Foundations I
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Voltage-Gated Ion Channels

I. Structure of ion channels

A. *Channels* consist of a hollow membrane bound protein coated with oligosaccharides (i.e., a glycoprotein) on the inner surface and filled with water.

B. The *selectivity filter* is engendered by a narrowing of the aqueous pore to restrict entry of ions based on the size of the hydrated ion. Water forms shells around ions. In a permeant ion channel, the pore wall itself is charged and solvates the ion (takes the place of the water molecules). Thus a pore is permeant to an ion if the pore diameter exactly matches the diameter of one of the spherical water shells around the ion. This is how a channel can be selective for K+, but not allow a smaller ion like Na+ in

C. The maximum channel conductance is a limited by (among many other things) the length of the pore itself.

Ion channels are not absolutely specific for certain ions. Thus the sodium channel is permeable to Na^+ ~Li^+>Tl^+>K^+>Rb^+>Cs^+. It's also slightly permeable to some small organic cations like ammonium. Consideration of the sizes of the hydrated forms of these ions suggests a minimum pore size of 3x5 Å for the sodium selectivity filter. The potassium channel is permeable to Tl^+>K^+>Rb^+>NH_4. The relative permeabilities depend on the type of K channel (see below). Calcium channels are permeable to Ca^{2+}, Sr^{2+} and Ba^{2+}.

D. *Gating Proteins* - If squid axon is perfused for a short time with certain proteolytic enzymes, most notably pronase, and then depolarized, the Na current comes on with its normal time course but does not inactivate. A similar phenomenon happens with perfusion with aldehydes or tannic acid, fixatives that cross-link proteins. Longer treatment with these agents eventually block activation of I_{Na} as well. Thus, the m and h gates appear to be physically as well as functionally distinct and composed at least partly of proteins. Furthermore, since only internal perfusion with these agents blocks the inactivation, the h gate is likely to be near the axoplasmic end of the channel rather than the extracellular end.

II. Voltage-Sensitive Na Channels and Conductances

A. Fast Na Channel (Na_v 1.1-1.9)

1. Structure - Each channels formed by 1 α and 2 β subunits. Each a subunit has 4 identical transmembrane repeat segments.
2. Density - evidence from radioligand binding studies suggests axonal density in the range of 35-500 channels/μm^2 (except at the nodes of Ranvier where it may be as high as 23,000/μm^2). Higher densities are associated with larger axons.
3. Conductance - on the order of 4-18 pS.

4. Blocked by tetrodotoxin (TTX - isolated from the venom of the puffer fish, and saxitoxin (STX)- isolated from marine dinoflagellates with Kds of 0.5 - 10 nM) or local anesthetics, e.g., cocaine, lidocaine. Blockade is use-dependent. The TTX has to get into the channel pore to block so the blockade requires the firing of several spikes.

B. **Molecularly defined a subunits**

1. Na_v 1.1-1.9
2. Channels containing Na_v1.5, 1.8, 1.9 and Na_x are TTX resistant
3. Persistent Na channel (non-inactivating)

III. Voltage-Sensitive Potassium Channels and Conductances

A. K+ channels- are much more diverse than Na channels. Several different K+ channels were originally identified in clones from drosophila mutants. These form the basis for 4 families, called Kv1 (shaker), Kv2 (shab), Kv3 (shaw) and Kv4 (shal). Channels consist of homo- or heteromers (within same family) of 4 pore forming a subunits and 4 b subunits.

1. Outward (Delayed) Rectifier - The original K channel (I_K) found in squid axon called was called the delayed rectifier channel, because it opened upon depolarization with a delay. The rectification means that the conductance of the channel depends on the membrane potential, i.e., conductance is increased by depolarization. This is seen empirically in IV plots where injections of positive current cause smaller voltage deflections than equal amplitude injections of negative current. I_K can be blocked by tetraethylammonium (TEA), 4-Aminopyridine (4-AP) or Cs^+ or Ba^+ ions. I_K does inactivate, like I_{Na} but with an extremely long time constant, ranging from 600 ms to several seconds in different membranes. I_K functions to limit the duration of the action potential

a. Density - evidence from patch clamp studies suggests a lower density of K channels, on the order of 7- 240/μm^2 except at nodes where it may be as high as 1100/μm^2.
b. Conductance - on the order of 2.4- 230 pS.
c. Kv 2.1/2.2 and Kv 3.1/3.2

2. Inward (Anomalous) Rectifier - Many neurons exhibit an outward current that increases with hyperpolarization instead of decreasing, hence the adjective "anomalous". At least in some systems, their voltage dependence seems to depend on $[K]_o$, something that is very different than I_K. The inward rectifier can be blocked by TEA, Cs^+ or Ba^+. This conductance appears to allow for long depolarizing episodes, since it closes upon depolarization. It appears that voltage-dependent blockade of the K_{ir} channel pore by intracellular Mg^{2+} and polyamines plays a critical role in the inward rectification. The channels form from K_{ir} 1.x – 5.x subunits.

