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 required 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.

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 are derived mainly from our previously published recordings of the K+ currents in these chondrocytes. Other information, summarized diagrammatically in Figure 1 below is obtained from published literature on isolated chondrocytes from rabbit, canine and equine tissue.

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 standard. This information leads 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.

Results

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

i) Background Currents

Output from this initial modeling is shown in the current voltage 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 which has been scaled to achieve a steady-state intracellular Na+ concentration of 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.

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 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.

iii) K+ Currents

This model incorporates mathematical expressions for a total of 4 distinct K+ currents that have been reported 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 incorporated.

a) Delayed Rectifier K+ Current: IK-DR

A time- and voltage-sensitive potassium 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 potassium conductances denoted Kv1.X. Panel A of Figure 3 shows a current voltage relationship generated under physiological conditions, that is, a normal electrochemical gradient for potassium. 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.

b) 2-Pore Potassium Current:

Our recent work has identified recording conditions under which an additional potassium current generated by what is believed to be the TASK family of 2-pore potassium channels. These channels show no detectable time dependence. An additional characteristic of this subclass of 2-pore channels is their significant increase in current generation in response to decrease pH or acidification 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 7.4 to 6.0.

Note that these recordings were made under conditions of elevated potassium 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 potassium with the expected reversal potential of 0 mV together with the corrected data assuming external potassium to be 5.4 mM with a corresponding reversal potential of approximately -85 mV.

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

c) Calcium Activated Potassium Current

Virtually all recordings of global potassium currents in human chondrocytes exhibit a significant outward current at depolarized potentials (that is in quadrant 1 of the current voltage relationship). The spontaneous fluctuations of this current suggest that it is generated by the so-called large conductance variant of the calcium activated potassium current family. We have not explored the biophysical properties of this current or its pharmacological profile in any detail. However, as mentioned it is consistently present in our recordings and a calcium activated potassium current has also been described in isolated chondrocytes from rabbits, dogs and horses. Figure 5 below shows represented data. The formulation for the calcium activated potassium current in this model is given by the set of equations below.

d) ATP-sensitive Potassium Current

An ATP-sensitive potassium 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. It is also possible however that the hypoxia serves to activate or enhance the delayed rectifier current described in A above.

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 sodium and calcium. 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).

v) Connexin Mediated Current Flow

The chondrocyte from adult humans functions as an isolated cell. As a result it would 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.