

1. *Recently in the news, it was announced that two patients died in Calgary when they had their blood dialysed against KCl instead of NaCl.*

Why is KCl lethal when our cells are normally full of K⁺ ions?

K⁺ is kept high inside cells and low outside by the Na⁺/K⁺ ATPase. When large amounts of K⁺ are added to extracellular fluids such as blood, this depolarizes membranes, and causes Na⁺ channels to open all at once. The resulting ion imbalances stop the heart.

If somebody murders their rich aunt by intravenous injection of KCl, why is this difficult for the coroner to detect?

After death, the Na⁺/K⁺ ATPases that maintain ion gradients cease action so that ion gradients dissipate and the electrolyte concentration inside and outside cells equalize. It would be hard to determine that K⁺ ions present had been introduced illicitly rather than simply leaking out of cells.

2. *Your lab has discovered a protein that allows Ca²⁺ to cross membranes. What experiments might you plan to try to determine the following:*

The following assumes that it is possible to isolate the transporter and reconstitute it in membrane vesicles.

a) is it active or passive transport?

Does transport activity depend on added ATP?

If ATPase activity is present, does presence of Ca²⁺ stimulate ATP hydrolysis?

A positive answer to either of these questions suggests an active transport system.

b) is it an electrogenic transporter?

An electrogenic transporter produces a net change in charge moved across the membrane for one complete transport cycle. An electrogenic transporter moves its substrate in response to membrane potential as well as concentration gradients.

If a membrane potential is applied to a membrane containing the transported, can this make Ca²⁺ move from low to high concentration?

If Ca²⁺ is introduced at different concentrations on either side of a membrane that has no initial potential, does movement down the concentration gradient induce a membrane potential.

A positive answer to either of these questions suggests an electrogenic transporter.

c) is it a transporter or an ion selective channel?

A transporter binds its substrate and then has to undergo conformational change to release each substrate on the other side of the membrane. It therefore follows Michaelis-Menten kinetics. An ion channel passes ions through a selectivity filter; ions have to interact with ligands of the selectivity filter, but pass through without having to stay bound. If the channel is in the open state no conformational change of the protein is needed for passage of each ion, and large numbers of ions pass in succession. Kinetics follows the electrochemical gradient in a linear fashion.

Do experiments to determine whether the transporter is saturable and has a K_M and V_{max} .

Can you explain the difference between an ion pump, an ion uniporter and an ion channel?

A pump is a transporter that uses an external energy source such as ATP hydrolysis to force conformation changes of the transporter to occur. These conformation changes change which side of the membrane the transporter faces, and may change the affinity of the binding site to bias substrate release in one direction.

A uniporter transports its substrate in the direction dictated by the electrochemical gradient. The substrate must enter to a binding site, then the protein changes conformation to expose the binding site of the opposite side: $\text{P} \leftarrow \text{S}$ becomes $\text{P} \rightarrow \text{S}$. The protein must actually change conformation twice to complete the cycle, so the empty binding site can be exposed once more on the high side of the electrochemical gradient $\text{P} \leftarrow \text{S}$. In many cases only one ion or molecule passes through for each conformation cycle, so the uniporter follows Michaelis-Menten type saturation kinetics (hyperbolic curve for rate of transport)

An ion-selective channel is more like a hole through the membrane which can accommodate large numbers of ions simultaneously (so is not easy to saturate). There is one point in the channel with a constriction that determines selectivity. However, ions pass through rather than binding to the selectivity filter. No conformation change is necessary for the passage of a single ion, although conformation changes may determine whether the channel is in an open or closed state.

If a uniporter moves an ion from low to high concentration, is this necessarily active transport?

Uniport is dependent on the electrochemical gradient, which has two components, concentration difference and membrane potential. If some other substance establishes a membrane potential, then an ion can be made to move from low to high concentration if it moves towards the opposite charged side of the membrane. This is not active transport as such, if the uniporter is not directly coupled to an energy yielding reaction such as ATP hydrolysis.

- 3 *If protein kinase B inactivates glycogen synthase kinase, and glycogen synthase kinase inactivates glycogen synthase, what happens to glycogen synthase when insulin binds to its receptor?*

Glycogen synthase kinase (GSK) contributes to inactivation of glycogen synthase.

