10/16/12

Molly

It was nice to speak with you.

Attached please find mock-up drawings & the planned Figures for our Biophys J paper. This may help to guide our discussions with Harish.

In addition I have attached hand-written material for the remainder & Results and perhaps 2/3 & the Discussion. This will be transmitted to you by Friday (19th).

Please confirm that you have received the material

W. Eaton

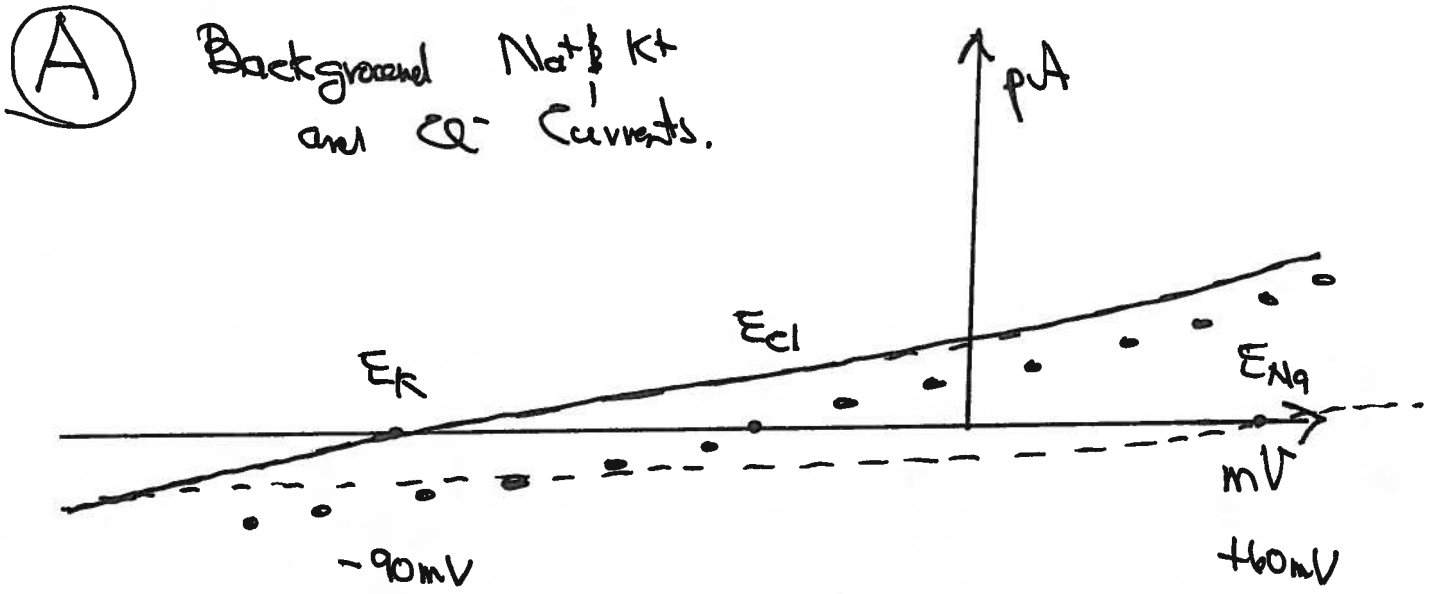
Fig. 1

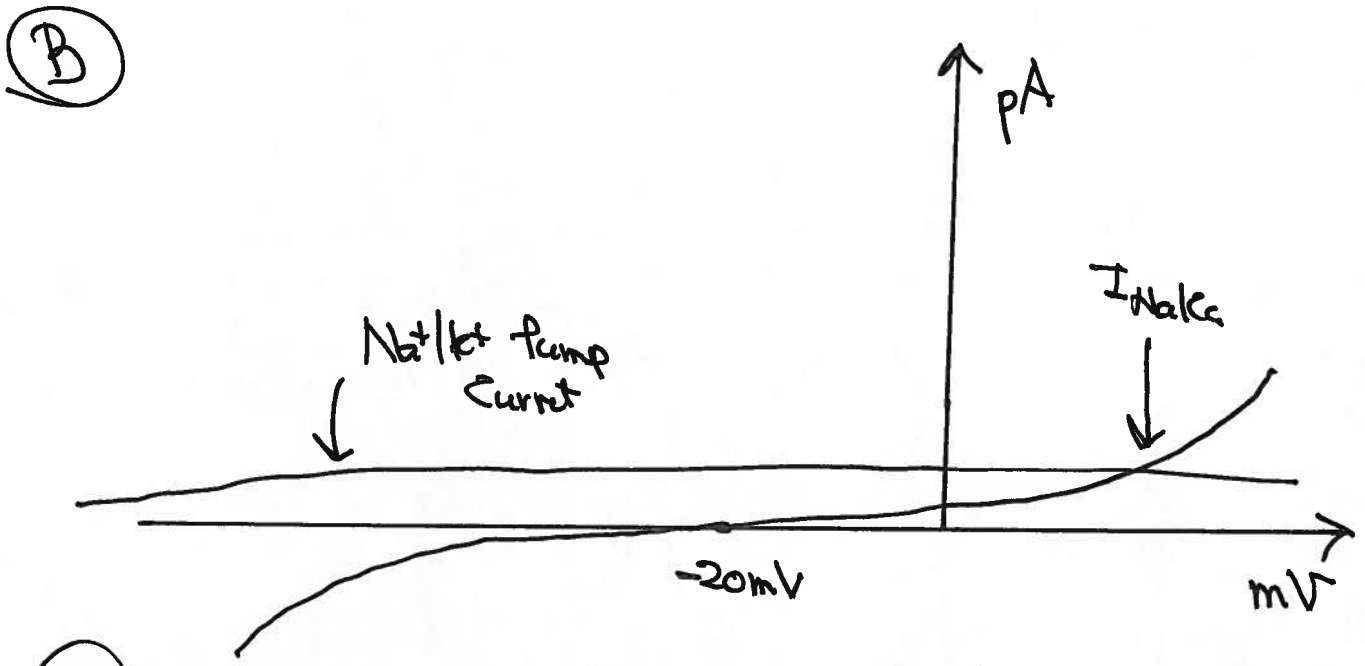
existing

3x larger labels

add Ca^{2+} pump

Fig. 2.


