580.439/639 Midterm Solutions, 2003

Problem 1

Part a) The suggested l.h.s has units moles/s and is the calcium flux through the entire cell membrane. The calcium current and the flux resulting from the pump have units Amp/m^2 or $moles/(s \cdot m^2)$, so need to be multiplied by the surface area S to get the flux for the whole cell.

$$Vo\frac{dCa_{in}}{dt} = \frac{moles}{\sec} = S\left[-\frac{I_{Ca}}{2F} - J_{pump}\right] \tag{1}$$

where the current is divided by $2F$ to convert current (coul/s) to moles/s and J_{pump} is the net transport by pumping, in $moles/(s \cdot m^2)$

$$J_{pump} = k_{out}Ca_{in} - k_{in}Ca_{out} \tag{2}$$

and both current and flux are positive in the outward direction. The differential equation is

$$\frac{dCa_{in}}{dt} = \frac{S}{Vo}\left[-\frac{I_{Ca}}{2F} + k_{in}Ca_{out} - k_{out}Ca_{in}\right] \tag{3}$$

To check the units;

$$\frac{moles}{m^3 s} = \frac{m^2}{m^3}\left[-\frac{coul/(m^2 s)}{coul/mole} + \frac{m}{s}\frac{moles}{m^3} - \frac{m}{s}\frac{moles}{m^3}\right] \tag{4}$$

Part b) The calcium current is given by:

$$I_{Ca} = P_{Ca}\, mV\, \frac{Ca_{in}e^{2FV/RT} - Ca_{out}}{e^{2FV/RT} - 1} \tag{5}$$

Note that $Ca_{in} << Ca_{out}$. In addition, at the resting potential, $\exp(2FV/RT)=\exp(2 \cdot (-60)/26)<<1$. Thus, to a good approximation the first term in both the numerator and denominator of the fraction in Eqn. 5 can be ignored, giving

$$I_{Ca} \approx P_{Ca}\, m\, Ca_{out}\, V \tag{6}$$

Part c) Substituting in the differential equation Eqn. 3:

$$\frac{dCa_{in}}{dt} = \frac{S}{Vo}\left[-\frac{P_{Ca}\, Ca_{out}\, m}{2F}V + k_{in}Ca_{out} - k_{out}Ca_{in}\right] \tag{7}$$

in the steady state, $dCa_{in}/dt=0$, so

$$\frac{dCa_{in}}{dt} = \frac{S}{Vo}\left[-\frac{P_{Ca}\,Ca_{out}\,m}{2F}V + k_{in}Ca_{out} - k_{out}Ca_{in}\right] = 0$$

(8)

$$Ca_{in}(ss) = \frac{1}{k_{out}}\left[k_{in}Ca_{out} - \frac{P_{Ca}\,Ca_{out}\,m}{2F}V\right] = \frac{1}{k_{out}}\left[k_{in} - \frac{P_{Ca}\,m}{2F}V\right]Ca_{out}$$

where $Ca_{in}(ss)$ is the steady-state internal calcium. Essentially, the resting membrane potential V and the outside calcium concentration Ca_{out} set the resting inside calcium concentration according to this equation.

Part d) The differential equation in Eqn. 8 can be written as:

$$\frac{dCa_{in}}{dt} = -\frac{1}{\tau_{Ca}}Ca_{in} + \frac{1}{\tau_{Ca}}Ca_{in}(ss)$$

(9)

where $\tau_{Ca}=Vo/(S k_{out})$ is a time constant (units s) and $Ca_{in}(ss)$ is given above. If V and Ca_{out} are constants, then this is a linear equation and the solution, for initial value $Ca_{in}(0) = C_0$ is

$$Ca_{in}(t) = Ca_{in}(ss) + (C_0 - Ca_{in}(ss))e^{-t/\tau_{Ca}}$$

(10)

Problem 2

Part a) In order to get into the internal cavity, the gate must be open. Given the gating model currently accepted for KcsA, when the gates are open, there is a wide cavity inside the channel that is continuous with the inside of the cell.

Part b) It should work only from the internal solution, since it cannot pass through the selectivity filter, which is on the outside of the membrane in KcsA.

Part c) It is likely to be a cation (i.e. + charge). K^+ is stabilized in the internal cavity of the KcsA channel by the negative ends of the dipoles of the P segment of the channel molecule. This electrostatic interaction would repel an anion from the cavity.

