

Question 1

Chapter 13

A pediatric patient is brought to the clinical research division of **St Jude's Children's Hospital**. The patient presents the following symptoms:

Symptoms:

1) Abnormal high plasma levels of the following enzymes: (these enzymes are never found in blood plasma of normal people)

alpha-L-Fucosidase Heparinase I Cathepsin 3 Cathepsin 6 Arylsulfatase
G/ARSG Iduronate 2-Sulfatase/IDS

2) Delays in growth and bone structure developmental

3) abnormal skeletal development,

4) restricted joint movement,

5) enlargement of the liver and spleen,

6) stiff claw-shaped hands

7) cornea clouding

8) recurrent respiratory tract infections,

.

A) What is your prognosis? What disease does this patient have?

B) What "key" enzyme is defective in this well-known disease and which cell organelle is the key enzyme a resident of?

C) What is the normal function of this "key" enzyme in normal individuals? Explain and draw a diagram that demonstrates how this enzyme works

D) What is the molecular cellular basis for the appearance of the alpha-L-Fucosidase Heparinase I Cathepsin 3 Cathepsin 6

Arylsulfatase G/ARSG Iduronate 2-Sulfatase/IDS in the patients blood serum. How did these enzymes end up abnormally in the blood?

E) What is the molecular cellular basis for the enlargement of the liver and spleen in these patients...how does the defect in the enzyme result in organ enlargement.

F) Design an experiment involving **western blotting (immunoblot)** and primary and secondary antibodies that proves the patient has the disease you have hypothesized they have.

Your western blot experiment should also prove the enzyme in that disease is not working. Draw out exactly what the western blot's expected results would look like based on the predictions you would expect if the enzyme is defective. Make sure you include control experiments with samples from normal unaffected patients versus those samples from affected patients. Make sure your drawing of the western blot has a loading control as well.... In your design description describe how..what method... you will use to get proteins from patient cell samples..., explain the method of making the gel and loading the gel....explain how you will transfer the proteins to a support to detect the transferred proteins...etc....western blot procedure should be detailed and clear. (research the actual antibodies you could purchase and use)

Question 2

Chapter 11

The ATP1A2 gene codes for the Na⁺,K⁺-ATPase aka the sodium potassium pump which is required in neurons for neurotransmissions involving propagated alterations in membrane potential. The Na⁺,K⁺-ATPase is a cation-transporting integral membrane protein involved in active transport of sodium and potassium ions against their concentration gradients across the cell membrane. For every molecule of ATP consumed, three sodium ions are exported from the cell in exchange for import of two potassium ions. The Na⁺,K⁺-ATPase serves many roles, including cell-volume homeostasis as well as maintenance of the membrane potential. In addition, the generated Na⁺ and K⁺ gradients drive various **secondary active transporters**. Since transport of Na⁺ and K⁺ across the cell membrane takes place against such steep concentration gradients, the enzyme consumes high amounts of energy: approximately 25–50% of cellular ATP is consumed by the Na⁺,K⁺-ATPase.

Mutations in the **ATP1A2** gene have been determined to be one the causes of **Familial Hemiplegic Migraine** types 1 and 2.....FHM & FHM2. Recent reports have suggested that patients with mutated ATP1A2 genes may be getting these migraines due to “synaptic fatigue” caused directly or indirectly by the mutated Na⁺/K pump. In these cases neuron to neuron synapses require pre-synaptic glutamate release and require glutamate binding to triggered channels (AMPA) on the post synapse leading to channel opening; letting Na⁺ into the post synaptic cell and also fostering Ca²⁺ entry via Nmda receptors. It is after these steps that “synaptic fatigue” potentially might occur at the presynaptic membrane due to the mutated Na/K pumps within the synapse region leading to migraines.