A
Background Na+/K+ and Cl- Currents.
pA
E_K
E_{Cl}
E_{Na}
mV
-90mV
+60mV


B
pA
I_{NaCa}
Na+/K+ pump Current
-20mV
mV

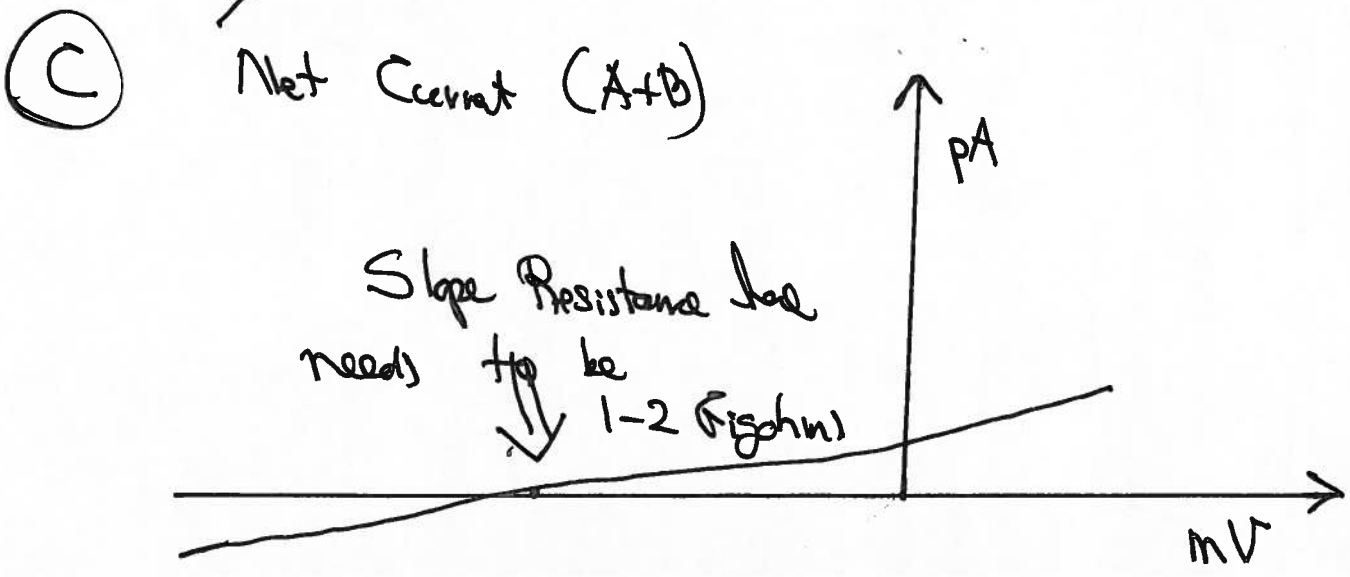
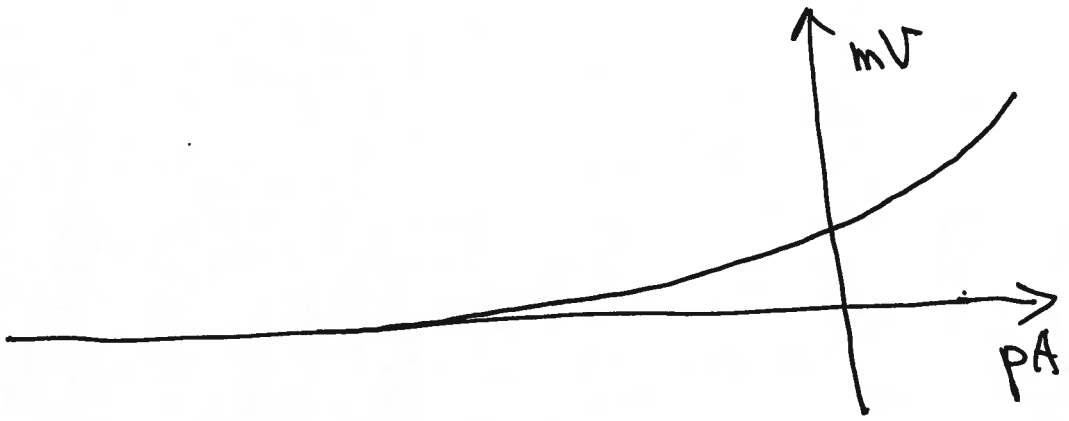
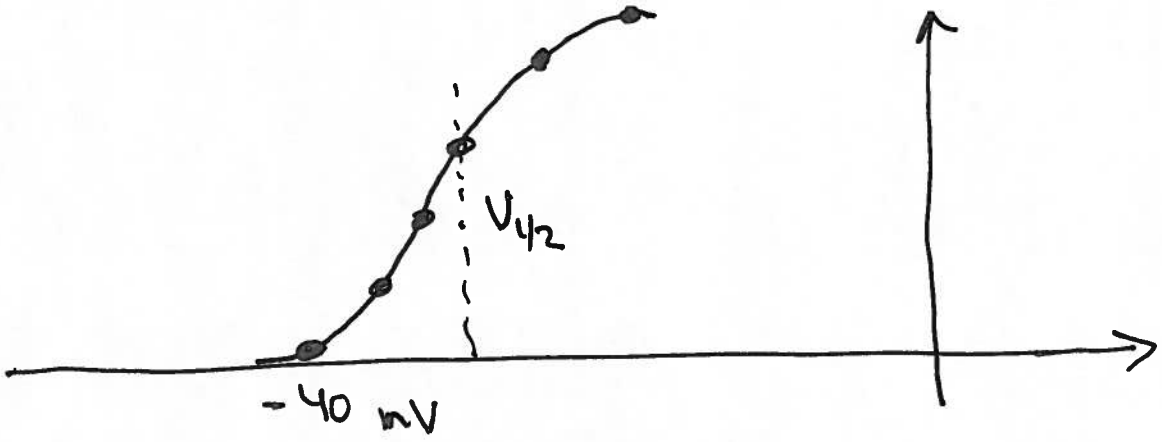

C
Net Current (A+B)
pA
Slope Resistance has needs to be 1-2 Gigohms
mV

Fig. 3

(A.) Delayed Rectifier K+ Current
(either peak or isothermal current)



