

Lecture notes courtesy of Wyan-Ching Mimi Lee. Used with permission.

2/11/04

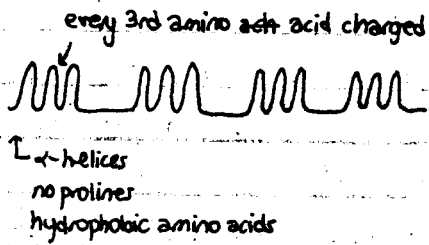
acetylcholine receptor - 5 subunits (2 α , 1 β , 1 γ , 1 δ): 4 homologous genes

- when 2 acetylcholines bind, opens, lets in Na^+

- 5 bent α -helices inside, as shown by crystal structure

- when ligands bind, α -helices swivel so channel opens; also forms polar environment to let ions through

voltage-gated Na^+ channel - every 3rd amino acid charged lysine or arginine: S5/S6 make channel, S4 moves through membrane (on outside of membrane in excited cell, inside in resting cell)



α -helices
 no prolines
 hydrophobic amino acids

↑ when positive inside: opens channel

gating of ion channels selective:



(Li smaller, Rb and Cs bigger)

- size filtration w/ naked ions lets Na^+ through, K^+ out

- but ions don't exist naked in aqueous solution; have waters of hydration

- the smaller the naked ion, the bigger the shell of hydration, so smaller ions actually bigger

- to conduct naked ions through channel, must line up polar amino acids to replace/mimic shell of hydration

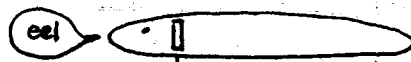
- let through naked K^+ (w/ polar proteins) but only sterically mimics shell of hydration of K^+ , Na^+ too small to be fooled (can't touch enough polar groups)

cloning membrane proteins:

1. purify, sequence, read genetic code backwards to make oligonucleotides, probe cDNA library

- but hard to purify b/c lipid proteins: need 2 tricks:

1. pick animals full of channel proteins, eg torpedo torpedo and Electrophorus electricus (electric eels and rays): electric organs full of channel proteins



degenerate muscle cells called electroplaques: no actin/myosin, but one side innervated w/ tons of synapses, dense in ACh receptors, also voltage-gated Na^+ channels

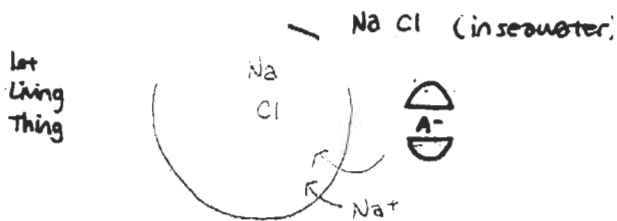
} almost 100% protein is these channels

receptor

2. cobra toxin binds to ACh channel and blocks, you can't breathe, etc

Krait (seasnake, Bungarus multistriatus multistriatus) makes bungarotoxin, principle component is

- α -bungarotoxin: like cobra toxin but tighter binding (use for ACh receptor binding)
- get toxin, label w/ radioactive iodine, bind to electric animal homogenate, etc., use Edman degradation to get oligonucleotide to probe libraries
- inject cDNA into *Xenopus* oocytes (lots of ribosomes etc, waiting to be fertilized so can start translating protein)
 - if put in foreign DNA, can be made + trafficked right away
 - clone all 4 genes for 5 subunits, put in oocyte, express channel protein (test by patch clamp w/ ACh in microelectrode, look for currents of right size)
- this system also worked for voltage-gated Na^+ channel, but need different toxin
 - pufferfish fugu makes tetrodotoxin, causes action potentials to disappear by blocking Na^+ channels
 - liver has a lot of tetrodotoxin
- 2. once you get some channels, others often homologous (eg Na^+ homologous to Ca^{2+} channels)
- 3. if no high-affinity toxins, take mutants (eg shaker), map mutation, clone gene positionally, look for membrane-spanning regions, test in oocytes



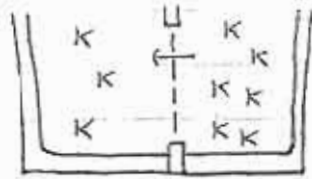
did fine until it wanted to eat
ate proteins (negative charge),
 Na^+ went in to balance charge,
more ions inside \rightarrow osmotic pressure,
1st living thing blows up

- need to take control of own ionic destiny: fill self up w/ different type of ion so can balance ion conc concentrations inside, outside; eg w/ Na^+/K^+ pump (K^+ uncommon in seawater)
- blood similar to seawater, more NaCl in it than in cells, more K^+ in cells
- Ca^{2+} very low in cell, b/c used as messenger (hide it eg in mitochondria, rise only when you want to have signal, et eg synaptic vesicle fusion)

$\text{Na}^+ \text{K}^+$ ATPase pumps K^+ in, Na^+ out: makes 2 gradients

ouabain, digitalis toxins that block this pump

- gradients + channels give you energy:



flow left will give voltage
w/ left side positive

membrane permeable only to K^+
if holes closed, can have more K^+ on one side,
when open, will run down gradient

- will not flow until equal: when equilibrium reached, energy gained by going down concentration gradient will balance energy lost by going up voltage gradient: Nernst equilibrium
- can find voltage w/ Nernst equation

- At Nernst equilibrium, for an ion crossing barrier: $\Delta E_{chem} = \Delta E_{electrical}$

$$G \text{ (free energy)} = G^* + RT \ln [K^+]$$

- ↳ energy in stuff itself
- ↳ energy to squash K^+ in concentrated form
- b/c hydrated, act like ideal gas

$$\Delta E = RT \ln [K^+]_{initial} - RT \ln [K^+]_{final} \quad \text{subtractive logs divide}$$

$$\Delta E = RT \ln \frac{[K^+]_i}{[K^+]_f} \quad \text{(from definition of Gibbs free energy)}$$

voltage - work done transporting charge up potential gradient divided by charge

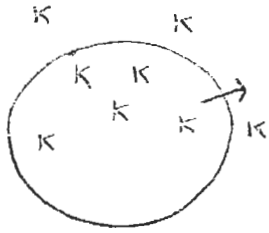
$$\Delta E_{elec} = qV$$

$$\text{for mole of electrons, } = zFV$$

- ↳ Faraday constant
- ↳ charge (eg +2 for Ca^{2+} , +1 for K^+)

$$RT \ln \frac{[K^+]_i}{[K^+]_f} = zF$$

$$\text{solve for voltage: } V = \frac{RT}{zF} \ln \frac{[K^+]_i}{[K^+]_f}$$



inside K^+ -rich

cell membrane only permeable to K^+ . (not quite true, but approximate)

- net positive charge \rightarrow outside, so inside negative

- practically, at 25°C, $V = 58 \text{ mV} \log \frac{[K^+]_o}{[K^+]_i}$

$$58 \text{ mV} \log \frac{[20]}{[400]}$$

$$= 58 \log \frac{1}{20} = 58 (-\log 20)$$

$$= \cancel{58 (-1.2)} \quad 58 (-1.2) = -69 \text{ mV}$$