A. Hypothetically suggest “in detailed description” two (2) possible models that would explain how defects/changes in the amino acid sequence of the Na⁺,K⁺-ATPase membrane protein (due to mutation in the gene) might result in “synaptic fatigue”. In at least one of these 2 proposed models explain how the mutant Na/K pump together with normal synaptic localized Astrocyte activity might be co-involved in causing the abnormal outcome of chronic “synaptic fatigue” associated with and exhibited in these FHM patients. (hint look up role of astrocytes at synapses that release glutamate)

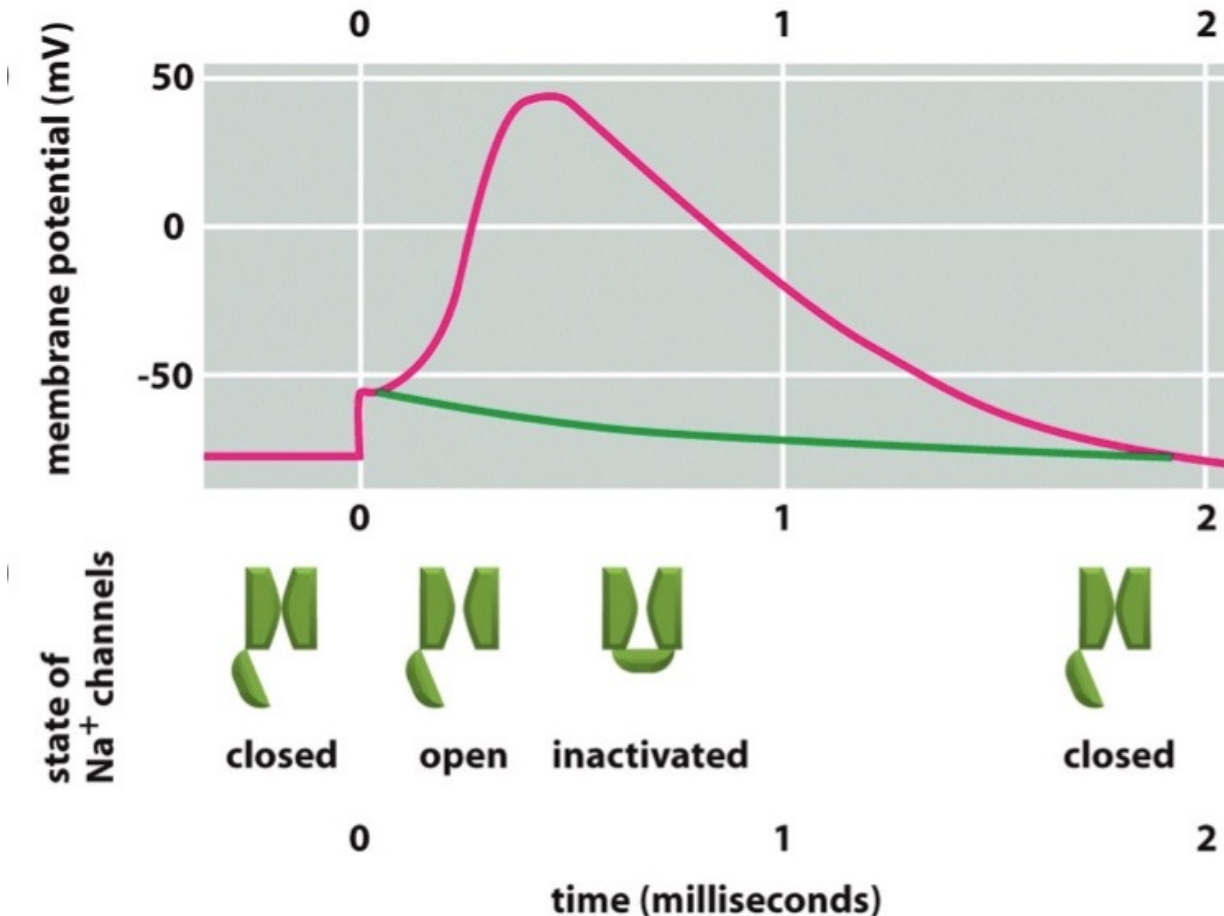
B. Suggest an experiment or experiments to validate or falsify your hypothesis...for one of your posited models.

