extracellular pH gradient can be identified.

[Table 1 near here]

RESULTS:

Model development has proceeded in defined stages starting with implementation of the minimal requirements for generation of a resting potential.

i) Background Currents

Output from our initial simulations is shown in the current voltage (I-V) relationship in Figure 2. This I-V curve illustrates what are termed 'background currents'. In this model these include the resting Na+, K+, and Cl- fluxes. Also shown is the small outward current that is generated by the electrogenic Na+/K+ pump that has been scaled to achieve a steady-state intracellular Na+ concentration of 10-12 mM. The remaining background currents, shown in Figure 2B, are those that are generated by the Na+/Ca2+ exchanger under resting and steady-state conditions corresponding to an intracellular Na+ concentration of 12 mM, and assumed free intracellular Ca2+ concentration of 3 x 10-8 M. In the mammalian chondrocyte there is also evidence for a background flux of Na+ and H+. Equations which capture this electroneutral ion transfer are included since it is anticipated that pH regulation and regulation of intracellular Na+ levels are of importance in both physiological and pathophysiological settings.

The scaling of current densities is dictated by the known input resistance of the cell which is approximately 2 Gigohms. This value is denoted on the I-V curve by the thickened trace in the region of the range of resting membrane potentials that has been reported.

[Figure 2 near here]

ii) Intracellular Ca2+ Levels

The intracellular Ca2+ is maintained at this level based on the assumption of a Ca2+ pump which is electro neutral. The intracellular Ca2+ buffering in this model is assumed to be due mainly to calmodulin within the cytosol. This Ca2+ buffering, and the mathematical expression for the Ca2+ pump have been developed in accordance with the expressions in our previous models (REFS).

iii) K+ Currents

This model incorporates mathematical expressions for a total of 4 distinct K+ currents that have been identified from electrophysiological studies done on mammalian chondrocytes. Two of these, a delayed rectifier K+ current which we denote IK-DR; and a K+ current due to a 2-pore K+ channel which we denote IK-2P have been studied in detail in our laboratory. The remaining two K+ currents, a Ca2+-activated K+ current (IK-Ca) and an ATP-dependent K+ current (IK-ATP) are also included.

a) Delayed Rectifier K+ Current: IK-DR

A time- and voltage-sensitive K+ current has been identified in the human atrial chondrocyte. 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.X. Panel A of Figure 3 shows a I-V relationship generated under physiological conditions, that is, a normal electrochemical gradient for K+. Panel B of this Figure shows the steady-state activation curve and Panel C illustrates the kinetics of activation and deactivation within this range of membrane potentials. The fits to this data provide the basis for the simulation of this current in our model.

[Figure 3 near here]

b) 2-Pore K+ Current:

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. These channels show no detectable time dependence. An additional characteristic of this subclass of 2-pore channels is their 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. Figure 4B illustrates the enhancement of this current when extracellular pH was changed from XXXXX.

Note that these recordings were made under conditions of elevated K+ so that the size of the currents could be increased to the level that they could be detected and biophysical properties could be resolved. Before this data can be incorporated into this mathematical model it needs to be corrected appropriately. The correction which has been employed is based on the Eisenman principle that the conductance of an ion selective channel scales according to the square root of the concentration of that permeant ion. The I-V curve in Figure 4C shows the original data recorded in isotonic K+ 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.

[Figure 4 near here]

Our experimental work also demonstrated that this particular K+ current was strongly inhibited by bupivocaine. An effective concentration of bupivocaine also resulted in a significant depolarization of the resting potential (see Discussion).

c) Ca2+ Activated K+ Current

Virtually all recordings of global K+ currents in human chondrocytes include a significant outward current at depolarized potentials. The spontaneous fluctuations of this current suggest that it is generated by the so-called large conductance variant of the Ca2+ activated K+ current family. 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 Ca2+ activated K+ current has also been described in isolated chondrocytes from rabbits, dogs and horses. Figure 5 below shows represented data. The formulation for the Ca2+activated K+ current in this model is given by the set of equations below.