(B.) Activation Curve



(C) Kinetics

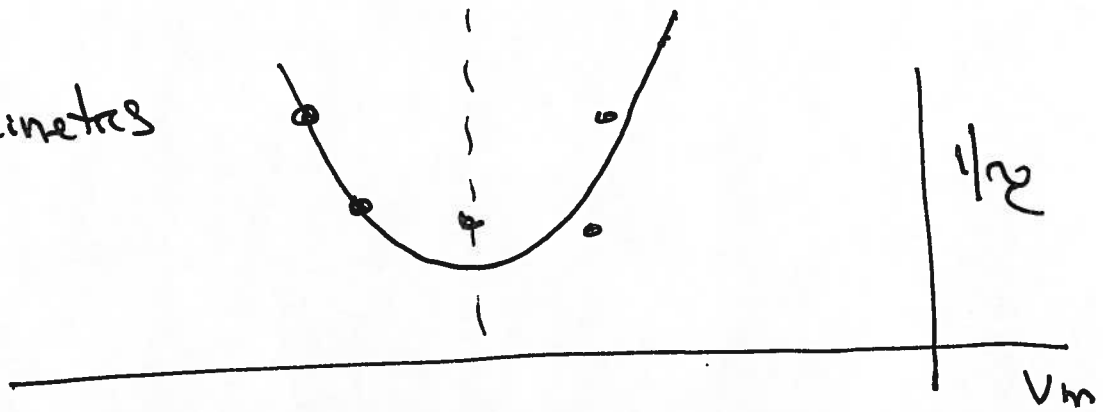
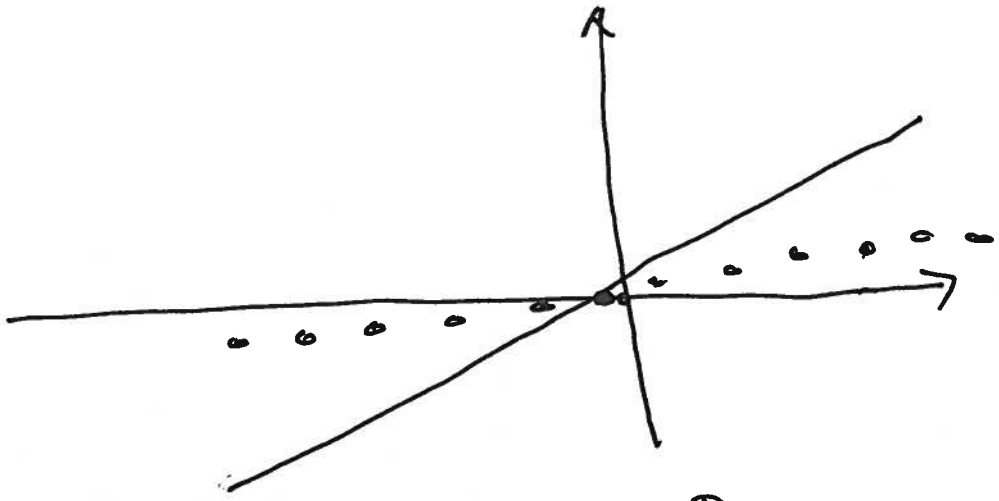


Fig. 4.