Question 3

Chapter 11

You are interested in understanding how the neuromuscular junction works. (see figure 11-39--5th ed)

Normally glutamate binding at the post synaptic membrane leads to post synaptic membrane depolarization via Na^+ entry into the muscle cell at the Acetylcholine gated cation channel which propagates to nearby voltage-gated Na^+ channels leading to more Na^+ entry. As the action potential propagates away from the channel and down the t tubules it ultimately causes Ca^{2+} release from the smooth ER which then Ca^{2+} binds troponin C leading to actin-myosin II contractions of muscle. The normal post synaptic voltage-gated Na^+ channel in the muscle cell membrane that responds to the depolarization caused by Na^+ entry at the Acetylcholine gated cation channel typically has the action potential characteristics depicted by the solid red line in (figure 11-29--5th ed below). In an experiment of your design you decide to replace this normal voltage gated Na^+ channel with a mutant voltage-gated Na^+ channel.....coded for by a mutant SCN1A gene



In your experiment you test and discover that this mutated voltage-gated Na^+ channel can bind to Na^+ ions with equal affinity as the wild-type gated Na^+ channel. But when the mutated post synaptic voltage-gated Na^+ channel's membrane potential is measured after adding acetylcholine it only reaches +20 mV at 0.5 ms (30 mV below the normal channel's max level at that time) (see figure 11-29--5th ed) and its downstream propagated depolarization event is severely delayed and hence triggers calcium release from the smooth ER 50ms later than normal (see figure 11-30 and 11-39--5th ed)

A. What might have this mutation done to the Na^+ channel to alter its ability to reach a max positive membrane potential of +50 mV at 0.5 ms.

Based on your answer above... how and why would this then delay the continued propagation of the "next wave" of depolarization needed for calcium release from the sarcoplasmic reticulum (SER)

B. If we use a lot more, acetylcholine to bind to and open the Acetylcholine gated cation channel at the synapse [see figure 11-39] could this somehow alter the action potential generated by the adjacent mutated voltage gated Na^+ channel returning it to normal max membrane potential levels of 50 mV levels at 0.5 ms and -20 mV levels at 1 ms? (see figure 11-29 and 11-30)

Question 4

Chapter 16

The movement of molecular motors depends on the conformational changes that the proteins undergo as they proceed through the cycle of nucleotide binding, hydrolysis, and release.

- A. Muscle contraction uses large amounts of ATP. What are the two main uses of ATP during muscle contraction?
- B. If you were to add a nonhydrolyzable form of ATP to myosin, at what point would you block its movement? How is this similar to or different from adding a nonhydrolyzable form of ATP to kinesin?

