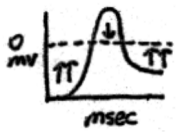
3/3/04

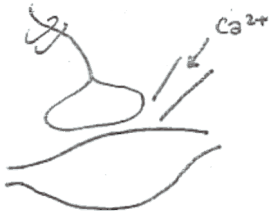
muscle AP 

reversal potential = 0 (or -15 mV, depending on muscle cell)
b/c AChR lets in Na^+ and K^+

- collagenase to get postsynaptic side clear, patch clamp (w/ ACh in blunt microelectrode), look at current flow (magnitude & direction) ↳ voltage clamp
  ($V_m < 0$; current negative = inward)
  $V_m > 0$, current are positive = outward)

- ionotropic receptors have reversal potentials
  - if $E_{reversal}$ above or below threshold, determines if excitatory or inhibitory
    if $E_{reversal}$ = threshold, will not affect either way

- stimulate extracellularly presynaptic, record intracellularly postsynaptic
  - need Ca^{2+} (4) for presynaptic transmitter release, EPSP
    - cobalt interferes w/ natural Ca^{2+} leak from slightly damaged muscle



Ca^{2+} must be near synaptic cleft, present around when AP reaches terminal (or right before reaches terminal)

treated whole system w/ TTX, increased stimulus intensity to passively conduct to terminal: found that pure voltage could mimic AP: evidence for voltage-gated Ca^{2+} channels
- intracellular presynaptic injection of Ca^{2+} also gives EPSP

- minis - correspond to small releases of ACh (b/c can affect w/ curare, neostygmine)
  - make up ~~m~~ EPSP: reduce Ca^{2+}, stimulate presynaptically, record postsynaptically
    - effect comes in quantal sizes (Mn)
  - Gaussian distribution around 0.4 mV ↳ direct evidence for quantal transmission
  - large number of vesicles w/ equal & ~~p~~ independent probability of release (from Poisson curve assumptions)

- make preparations of synapses, synaptic vesicles
  - synaptobrevin - V-SNARE (important in exocytosis)
    - target of botulinum toxin (BOTOX) & tetanus toxin
    - mutants homologous to yeast mutants w/ exocytotic defects
      - yeast cycle vesicles between inner & outer lamellae
      - bad cycling due to mutant v-snares & t-snares
      - vesicle endocytosis looked at in cell-free dog pancreas system; these same proteins showed up
  - synaptotagmin - Ca^{2+} binding domain: this is the calcium sensor for vesicle exocytosis

- neuromuscular junctions can be modulated
  (eg by chip screaming - potentiated by adrenaline)
  - potentiated by sympathetic stimulation (neuromuscular transmission)
    - Orbelli effect (controversy: presynaptic or postsynaptic?): turns out is both
      - do quantal analysis before & after sympathetic stimulation: more quanta released per action potential will give more double, triple, etc releases (average # releases), if presynaptic effect; if postsynaptic (eg more ACh Rs, or more sensitive ACh Rs, will ~~not~~ affect placement of peaks by shifting histogram right (eg 0.4 mV → 0.6 mV)
      - $P_{(x)} = e^{-m} \frac{m^x}{x!}$ (but there is better way)
        ↳ easy to tell 0's from 1's : $P_0 = e^{-m}$ $\left(\frac{m^{x=1}}{x!=1}\right)$

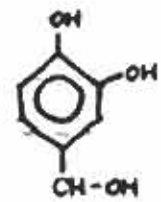
        so: if presynaptic → ↓ P_0 (increase mean quantal content) (P_0 decreases)
        measure fraction of failures, see if goes up or down
        ~~if postsynaptic~~
        if postsynaptic, will affect quantal size

- mean quantal content = average #quanta (vesicles) released per stimulus (not contents of vesicle)
  - measure fraction of failures, plug into $P_0 = e^{-m}$ no dimensions
    ↳ fraction of failures
    higher quanta release # = lower probability of failure

to measure change in quantal size, look at minis, look at size of mEPSPs
- this will increase if Orbelli effect postsynaptic
- quantal size $\overline{V}_1$ = average voltage displacement of quantum ~~of~~ or spontaneous miniature potential
  not physical size of vesicle (all ~ same size, same density of transmitter)

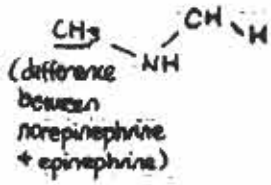
Orbelli effect: more ~~adrenaline~~ norepinephrine comes down sympathetic axons to neuromuscular junctions

- adrenal glands secrete another compound (cycles around your body): ~~norepinephrine~~ epinephrine
- these result in presynaptic enhanced quantal release (NE)
- also postsynaptic response from circulating hormone (EPI)



phenylalanine → tyrosine → tyramine → dopamine → noradrenaline

adrenaline ← (norepinephrine)
↳ NE



(difference between norepinephrine + epinephrine)

adrenaline from adrenal medulla (on top of kidney)

- epinephrine same in Greek
  ↳ EPI

norepinephrine & epinephrine both from phenylalanine, differ by methyl group

antagonist: β-propanolol ~~isoproterenol~~ (EPI antagonist) drug used for heart conditions ~~asthma~~ in hospitals, stage fright

- applying this gives no change in quantal size
- better music performance

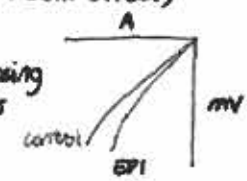
epinephrine - agonist = isoproterenol
(adrenaline) antagonist = β-propanolol (block adrenergic response on postsynaptic side)

presynaptic - look at number of failures (Cuba found less failures)

norepinephrine - agonist = norepinephrine
(noradrenaline) antagonist = clonidine (blocks presynaptic response, gives pure postsynaptic)

postsynaptic - look at quantal size (will be bigger w/ Orbelli effect)

- postsynaptic se increase actually from epinephrine closing leakage channels



depolarizing current leaks out less if R↑ (R input)
V = IR
so R↑, V↑
depolarizes more & longer

I from ionotropic AChRs: doesn't change, depolarizes cell, but leaks out b/c muscle is big: plug up leakage holes in muscle, increase R

- modulated synapses - have volume control
- norepinephrine + epinephrine are modulatory transmitters (gain control)