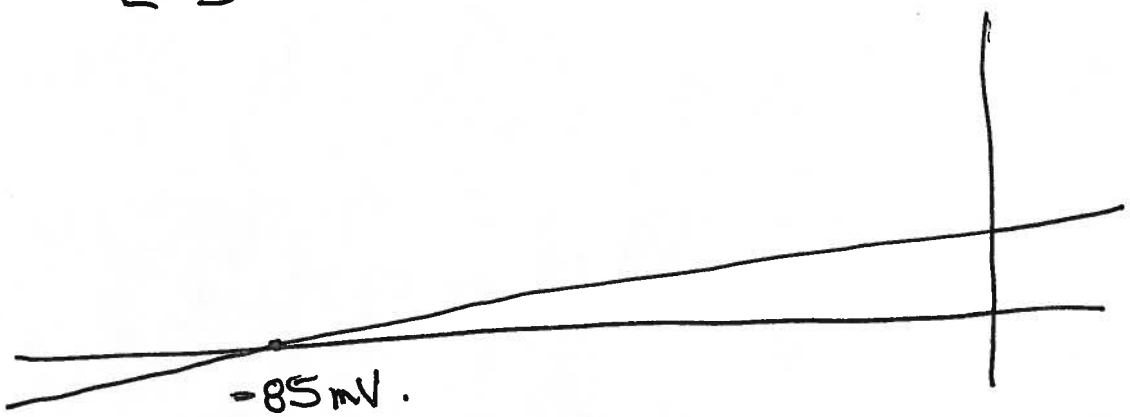
2-Pore K^+ Current

(A.) Data obtained / recorded in isotonic [K^+]



Control showing data in Bupivacaine

(B.) Data in (A) corrected for physiological [K^+]



(C.) New unit current

ie Fig 2C plus Fig 4B.

Fig. 5.