[Figure 5 near here]

d) ATP-sensitive K+ Current

An ATP-sensitive K+ current has been reported in chondrocytes from a variety of mammalian species. None of our experimental work has addressed this possibility and there are no reports of this current being activated in human chondrocytes. However, the hypoxic environment in which the chondrocyte is placed makes it plausible that this current could be turned on during normal biomechanical activity.

[Figure 6 near here]

iv) Transient Receptor Potential or TRP Current

Ligand gated channels which exhibit properties that correspond to some of those of TRP ion channels are expressed in mammalian chondrocytes. The TRPV4 family is prominently expressed in mouse chondrocytes. For that reason this type of ion channel has been incorporated into this initial model of the human chondrocyte. The rationale for doing this is based on the likelihood that this type of conductance is needed to explain electrophysiological responses to naturally occurring paracrine substances (e.g., ATP) or to cytokines which are liberated in the setting of acute or chronic inflammation (e.g., XXX). 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+. Either or both of these cation species are likely to be 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 (see below).

[Figure 7 near here]

v) Connexin Mediated Current Flow

The chondrocyte from adult humans functions as an isolated cell. As a result it may seem that consideration of connexin function is irrelevant. However data from humans during early adolescence suggest that the growth plate of articular joints is characterized by small groups of directly opposed chondrocytes with expression of selected members of the connexin family being detectable using standard immunohistochemical approaches. Moreover it is now known that even in the case of adult isolated cells prominent ATP release can be observed. One plausible mechanism for this chemical or mechanical release is transient opening of HEMI channels due to expression of either pannexin or connexin subunits. For these reasons our model incorporates a connexin mediated conductance which, however, under physiological conditions is shut off or has a value of 0 pS.

DISCUSSION:

i) Practical Applications of this Model

In the present state of its development, our model represents an original but only semiquantitative tool that can be used as one part of our multidisciplinary studies of electrophysiological and biophysical principles of the human chondrocyte. Our initial emphasis was on investigating the basis of the resting potential (Em) since our data set consists mainly of K+ current measurements in this non-excitable cell. Moreover, it is known that even very small changes in the resting membrane potential can strongly modulate factors that govern intracellular Ca2+ signaling and homeostatic mechanisms. There is also evidence that relatively small alterations in membrane potential contribute to dynamic regulation of cell volume. Both the strength and duration of ligand gated conductance changes are dependent upon membrane potential (Em) since this sets the electrochemical driving force.

The computations shown in Figures 2 through 6 in fact do not fully explain the ionic basis for the resting membrane potential in the human chondrocyte. Rather, they serve to illustrate that a range of membrane potential values is to be expected. This is because the net outward current that sets the resting membrane potential is very small even though it is the algebraic sum of background conductances as well as currents due to the electrogenic Na+/K+ pump and Na+/Ca2+ exchanger. In many recording situations the leak current through the patch pipette seal resistance can influence (and could even dominate) the observed 'resting potential'. In addition spontaneous changes in this seal resistance can give the impression of an unstable resting potential in these cells.

Nevertheless, our computational work does provide a basis for addressing the question, 'what are the physiological roles of each of the four K+ currents that have been identified and characterized in human chondrocytes'? At the outset one can appreciate from the computed I-V curves that any, or all of these currents could: i) significantly hyperpolarize the resting potential or ii) repolarize the chondrocyte after it had been depolarized by e.g., the effects of mechanical activity or by a ligand gated conductance.