3. A current (I_A) I_A–type channels (there are several) open upon depolarization *after* from a hyperpolarized holding potential. That is, it appears to be inactivated at rest. The conductance is differentially sensitive to TEA and 4-aminopyridine in different tissues. It serves as a mechanism for sustaining repetitive firing at low frequencies by slowing the ramp depolarization that occurs due to the decay of I_K following an action potential. K_v4.1, 4.2 and 4.3 subunits make up A channels as tetramers.

There is a "slow A" current in striatal neurons made up of K_v 1.2 subunits that activates and inactivates more slowly than K_v4.x channels. It can be blocked by dendrotoxin.

4. Fast Outward Rectifier (K_v 3.1-3.4)- K_v3.1 and 3.2 are fast activating, persistent channels. K_v3.3 and 3.4 are fast activating, rapidly inactivating. These channels are characterized by a very depolarized activation range (~40% at about +10 mV) and are often expressed by fast-spiking GABAergic interneurons that fire sustained trains of spikes at high frequencies. These interneurons often express the calcium-binding protein, parvalbumin, as in hippocampal basket cells, cortical fast-spiking interneurons and striatal GABAergic aspiny fast-spiking interneurons. Kv 3.x are blocked by TEA and 4-AP.

5. Calcium activated potassium conductance (K_{Ca}) - K_{Ca} channels are activated by intracellular levels of Ca. Their voltage dependence arises partly from the voltage dependence of Ca entry, but also because this conductance increases directly with membrane depolarization. There are 3 families of $I_{K(Ca++)}$

a. BK or maxiK channels – B for big –large conductance (200-400 pS). Blocked by TEA at low micromolar concentrations and scorpion toxins (charybdotoxin, iberiotoxin). BK channels are also voltage dependent and their activation by Ca++ increases with depolarization. BK underlies the fast spike afterhyperpolarization.
b. SK - S for small conductance (2-20 pS). SK channels are unaffected by low concentrations of TEA but are very sensitive to apamin, a bee venom toxin. SK underlies the so-called medium spike afterhyperpolarization.
c. IK – intermediate conductance. Not present in neurons.

6. Hyperpolarization activated cyclic nucleotide gated (HCN) cation conductance (I_h)- this is a mixed cation conductance that activates in the voltage range of -90 to -60 mV. It is requires cAMP, hence the designation HCN. The somewhat unusual composition of the current, Na^+ and K^+, give it a reversal potential around -43 mV. I_h produces an upward "sag" in the IV curves of neurons in current clamp mode in response to hyperpolarizing current injections. The onset latency of I_h is variable, with τ ranging from a few hundred ms to 1-2 s depending on membrane potential. I_h tends to prevent the neuron from becoming too hyperpolarized, and contributes to the resting membrane potential. I_h also contributes to rhythmic burst firing by its deactivation during a burst, which would tend to produce a large afterhyperpolarization following a burst which would then reset the low threshold Ca^{++} conductance and re-initiate the burst (see below) forming a pacemaker action. ZD7288 is the most common specific antagonist.

There are 4 different gene coding for I_h called HCN1-4.

IV. Voltage-Sensitive Calcium Channels and Conductances

A. T- Channels - have a low threshold (> -70 mV), are *rapidly inactivating* and therefore **T**ransient and have small a conductance (8 pS). They are sensitive to blockade by low µM nickel, but are resistant to Cd^{2+} and dihydropyridines. There are no specific t-channel antagonists. Although originally identified in cardiac muscle, this is the current that

underlies the *"Low Threshold Calcium Spike (LTS)*" seen in many different types of CNS neurons. The genes coding for the t-current are known as $Ca_v3.1$- 3.3

B. L- Channels - have a high threshold (> -10 mV) are *non-inactivating* and therefore **L**ong lasting and have a large conductance (25 pS). They are blocked by dihydropyridines and Cd^{2+}, and are relatively resistant to nickel. The genes coding for L-channels are $Ca_v1.1$-1.4

C. N- Channels - have a higher threshold (> -20 mV), are *slowly inactivating* therefore **N**either transient nor of prolonged duration and have a larger conductance (13 pS) than T-Channels. They are sensitive to blockade by Cd^{2+}, less sensitive to blockade by nickel than T-Channels and resistant to block by dihydropyridines. This is the current that underlies the *High Threshold* Ca^{2+} spike seen after TTX and TEA in many CNS neurons. The genes doing for N-channels are $Ca_v2.1$-2.2

V. Calcium-Activated Chloride Channels (CaCC).

These have been known to exists in other cells but have only recently been demonstrated to exist in a variety of CNS neurons. A specific antagonist is niflumic acid. CaCCs have been show to be involved with subthreshold membrane oscillations, modulation of action potential waveforms and EPSPs.