Ca^{2+} - activated K^+ Current

calculated at
$[Ca^{2+}]_i = 10^{-7} M$

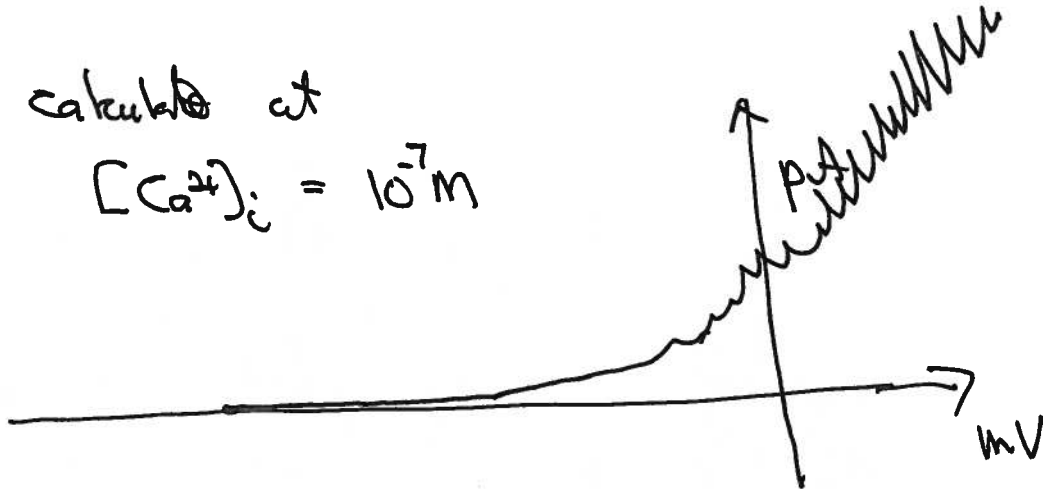


Fig. 6

ATP sensitive K^+ Current
