EXCITATION-CONTRACTION COUPLING IN SKELETAL MUSCLES[1]

Summary: The sequence of events from the movement of an AP moving down a neuron to the completion of a contraction is examined. These events are referred to as excitation-contraction coupling. Next we examine the importance of elasticity in the operation of muscles using Hooke's Law as a simple model of muscle elasticity. Both series and parallel elastic elements are discussed. These notes conclude with a brief introduction to the concept of tissue viscosity and viscous loss as an internal work component in muscles.

I. **EXCITATION-CONTRACTION COUPLING (E-C Coupling)**

A. Definition: **the events that translate an AP (Action Potential, a membrane-electrical event) into a mechanical event (contraction).**

B. The most important things to understand about EC coupling deal with regulatory events. There are essentially two sites of regulation in skeletal muscles:

1. **the membranes** -- both in terms of the conduction of the AP and also the control over sarcoplasmic (myoplasmic) Ca^{++}.

2. **the Ca^{++} binding proteins associated with the myofibrils**. In skeletal muscles this is a function of the behavior of troponin.

3. **NOTES ABOUT Ca^{++} REGULATION by the SR**

a. the membrane-bound Ca^{++} /ATPase that will transport 2 Ca^{++} into the interior of the SR (especially the cisternae) for every ATP hydrolyzed.

1. This pump's activity depends on the relative amount of work that needs to be done to transport a Ca^{++} ion up a concentration gradient as compared to the amount of free energy that is available due to the hydrolysis of ATP to ADP and Pi.

The work needed to move Ca^{++} up a concentration and electrical gradient is given as:

1. $$\Delta G = -n\,R\,T\,\ln\left\{\frac{[Ca^{++}]\text{ in SR}}{[Ca^{++}]\text{ in sarcoplasm}}\right\} - n\,F\,Z\,E$$

(see Packet Q&T-2: Metabolism and energetics). Likewise, the free energy of synthesis (opposite of hydrolysis) of ATP is given as:

2. $$\Delta G = \Delta G^{o'} + R\,T\,\ln\left\{\frac{[ATP]\,[H_2O]}{[ADP]\,[Pi]}\right\}$$

Thus, for transport to occur:

3. ΔG for ATP hydrolysis > ΔG for Ca^{++} transport into cisternae

! Why is all of this important? In resting muscles, there is normally far more ATP than ADP (thus eq. 2 predicts more than enough energy to move Ca^{++} up the 1000X fold gradient that often exists), there are

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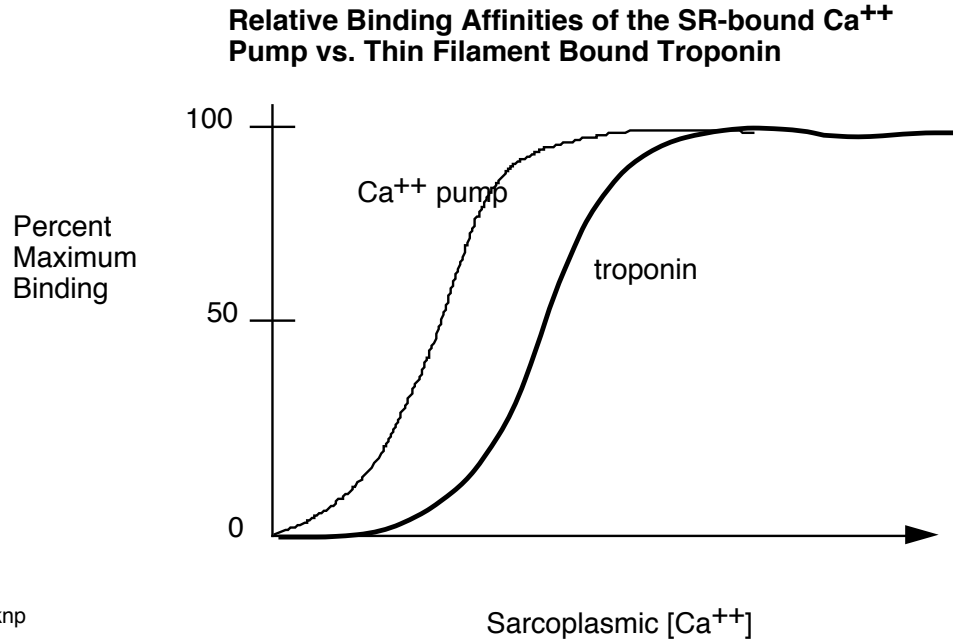
cases in muscles where the [ATP] drops so far that this is no longer possible. This represents one possible cause of fatigue (see later).

