2/11/04

acetylcholine receptor - 5 subunits (2α, 1β, 1γ, 1δ) : 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: Na^+ K^+ (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

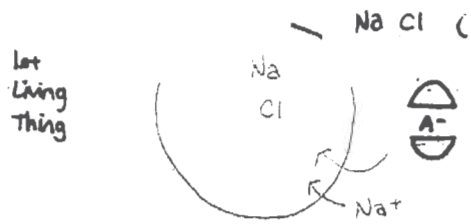
↳ degenerate muscle cells called electroplaques: no actin/myosin, but one side innervated w/ tons of synapses, dense in ACh receptors, also voltage-gated Na^+ channels } almost 100% protein is these channels

receptor

2 cobra toxin binds to ACh channel and blocks, you can't breathe, etc
Krait (seasnake, Bungarus multisigno multicinctis) 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



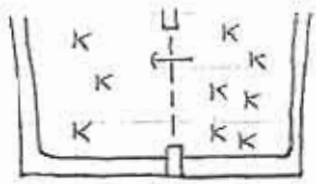
did fine until it wanted to eat ate proteins (negative charge), Na^+ went in to balance charge, more ions inside → osmotic pressure, 1st living thing blows up

- need to take control of own ionic destiny: fill self up w/ different type of ion so can balance ion cont concentrations inside, outside; eg w/ Na^+/K^+ pump (K^+ uncommon in sea water)
- 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)

Na^+ K^+ ATPase pumps K^+ in, Na^+ out: makes 2 gradients

~~ouabain~~, digitalis toxins that block this pump
ouabain

- gradients + channels give you energy:



membrane permeable only to K^+

if w holes closed, can have more K^+ on one side, when open, will run down gradient

flow left will give voltage w/ left side positive

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

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

- G (free energy) $= G^* + RT \ln[K^+]$
  - G^*: energy in stuff itself
  - $RT \ln[K^+]$: energy to squash K^+ in concentrated form b/c hydrated, act like ideal gas

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

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

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

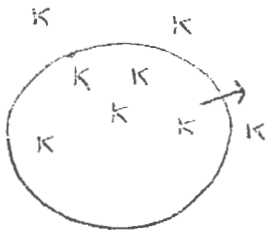
$\Delta E_{elec} = qV$

for mole of electrons, $= zFV$

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

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

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 → outside, so inside negative

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

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

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

$$= 58(-1.2) = -69mV$$

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