Part d) The usual barrier model is drawn at right and labeled with the relevant variables. S means the porestickalate concentration in solution, Ch is concentration of channels with porestickalate bound. The rate constants are determined by G_1 and G_2. G_2 sets the equilibrium binding level. λ_1 and λ_2 are the fraction of the membrane potential seen by the ion at the energy peak and in the binding site. In general, both of these are less than 1 since the blocking ion does not cross the whole membrane when it binds to the channel.
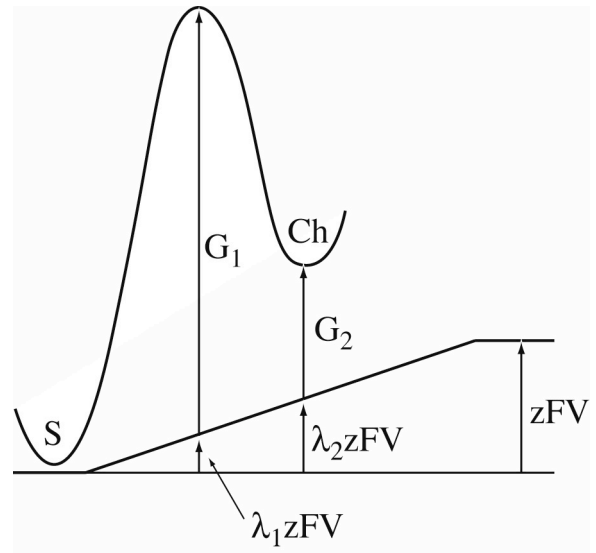
Part e) The expected behavior is shown at right. This is actually the behavior of K^+ channel block by nonyltriethylammonium (C_9), from Fig. 3.5 of Hille. At the resting potential, most of the channel is closed, so there is no current. In addition, there are no blocked channels because the channels are closed. Following the voltage clamp, channels open, giving transient currents, which then decline toward zero as C_9 enters and blocks the channels. The duration of the transient currents is determined by the time constant for binding of porestickalate to the channel.

(A) K CURRENTS IN TTX

(B) BLOCK BY INTERNAL C_9
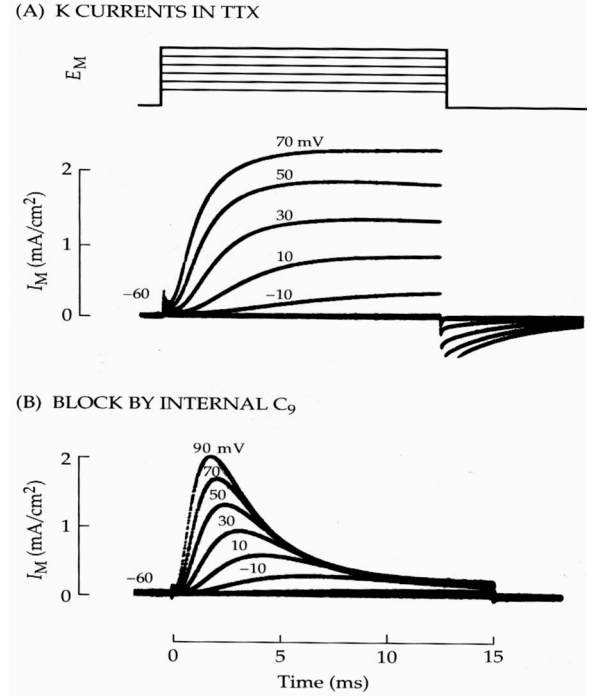
Part f) The differential equation for entry of the blocker into the channel in the case where all channels have open voltage-gates is:

$$\frac{dCh}{dt} = k_1 S (Q - Ch) - k_2 Ch \tag{11}$$

where Ch is the concentration of channels that are blocked and Q is the total concentration of channels. k_1 and k_2 are the forward and reverse rate constants for moving over the barrier. In the steady state, $dCh/dt=0$ and the steady state distribution is given by the following.

$$Ch = \frac{k_1 S}{k_1 S + k_2} Q \tag{12}$$

Membrane potential enters this equation through the rate constants, which are given by the following, from the energy diagram above:

$$k_1 = (const)e^{-(G_1 + \lambda_1 zFV)/RT} \quad \text{and} \quad k_2 = (const)e^{-(G_1 - G_2 + (\lambda_1 - \lambda_2)zFV)/RT} \tag{13}$$

The condition for 50% block is $Ch/Q = 0.5$, so that

$$0.5 = \frac{e^{-(G_1 + \lambda_1 zFV)/RT} S}{e^{-(G_1 + \lambda_1 zFV)/RT} S + e^{-(G_1 - G_2 + (\lambda_1 - \lambda_2)zFV)/RT}} \tag{14}$$

$$0.5 = \frac{S}{S + e^{(G_2 + \lambda_2 zFV)/RT}}$$

or

$$G_2 + \lambda_2 zFV = RT \ln S \qquad (15)$$

Note that this condition resembles equilibrium between the states in the barrier diagram.

