Lecture 5: Myofilaments

- Tropomyosin and troponin overview
  - Tropomyosin and troponin physically prevent myosin and actin from interacting
    - Tropomyosin is bound to the actin
    - Troponin
  - **Troponin complex has 3 proteins**
    - 1. **Troponin T** (anchors to the tropomyosin)
    - 2. **Troponin C** (for Ca++ binding)
    - 3. **Troponin I** (inhibitory element)
      - Folds in a way that creates an inhibitory region that prevents interaction between myosin and actin
    - Process of removing inhibition
      - Ca++ binds to troponin C
      - Conformational change of troponin moves Troponin I out of the way
        - Now, there is a higher probability that actin and myosin will interact
  - After the troponin inhibitory element is out of the way, the myosin head pushes the tropomyosin out of the way and interacts with the actin
    - Tropomyosin are linked, so pushing one out of the way helps move the other tropomyosin out of the way too!
      - This makes it easier for other myosin heads to bind to the actin filament
      - This effect doesn't influence the all the tropomyosin and myosin heads, but extends to a couple neighbouring tropomyosins
    - So binding one myosin head to the actin filament helps the other myosin heads bind too!
- Slide 6. How myosin head flexing can create muscle cell contraction
  - 1. **Blocked state**
    - Ca++ levels are low, so troponin I prevents myosin head from binding to actin
  - 2. Ca++ levels increase
    - Troponin I moves out of the way when Ca++ binds to Troponin C
    - There is still a chance that the myosin head doesn't bind to the actin
    - Ca++ can unbind, and then we go back to the blocked state
  - 3. Myosin head binds
    - It's a **weakly bound state** though [A] , and the actin-myosin contraction hasn't occurred yet
    - Maybe only a few amino acids are holding the myosin and actin together
  - 4. **Strong state** [B]
    - More and more points of interaction occur between the actin and myosin
    - The tropomyosin is pushed further out of the way
  - 5. Myosin head flexes
    - The ATP molecule in the myosin head is broken
    - The energy released propels the myosin head towards the center of the cell
      - The actin-myosin interaction is still there!
      - So when the myosin is propelled towards the center of the cell, the actin filament is also pushed towards the center of the cell
      - If the other end of the actin filament is anchored to the cell membrane, then moving the actin filament to the center of the cell contracts the cell
- Regulating myofilaments
  - **PKA cascade**
    - All actions of PKA favour muscle contraction and heart rate/stroke rate increase
    - Troponin I phosphorylated by PKA,
      - Between S23 and S24 residues
      - Helps the myosin head release faster (rate of relaxation enhanced)
        - Myosin can grab and pull actin more often and faster
    - L type Ca++ channels
    - Ryr phosphorylation (keep RyR more open, allow Ca++ to come out)
    - PLB phosphorylation (less likely to bind and inhibit SERCA, SERCA can pump more Ca++ back into the SR)
  - Disease state
    - If contraction strength is decreased (heart isn't pumping the blood forcefully enough), the heart can compensate by increasing heart rate
      - However, if you pump too fast, the heart chambers don't get to fill up properly
      - This is very ineffective
    - A lot of drugs focus on increasing Ca++ levels to increase heart rate
      - But this stresses the cells, and kills them off
      - Then you try to push the remaining cells harder, killing them off even faster
      - However, a Ca++ sensitizer drug wouldn't increase the Ca++ levels
        - So you wouldn't be over-stressing the cells, but would be able to sensitize to cell to contract harder to lower Ca++ levels
  - **The promise of Ca++ sensitizers**
    - ???
    - Pimobendan was causing more mortality in humans…???

- But was effective in dogs
- More investigation is needed

- Levosimendad
  - It was decreasing a biomarker of heart failure
  - But not mortality…
- Are Ca++ sensitizers solving the issue, or masking it?
  - If the problem is that the myofilaments are insensitive to Ca++, but the Ca++ levels are normal, a sensitizer solves the issue
  - If the problem is that the Ca++ levels are low, using a sensitizer only masks the issue
    - If you administer a sensitizer, the Ca++ levels might drop even further!
- In Doberman Pinschers, there is a big issue with Ca++
  - This dog breeds' hearts are
    - Slow to activate and have poor force generation, but there are no issues in relaxation
  - About half to a third of the normal Ca++ is entering the cardiac muscle cell..
  - **Deoxy-ATP**
    - Helps push the weakly bound state [A] of myosin-actin to the strong state [B]
    - Composes ~1% of ATP stores
      - The other 99% are regular oxy-ATP
    - Gene therapy to increase dATP to 2%
      - Doesn't impact the regular ATP stores significantly (98%)
      - This therapy was given to pigs 2 weeks after a heart attack
      - Showed great results!! (slide 16)
- **Myosin activator omecamtiv mecarbil**
  - Favours the strongly bound state [B] from the weakly bound state [A]
    - This is the rate determining step, so speeding it up greatly speeds up contraction
    - It doesn't speed up the velocity of myosin head flexing
  - Only targets cardiac myosin, not affecting other cells
  - Slide 17
    - Left image: the drug is targeting the myofilaments directly by making them contract (shrink) more
    - Right image: the drug is not making Ca++ levels increase!
      - Stays true as a Ca++ sensitizer, no added cell stress!
  - Slide18
    - Modest decline in Heart Rate, and substantial increase in Stroke Volume
  - Slide 19
    - A short infusion of omecamtiv mecarbil has significant improvement in humans
      - Won't be tested on the details
  - There is testing for this drug in oral form, and there seem to be good effects

- Titin
  - Very elastic protein that contributes a big passive force in cardiac myocytes
    - Unwinds and stretches out when the heart relaxes
    - Coils up during contraction
      - The coiling up provides some force for the contraction phase!! (very little tho)
  - Binds to myosin and the Z line (boundaries of the sarcomere)
  - Stiff titin makes it harder for the heart to relax
    - It fills will less blood! We want relaxed titin
  - **Phosphorylate titin by PKA and PKG**
    - The cell is able to stretch a little more easily (titin is more stretchy)
      - And so the passive force goes down
  - **Phosphorylate titin by PKC**
    - The cell has a harder time stretching because the passive force increased
      - Titin is stiffer
    - In failing hearts, we see an increase in PKC activity!!
      - This is no good, because the heart is becoming more stiff
  - Ideally, we should phosphorylate stiff cells with PKA and PKG to relax it
    - **2 pathways to increase PKA levels**
      - 1. **Increase β-adrenergic receptor action**
        - Go thru cascade
        - Ultimately, there will be more PKA expression, which will phosphorylate titin at certain spots (residue S469) to make it more stretchy
        - This isn't favourable, because the β-adrenergic cascade will have other effects, and is usually shut down in patients with heart failure
      - {2}. **Prevent cAMP breakdown by PDE** (using a PDE inhibitor)
        - cAMP levels will be higher, which will increase levels of PKA
    - Increase PKG levels
      - Similar method to {2}
        - See the similarities between the pathway diagram shown on slide 23 and 24
      - **Prevent cGMP breakdown by PDE-5** (using a PDE-5 inhibitor)

    - cGMP levels will be higher
- **Sildenafil** (a PDE-5 inhbitor) will increase levels of cGMP, and PKG
    - Administration of sildenafil decreased ventricular stiffness in patients with heart failure
    - But…
        - There weren't any clinical benefits
        - Much like the SERCA clinical trials