Lecture 2: Patch Clamping

I. Useful modifications of Ohm's law for calculating the amount of current flowing through a membrane and the conductance

A. Ohm's law is: V = IR

1. Where

2. V = voltage

3. I = current

4. R = resistance

a) The inverse of resistance is conductance (abbreviated as a "g" [some sources use a capital G as the abbreviation])

B. Ohm's law using conductance: $V = \frac{I}{G}$

1. Solving for I: $\mathbf{I = gV}$

C. The voltage that produces currents in cells is the difference between an ion's equilibrium potential & the membrane potential ($V_m - E_x$), where "x" indicates the ion in question

1. Substituting into Ohm's law: $I_x = g_x (V_m - E_x)$

2. Note that for a typical negative resting potential that if E_x is negative (like for K^+) I_x is positive, while if E_x is positive (like for Na^+ or Ca^{++}) I_x is negative

3. This is very important when considering voltage clamping

D. ***Rearrange the above equation to solve for conductance:*** $g_x = \frac{I_x}{(V_m - E_x)}$

1. ***This is useful during patch clamping because different channels will have different conductances***

a) **You determine ion selectivity by either changing the equilibrium potentials or using selective toxins that block certain classes of channels**

b) **There are often several different channels for a particular ion**

(1) *You can tell them apart by their conductances*

II. **We can observe the behavior of individual channels in biological membranes by use of a technique called <u>patch clamping</u>**

A. **Start with an electrode similar to a glass microelectrode except tip is may be 1-3 μm in diameter**

1. **Tip is firepolished to make sure it's very smooth**

B. **Place electrode tip on clean surface of a cell**

1. **Apply suction & if lucky a gigohm (10^9 Ω) seal will develop**

C. **The membrane encircled by the tip of the electrode is called a patch**

D. **Because of gigohm seal, very little current generated in the patch will leak out between the electrode and the membrane**

1. **Most of the current is then forced to flow through the electrode**

E. **In any one patch there one or a few channels**

F. **During patch clamping the potential across the membrane is held constant**

1. **This allow the measurement of the amount of current flowing through the channel**

a) ***This technique is called voltage clamping***

(1) The term "clamp" refers to keeping that parameter constant

(a) In voltage clamping, we keep the voltage constant and measure the current flowing through the membrane

G. There are several configurations for patch clamping

1. Cell-attached patch (single channel resolution)

2. Excised inside-out patch (single channel resolution)

a) After forming a cell-attached patch, the electrode is pulled away from the cell

(1) Eventually the membrane will separate from the cell and form a vesicle

(2) Vesicle is either exposed to air or a low calcium saline

(a) This ruptures the vesicle outside the pipette

(3) The pipette is left with a single layer of membrane spanning the opening

(a) The membrane surface inside the pipette was the outside surface of the cell

(b) The surface exposed to the outside saline was the cytoplasmic face of the membrane

3. Whole cell clamp

a) Start with cell attached configuration

(1) Apply suction in pipette until membrane is ruptured

b) Records are very similar in appearance to traditional voltage clamping because the activity of many channels is recorded

c) Advantages

(1) Often technically easier than traditional voltage clamping

d) A variation on this is the perforated patch, which uses a pore-forming compound such as nystatin

(1) Advantage is that the pores are small enough that most of the intracellular stuff stays in the cell (prolongs channel "life")

4. Excised outside-out patch (single channel resolution)

a) Start with whole cell clamp configuration

b) After rupture of membrane, electrode is pulled away from cell until membrane separates

(1) Ruptured membrane will seal so that outside membrane surface in on outside of electrode

(a) Membrane surface inside electrode is cytoplasmic face

H. Patch clamp records show the following characteristics

1. Channel opens or activates abruptly

a) Indicate opening and closing

(1) Explain inward current and downward deflection

2. Channel shows constant open conductance

3. Channel closes or inactivates abruptly

4. The open time varies

5. Channels often show flicker **where they briefly open or close**

6. Channels can flicker between the open state and partially closed states

a) This indicates that there may be substates between fully open and fully closed states

7. The noise level during the open state is higher than during the closed state

a) Greater noise probably the result of slight variations in the number of ions passing through the channel

I. Channel opening is stochastic

1. When the individual channels open is random & not predictable

J. Channel gating is Markovian

1. The probability of a channel transition is always constant – it does not depend on channel history (what it has just been doing has no impact on what it's about to do)

a) Applies to both opening & closing

K. The amount of open and closed time of the channel can depend on:

1. The voltage the patch is clamped at (for voltage sensitive channels)

2. For chemically sensitive channels, the presence and amount of the chemical

a) For example, the chemical could be a neurotransmitter, a second messenger or the ionic composition of the medium

L. Open probability time:

$$P_{open} = \frac{Time\ spent\ in\ open\ state}{Time\ spent\ in\ open\ state + Time\ spent\ in\ closed\ state}$$

M. Channels can have different kinetics, but the same open probability

III. Current-voltage relationships (I-V curves) of single channels

A. If you control the ionic concentrations on both sides of the patch, you can specify the equilibrium potential at any value you want

B. By varying the voltage across the patch you can examine voltage dependence of the channel

1. Plot channel current vs. transmembrane voltage to see

2. Also allows you to see reversal potential