2. **The Ca^{++} pump has a higher affinity for Ca^{++} than does troponin and can out-compete troponin for Ca^{++}**

3. The affinities of the Ca^{++} pump vs. troponin can be visualized by a graph:



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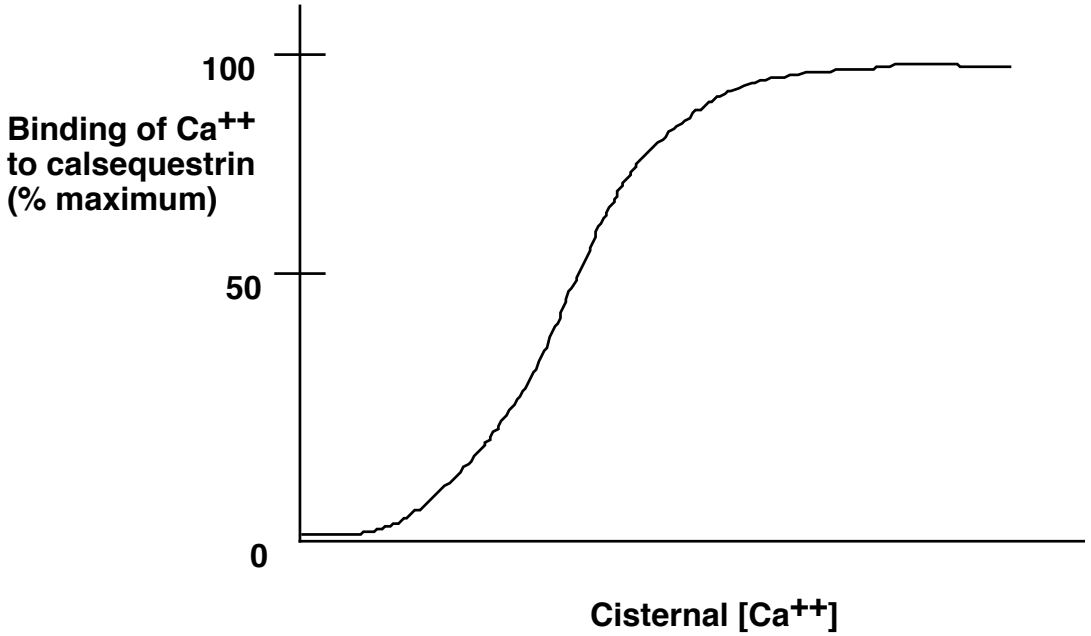
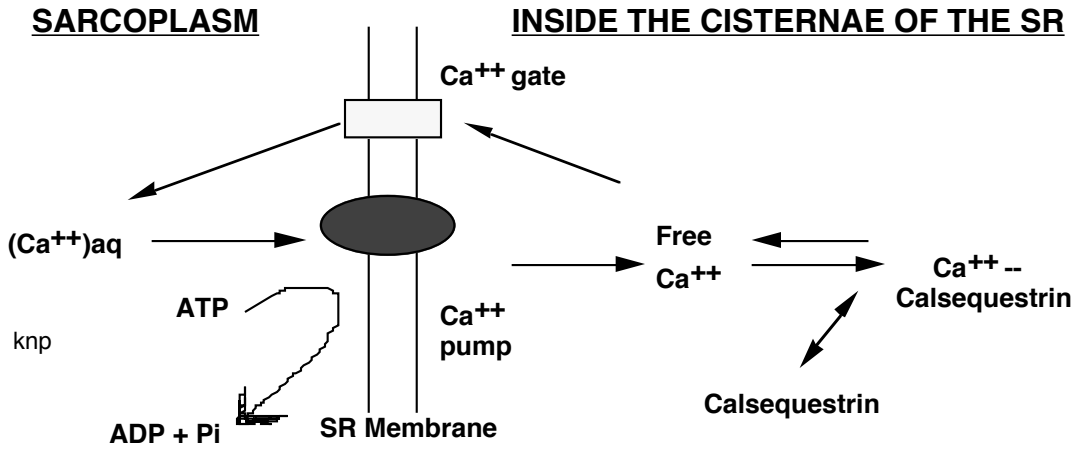
b. the assistance of a protein called **CALSEQUESTRIN** that is found inside the cisternae. It follows a binding curve that is also similar to hemoglobin and to the pump and troponin:



the fact that it performs a different function is related to the fact that it is in a different location and has different affinities than these other proteins.

1. Large amounts of Ca^{++} bind to this protein and are thereby removed from solution inside of the SR. Thus, the $[Ca^{++}]$ in the SR is lowered and this produces a more favorable (i.e., less unfavorable) gradient for pumping Ca^{++} into the SR.

2. The Ca^{++} bound to calsequestrin is **in equilibrium** with the Ca^{++} in solution, thus it ***BUFFERS*** **SR $[Ca^{++}]$** and keeps it from getting too high and also acts as a reservoir if large amounts are released. **Thus, the action of calsequestrin is like that of Hemoglobin in blood** in terms of how it behaves with respect to O_2 and CO_2.