An example of this, illustrated in Figure 7, is based on simulation of the electrophysiological response observed following activation of the TRP channels that are known to be expressed in human chondrocytes. A characteristic TRPV4 I-V curve is shown in Figure 7A. The effect of this channel mediated current on the resting membrane potential can be deduced by adding this current to the steady-state I-V relation illustrated in Figure 7C. The resulting net current is shown in Figure 7B. From this it is clear that activation of TRP channels results in a transient depolarization of the chondrocyte. However and possibly more importantly, it is also apparent that TRP channel activation results in a significant influx of Ca2+ and Na+ under circumstances in which the chondrocyte membrane potential is negative to approximately -40 mV. The TRIP channel induced depolarization is limited in magnitude and eventually transformed into a repolarization by the voltage dependent activation of the delayed rectifier K+ current, IK-DR. It follows that under circumstances in which the TRP channel results in a significant increase in intracellular Ca2+, the Ca2+ dependent K+ current, IK-Ca will also be activated. This will hyperpolarize the membrane potential and this potential will be maintained until intracellular Ca2+ and/or Ca2+ dependent signaling mechanisms reset to resting values.

[Figure 8 near here]

Consideration of the physiologic milieu within the articular joint identifies conditions under which some of these K+ currents may be modulated significantly. For example, the extracellular fluid is hypertonic. The effects of osmolarity on voltage gated K+ currents have been studied extensively. In brief, as a consequence of changes in surface charge shielding alterations in superfusate tonicity can significantly shift the steady state gating of currents such as IK-DR. Our experimental work is done under fixed isotonic conditions. The literature would suggest that an increase in osmotic strength would cause a shift to the right, in the depolarizing direction by perhaps 5 mV. This biophysical correction would make it unlikely that IK-DR makes any significant contribution to the resting potential. However, this alteration would position this current to initiate prompt repolarization following any transient depolarization, while also ensuring that the input resistance of the cell at the resting potential remained very high. This is a critical factor since a minimum of ion transfer is desirable in the relatively hypoxic milieu of the articular joint.

ii) Limitations of this Model

As presented, this mathematical model of chondrocyte electrophysiology provides a reliable platform for explaining and evaluating the experimental data in this field. This first order model also has proven utility for rationalizing and integrating genomic data from expression array profiles and as part of ion channel/antiporter drug target initiatives. Iterations based on the semiquantitative approach that is made possible by this model can also be useful for designing new experiments that are aimed at revealing cellular mechanisms that govern/modulate excitation-secretion coupling. Finally, given that the chondrocyte is in a unique, but yet not completely defined microenvironment our model provides a basis for explaining: i) the known biophysical effects of alterations in ionic strength of synovial fluid on ion channel voltage-dependent gating (zeta potential effects), ii) one common approach in attempts to account for the effects of cyclic stretch on ion channels - strain dependent alterations in channel gating voltage dependence or kinetics (cf. YYYY), and iii) much needed approaches for detecting and determining the limitations of present patch clamp technology that can bias, if not dictate, resulting data sets and influence their interpretation.

We recognize that at this stage our model has significant limitations. These include but are not limited to:

a) The absence of any comprehensive account or set of mathematical descriptors for intercellular Ca2+ homeostasis. Extension of the model to include simulations of the Ca2+ is needed before it can be used to explore the roles of Ca2+-activated K+ and/or Cl- currents.

b) Essential aspects of excitation-contraction coupling including the sources for the Ca2+ which triggers and supports secretion and the reuptake or extrusion mechanisms for this Ca2+.

c) Mathematical expressions that would allow rationalization of what has been termed 'the AM and FM modes of Ca2+ signaling' which will require consideration of intracellular Ca2+-dependent phosphorylation and dephosphorylation reactions and consideration of the Ca2+-dependence involved in transcriptional regulation of ion channel, antiporter and pump target molecules.

d) An improved understanding of and development of mathematical approaches that may account for the response of the chondrocyte to the ambient hypoxia within the articular joint. Initially this is likely to require formulation of equations for hypoxia-activated K+ channels.

e) Adaptation of published mathematical formulations for alteration of ion channel activity arising from cyclic biomechanical stretch or from transient changes in shear forces that impinge upon the chondrocyte.

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CONFLICT OF INTEREST:

None of the authors have any conflict of interest items to declare.

APPENDIX:

Our model of seal resistance re: membrane potential

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