Hypothetical - would have no dat

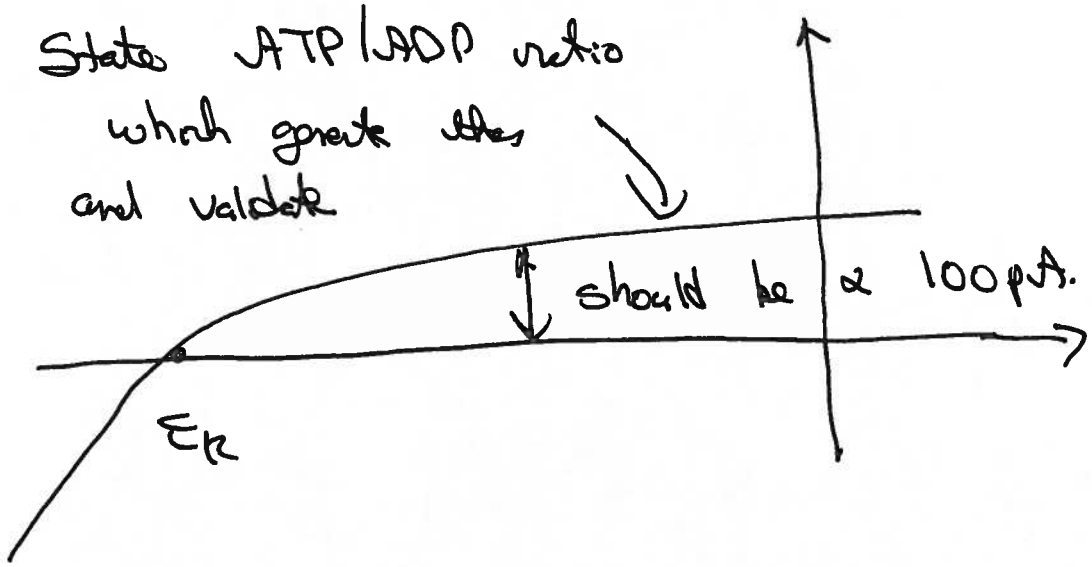
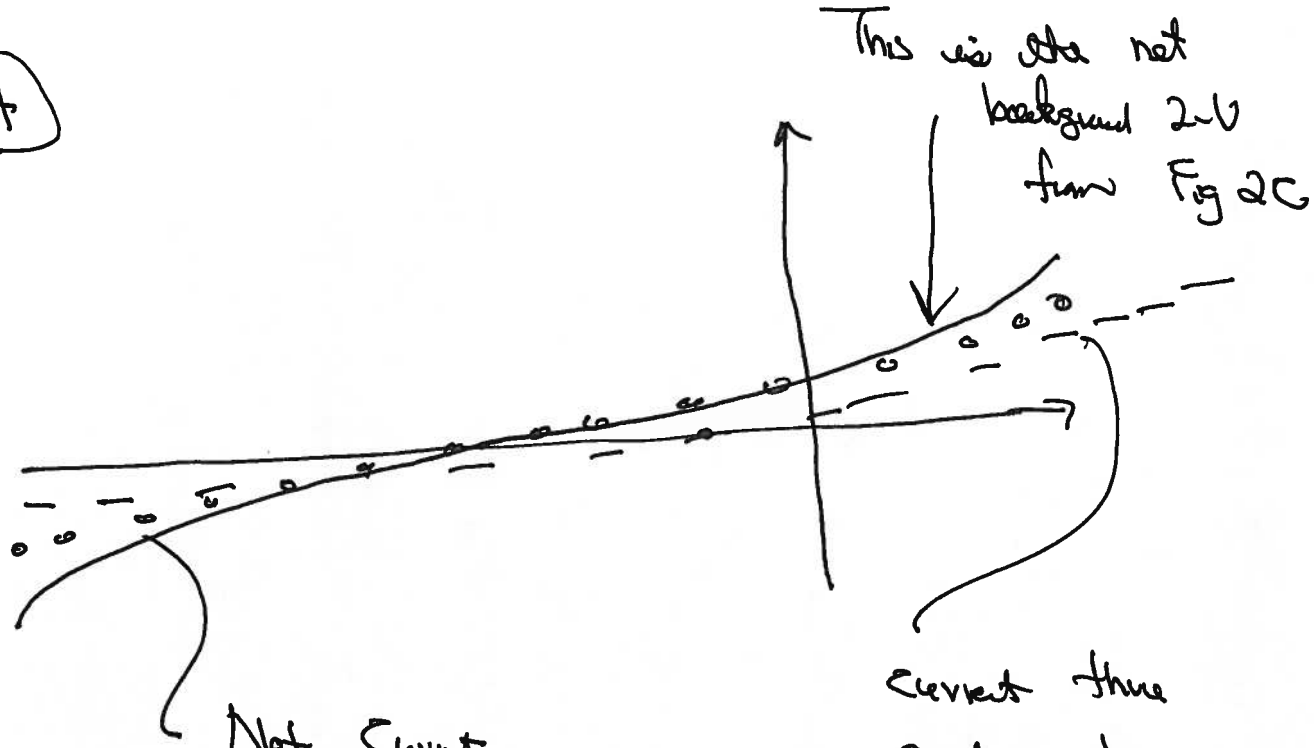


Fig. 7.