Problem 3

Part a) The three state variables in this system are internal calcium (Ca_{in}), membrane potential (V), and the HH gating variable (m). Other HH variables are assumed away by being combined into G_M and E_M. The external calcium concentration could also be a state variable, but was assumed constant in problem 1.

Part b) The Kirchoff's current law equation is:

$$C\frac{dV}{dt} = I_{ext} - I_{Ca} - G_M(V - E_M) - 2F\,k_{out}Ca_{in} + 2F\,k_{in}Ca_{out} \qquad (16)$$

There are two extra terms in this equation, which express the current carried by the ion pumps. The external current I_{ext} is irrelevant to this problem and is set to 0 below. The calcium current I_{Ca} was worked out in Eqn. 6; substituting that value:

$$C\frac{dV}{dt} = -P_{Ca}Ca_{out}m_\infty(V)V - G_M(V - E_M) - 2F\,k_{out}Ca_{in} + 2F\,k_{in}Ca_{out}$$

$$\frac{dV}{dt} = -\frac{P_{Ca}Ca_{out}m_\infty(V) + G_M}{C}V - \frac{2F\,k_{out}}{C}Ca_{in} + \frac{G_M E_M + 2F\,k_{in}Ca_{out}}{C}$$

$$(17)$$

Note that the units of calcium current (Amp/m^2) are the same as those of $G_M(V\text{-}E_M)$, because G_M is defined as mho/m^2 and similarly for the pump terms. The HH variable m has been set to $m_\infty(V)$, under the usual assumption that its time constant is fast, compared to the other time constants in the system (of course, this has not been verified).

Part c) At the equilibrium point, both time derivatives must be zero. Rewriting $Ca_{in}(ss)$ from Eqn. 8 and writing the condition for $dV/dt=0$ from Eqn. 17 gives

$$Ca_{in}(ss) = \frac{1}{k_{out}}\left[k_{in}Ca_{out} - \frac{P_{Ca}m_\infty(V)Ca_{out}}{2F}V\right]$$

$$\frac{P_{Ca}Ca_{out}m_\infty(V) + G_M}{C}V(ss) = -\frac{2F\,k_{out}}{C}Ca_{in} + \frac{G_M E_M + 2F\,k_{in}Ca_{out}}{C}$$

$$(18)$$

The second equation can be simplified by canceling the C terms and substituting $Ca_{in}(ss)$ from the first equation.

$$\left[P_{Ca}\,Ca_{out}\,m_\infty(V) + G_M \right] V(ss) =$$

$$- 2F\,k_{out}\,\frac{1}{k_{out}}\left[k_{in}Ca_{out} - \frac{P_{Ca}\,m_\infty(V)\,Ca_{out}}{2F}V(ss) \right] + \left[G_M E_M + 2F\,k_{in}Ca_{out} \right] \quad (19)$$

Inspection of this equation shows that several terms cancel (1st and 4th, 3rd and 6th), leaving

$$V(ss) = E_M \quad (20)$$

This result can be obtained more simply by considering the following argument. There are four ways for current to pass across this membrane: 1) Ca channels; 2) Ca pumps; 3) resistor/battery G_M, E_M; and 4) Capacitor C. In steady state at the equilibrium point, current 4) is zero by the steady-state assumption; currents 1) and 2) must add to zero if calcium is to be in steady state (Eqn. 8). Thus the only circuit element left is the battery-resistor pair and there can be no current through it since the sum of the other three currents is zero, so the potential across the system must be E_M.

Part d) The differential equations for this system can be written as follows (repeating Eqns. 7 and 17):

$$\frac{dV}{dt} = -A(V)V - B\,Ca_{in} + D$$

$$\quad (21)$$

$$\frac{dCa_{in}}{dt} = -E(V)V - F\,Ca_{in} + G$$

where the coefficients are defined as follows:

$$A(V) = \frac{P_{Ca}Ca_{out}m_\infty(V) + G_M}{C} \qquad B = \frac{2F\,k_{out}}{C} \qquad D = \frac{G_M E_M + 2F\,k_{in}C_{out}}{C}$$

$$\quad (22)$$

$$E(V) = \frac{S}{Vo}\frac{P_{Ca}Ca_{out}m_\infty(V)}{2F} \qquad F = \frac{S}{Vo}k_{out} \qquad G = \frac{S}{Vo}k_{in}Ca_{out}$$

In the $Ca_{in} - V$ plane, the isoclines are approximately straight lines with slopes $-A(E_M)/B$ and $-E(E_M!)/F$. The "approximately" holds because both A and E are functions of V. The slope for the V isocline is larger, by $G_M/(2Fk_{out})$. The phase-plane is sketched at right.