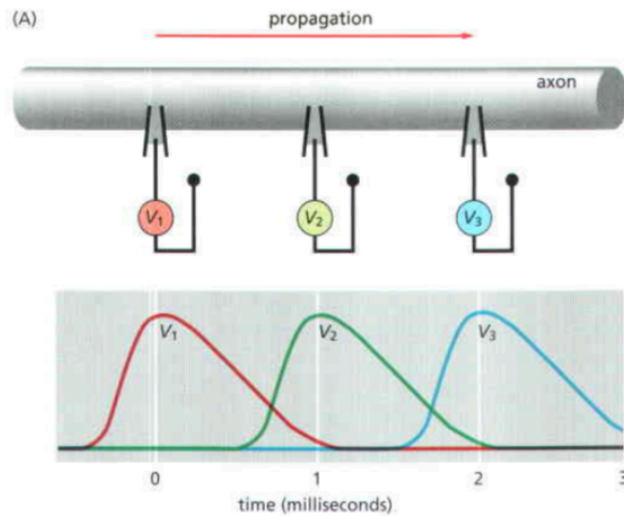
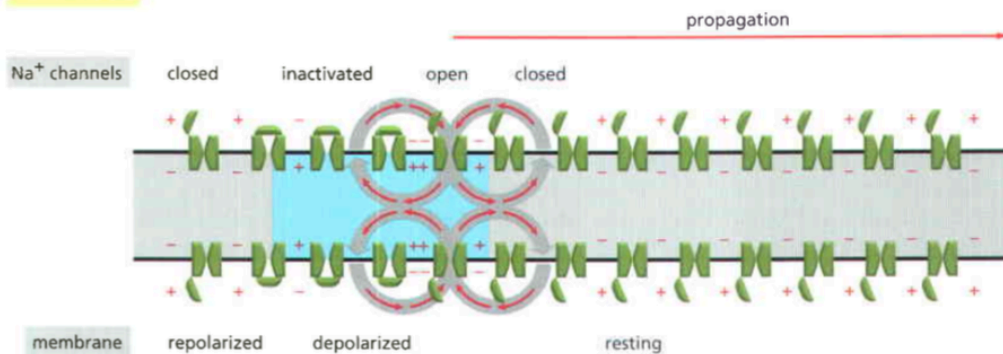


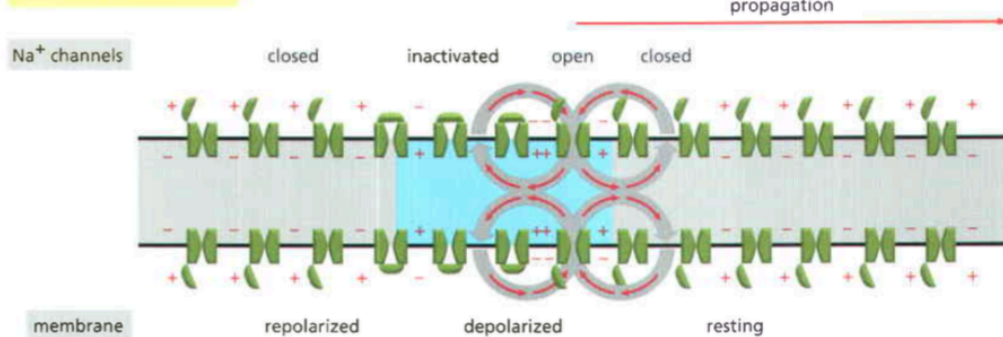
Figure 11–30 The propagation of an action potential along an axon. (A) The voltages that would be recorded from a set of intracellular electrodes placed at intervals along the axon. (B) The changes in the Na^+ channels and the current flows (orange arrows) that give rise to the traveling disturbance of the membrane potential. The region of the axon with a depolarized membrane is shaded in blue. Note that an action potential can only travel away from the site of depolarization, because Na^+ -channel inactivation prevents the depolarization from spreading backward.

(B)

view at $t = 0$



view at $t = 1$ millisecond



This description of an action potential applies only to a small patch of plasma membrane. The self-amplifying depolarization of the patch, however, is sufficient to depolarize neighboring regions of membrane, which then go through the same cycle. In this way, the action potential sweeps like a wave from the initial site of depolarization over the entire plasma membrane, as shown in Figure 11–30.

with them. The resulting influx of Na^+ causes a local membrane repolarization.

3. The local depolarization of the muscle cell plasma membrane opens voltage-gated Na^+ channels in this membrane, allowing more Na^+ to enter, which further depolarizes the membrane. This, in turn, opens neighboring voltage-gated Na^+ channels and results in a self-propagating depolarization (an action potential) that spreads to involve the entire plasma membrane (see Figure 11–30).

Figure 11–39 The system of ion channels at a neuromuscular junction. These gated ion channels are essential for the stimulation of muscle contraction by a nerve impulse. The various channels are numbered in the sequence in which they are activated, as described in the text.