Influence of Patch Pipette Seal Resistance

(A)



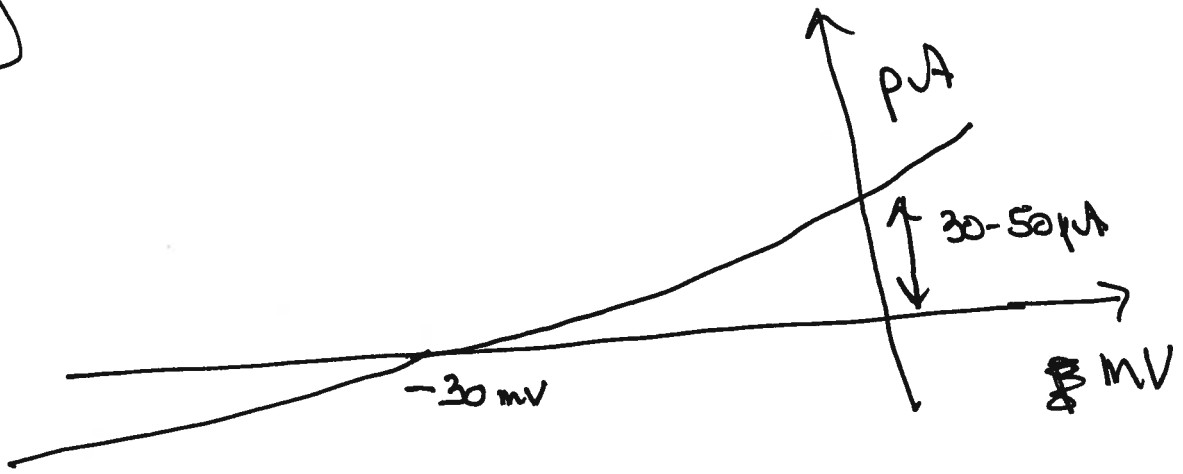
(B) Repeat of ω 2-Pore kt Current Activated

ie net from above plus I_{K-2D} from Fig. 4B.

Figure 8

- placed in the Discussion section
- demonstrate effects of ligand e.g ATP activation of TRPV4 channel

(A.)



(B.) Assume $p^{Na} = pCa$ and calculate Na^+ and Ca^{2+} influx and $\Delta [Na^+]_i$ and $[Ca^{2+}]_i$ if channel is open for 500 msec; or one event second for 2 min.

(C.) Assume that stretch increases the conductance 3-fold and repeat calculations in (B) above

Discussion

Utility of the First Order Model

In its present state of development the model represents a useful semi-quantitative tool as part of our multidisciplinary studies of human chondrocyte electrophysiology / biophysics. Our initial emphasis was the resting potential since our data consists mainly of current measurements and the chondrocyte is a nonexcitable cell. In such circumstances it is known that even very small changes in resting membrane potential can strongly

modulate intracellular Ca^{2+} homeostasis and signalling. Small changes in membrane potential can also contribute to the regulation of cell volume. The strength and ~~the~~ duration of ligand-gated conductances also change significantly as a consequence of changes in membrane potential and their net electrochemical driving force.

The computations shown in Figs. 2 through 6 do not explain the ionic bases of the resting membrane potential in the human chondrocyte. Rather they illustrate that a range of values is to

be expected based on the net current that results from the background conductances, as well as the pump and other events. In many recording situations the leak current though the seal resistance will influence and could dominate the observed 'resting potential' In addition, changes in seal resistance could, in the cell, give the impression of an 'unstable' resting membrane potential.

The question arises: What are the physiological roles of the 4 K+ currents which have been

identified and characterized to the extent that the available experimental data can justify. At the outset it is clear that information from the mathematical model cannot provide any such unequivocal insights / answers. Nevertheless, the computed rate and net I-V curves make it clear that any & all of these scenarios could (i) significantly hyperpolarize the resting membrane potential and/or (ii) depolarize & characterize that has been depolarized by neuronal activity or a ligand-gated conductance.