Calcium ion movement in skeletal muscle. There are two aqueous pools of Ca^{++} **-- one in the sarcoplasm which is in equilibrium with the Ca^{++} pump and with troponin (they compete for it) and which also receives Ca^{++} from the cisternae via gated channels in the cisternal membranes. The other free Ca^{++} pool is in the cisternae where it is in equilbrium with the storage binding protein** calsequestrin **and also with the sarcoplasmic pool when the gate is open.**

c. Ca^{++} can enter the sarcoplasm either via the SR or from the sarcolemma.

1. In skeletal muscle cells, the SR is by far the most important route, but in other types of muscles the role of the sarcolemma may become the most important.

2. Ca^{++} enters by specialized gates, we will not worry about their exact nature at present.

d. In the sarcolemma and perhaps the t-tubules, Ca^{++} is allowed to enter the cell in direct response to an AP: a slow Na^{+}/Ca^{++} channel opens.

e. In the SR, there is probably no AP, so Ca^{++} release is by some other factor that was set-off by the AP that entered the cell at the T-system.

C. EVENTS OF EC-COUPLING IN SKELETAL MUSCLE DURING A SINGLE TWITCH[2]:

1. **AP arrives at the presynaptic end of the Axon, Ca^{++} influx into axon causes the release of ACH**.

2. **ACH binds with the neuromuscular ACH receptor** and **causes Excitatory Post-Synaptic Potentials** (**EPSPs**), these sum to produce an AP. Meanwhile the **ACH is degraded by acetylcholinesterase**.

3. An **AP is conducted along the muscle fiber**.

4. The **AP is also conducted into the cell via the opening to every t-tubule**.

5. Once inside, the **AP indirectly triggers the release of Ca^{++} from the cisternae**. It flows down a [gradient] into the sarcoplasm and the sarcoplasmic [Ca^{++}] increases rapidly above the resting level of 10^{-7} M. ***Note that the Ca^{++} pump continues to be active the whole time but Ca^{++} enters the sarcoplasm at a much higher rate than it can be pumped back.***

6. **The Ca^{++} binds to troponin**, this induces a conformational change in the troponin that results in the troponin moving the tropomyosin away from the myosin binding site on actin.

7. **Myosin * ADP * Pi complex binds to actin, forming a CROSS-BRIDGE** and causing the release of ADP and Pi. A-M complex has a low affinity for ATP.

8. The myosin head rotates and pulls the thin filament towards the center of the sarcomere. When this is complete, the myosin in the A-M complex has a high affinity for ATP.

9. ATP binds to the myosin on the A-M complex, causing the cross-bridge to break and yielding A + M-ATP.

10. The myosin ATPase partially hydrolyzes the ATP in the M-ATP complex to yield M * ADP * Pi. This complex is now primed to form a new crossbridge.

11. Meanwhile the sarcoplasmic [Ca^{++}] is rapidly decreasing via the action of the Ca^{++} ATPase. Since the Ca^{++} bound to the troponin is in equilibrium with the dissolved sarcoplasmic Ca^{++}, and since the pump has a greater affinity (lower Km) for Ca^{++} than troponin, the amount of Ca^{++} on troponin decreases.

12. As more and more Ca^{++} is re-sequestered, the troponin changes shape back to a conformation that moves the tropomyosin back over the binding site for myosin on actin. Steric hindrance is restored. Note: even if a crossbridge exists at the time that the troponin tries to change shape (and prevents it from moving the tropomyosin over the actin), you can visualize the tropomyosin as "leaning on" the myosin. As soon as the crossbridge breaks, the troponin will slip the tropomyosin into inhibitory position.

13. At this point the contraction is over. Note the following:

a. the conditions now exist such that the muscle is primed for its next contraction -- Ca^{++} is stored and ready to flow down its concentration gradient and each myosin head has partially hydrolyzed its ATP.

b. Note that the direct use of ATP in muscle contraction is to BREAK-UP THE CROSSBRIDGE. The energy needed for the next contraction is stored in the myosin before the crossbridge is formed. No constant input of energy is needed to maintain the formation of crossbridges -- in the absence of ATP the bridges are stable.

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[2] A **twitch** can be defined as the contraction that results from a single AP.

? What processes involved in EC-coupling use ~P directly? Indirectly?

What fuels the movements of the troponin-tropomyosin complex? How much free energy is potentially available for this process? (Assume sarcoplasmic [Ca^{++}] changes from 10^{-7} M to 10^{-6} M in a single twitch.) Compare that with the energy available from ATP (see the earlier problem on page 1 of these notes).

Rigor mortis is a well-known, "permanent" contraction of muscles in organisms that are truly feeling no pain.

Explain in detail the development of *rigor mortis* and its maintenance.

The change in the shape of troponin obviously involves the doing of work and therefore must have an energy source. What is this energy source? Explain.

14. We will review some of the differences between contraction in striated and smooth muscles in the next class..