An example illustrated in Fig. 7, is the electrophysiological response to activation of the TRP channels that are known to be expressed in chondrocytes. A characteristic TRP V_4 I-V curve is shown in Figure 7A and the effect on membrane potential can be deleted by adding the current (- - -) to the I-V relation first computed in Fig 5C. It is clear that the ability of the receptor to deliver a significant influx of Ca^{2+} and Na^+ depends on, in fact, requires the chondrocyte to have a resting membrane

potential in the range of ~50 mV. The TRP channel induced depolarization will be limited and eventually transformed into a repolarization by activation of the delayed rectifier K+ current I_{K-DR}. In addition if TRP channel activation results in a significant increase in intracellular Ca^{2+}, the activation of I_{K-Ca} is expected.

[Figure 7 near here]

Consideration of the physiological milieu within the extracellular joint also suggests circumstances under which the identified K^+ currents may be important for function. Thus the extracellular fluid is hypertonic. The effects of osmolarity on voltage-gated K^+ currents have been studied extensively. In brief, a reasonable of several these effects (shielding) a hypertonic medium produces a depolarizing shift in steady-state gating of voltage sensitivity. Since our experiments were performed in an isotonic medium,

the required concentration would make the steady-state activation curve for 2_K-DR shift 5 mV in the depolarizing direction. This would make it unlikely that the cell could saturate to the resting potential, but position it well to ensure prompt repolarization while maintaining a high resting input resistance.

The fact that the channels are bathed in a milieu in which the pH is approx 7.0 is of interest with respect to the functional role of 2-pore K^+ channels of the TASK family. These K^+

channels are known to be sensitive to pH. In fact ~~the~~ a current is strongly activated by acidification of the extracellular medium. Our experiments were done at pH 7.4 Accordingly, the recorded current would be ~~expected~~ expected to be larger at pH 7.0 and ~~the~~ would add a significant hyperpolarizing current. This prediction is plausible since it is known that a ~~selective~~ potent TASK channel blocker, bupivacaine, significantly depolarizes isolated chemoreceptor preparations, including the human chemoreceptor.

Limitations

As presented the mathematical model of cardiac electrophysiology provides a reliable platform for explaining and evaluating experimental data on the field which relate to the resting potential or some aspects of plane repolarization. The first order model is also useful for rationalizing and integrating generic data from expression array profile on ion channel / antiporter drug screening initiatives. However based on the semi-quantitative approach which is made possible by the model

also is very useful for designing
new experiments aimed at revealing
cellular mechanisms which govern/ modulate
excitation - secretion coupling. Finally,
given that the channelopathies are in
a unique, but yet not completely
defined environment, our model
provides a basis for explaining
(i) the known biophysical effects &
altered ionic strength on ion channel
voltage dependent gating (zeta potential
effects) (ii) are currently made for
accounting for the effects &
cyclic stretch on ion channel -
alteration in channel gating kinetics
(cf) and

(iii) unsupervised approaches for detecting and determining the ways in which limitations of path clamp technology (and associated values) can bias, if not dictate, resulting data sets and their interpretations.

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

(1) The absence of any comprehensive account / set of descriptors for intracellular Ca^{2+} homeostasis. This is needed for an improved understanding of the roles of Ca^{2+} activated K^+ and Cl^- channels, (ii) essential aspects of excitation secretion coupling and (iii)

an approach to what has been termed AM and FM Ca^{2+} signalling in the context of Ca^{2+} dependent phosphorylation/ dephosphorylation or other modes of transcription regulation of

(ii) An improved understanding of ~~and~~ mathematical appeal to the relative and subsidiary consequences of hypoxia: (a) O_2 sensitive ion channel activation (b) shifts in modes of metabolism

(iii) Intermittent censoring the electrophysiologic 'target' for mechanosensitivity. ~~and~~

Acknowledgements

Experimental work in the Gibb laboratory was supported by an Alberta Innovates - Health Solutions Scientist Award (WRG), an AI-HS starter grant, and Canadian Institutes for Health Research operating funds.

We are grateful to the Southern Alberta Tissue Transplant facility and its leader (Dr R. Krawetz) for supply of human tissues, articular joint (knee)

Ms Colleen Kuhl prepared the human chondrocyte samples and provided other essential technical assistance.

_____ was supported by Simons in conjunction with an AI-HS - funded postdoctoral award

M. Mabuka _____

References

Please modify ref list so that it is created with the required

Bigby J format.

Bob Clark will do the same re new refs at the end.