If glycogen synthase kinase is made inactive by protein kinase B, GSK can't inactivate glycogen synthase any more, so glycogen synthase will stay active. Any glycogen synthase that was previously phosphorylated and inactivated will be dephosphorylated and activated by protein phosphatase 1.

Protein kinase B	
^	
Glycogen Synthase Kinase	
⊥	
Glycogen synthase	

The ⊥ symbol is often used to represent inhibition or inactivation. This figure is part of a larger diagram representing metabolic effects of insulin that was shown in lecture 33.

In the absence of insulin, Protein kinase B is not active so glycogen synthase kinase is active and inactivates glycogen synthase.

In the presence of insulin, protein kinase B is active, so glycogen synthase kinase is not active, so can't inactivate glycogen synthase. The two negatives can't operate at the same time.

4. *Which do you think is more dangerous to an organism?*

A mutation of Ras that prevents GTP hydrolysis

A mutation of Ras that speeds up GTP hydrolysis

A mutation of Ras that prevents GTP from binding

A mutation of Ras that prevents GTP hydrolysis leaves Ras in a permanently active state, and this contributes to uncontrolled cell division and cancer.

A mutation of Ras that speeds up GTP hydrolysis would deactivate Ras too quickly. A cell that contained this mutation would not proliferate, therefore would not produce more cells with the same mutation. The individual cell would die eventually but it is unlikely that other cells in the organism would be affected.

A mutation that prevents GTP from binding would have either consequence depending on whether Ras stays in active or inactive conformation in the absence of bound GTP. The mutation would lead to tumors if Ras stayed in the active state with no GTP bound. The individual cell containing the mutation would die off if Ras without GTP remained in the inactive state, but the remaining cells in the organism with normal Ras would survive.

5. *How do cholera toxin and pertussis toxin increase activity of adenylate cyclase? Why are the symptoms of cholera and pertussis (whooping cough) different if the biochemical effect is the same?*

Cholera toxin modifies $G_s\alpha$ so that GTP hydrolysis is prevented and $G_s\alpha$ activates adenylate cyclase continuously.

Pertussis toxin modifies $G_i\alpha$ so it can't exchange out the old GDP and replace it with a new GTP. This leaves $G_i\alpha$ in a permanently inactive state.

Since most cells have many GPCR receptors of different kinds, the state of adenylate cyclase is actually a balance between all the positive signals induced by $G_s\alpha$ and negative signals induced by $G_i\alpha$. If $G_s\alpha$ is permanently on or $G_i\alpha$ is permanently off, adenylate cyclase will be pushed into a more active state.

The different response is determined by the particular cells that the pathogen encounters in its normal mode of infection, and how those cells respond to elevated cyclic AMP. Cholera enters through the intestinal system, whooping cough through the bronchial system.

If someone receives an extreme fright, high levels of epinephrine are released, and the intestinal cells do partly respond to this, resulting in sudden increase in fluid in the gut, and possible embarrassing consequences.

6. *Why do voltage gated ion channels in the nervous system use different mechanisms for opening and closing the channel?*

Channels open in response to a change in potential, e.g. for sodium channels, a rise from -60 mV to -40 mV is sufficient to partly open the channel, this accelerates the rise in potential until the channels are fully open.

Channels close by binding the inactivation domain into the channel. Having a different mechanism allows the channel to close while the potential still keeps the voltage gate open. This allows the channels to close very quickly, so that relatively few ions have time to cross the membrane and the ion gradients are not dissipated. The spike in potential lasts less than a millisecond, but is sufficient to send a signal down the nerve axon.

Why do nerve impulses travel in one direction?

It takes a few milliseconds for the inactivation domain to release, and until this happens, there is no effect of opening the voltage gate. This is called the refractory period.

Consider three adjacent channels A B C equally spaced along the axon. When A opens, potential around A rises and triggers its immediate neighbour B to open. By the time B is fully open A has closed and is in the refractory state. B triggers its neighbour C to open but A can't reopen yet. Therefore the potential spike travels in one direction $A \rightarrow B \rightarrow C$.

7. *What would happen to the normal cellular membrane potential of -60 mV inwards if K⁺ channels opened unexpectedly? Does this explain why there are many toxins that act on Na⁺ channels but none known that act on K⁺ channels?*

A sudden opening of the potassium channel in the resting axon would have little effect, since K⁺ is already the ion with greatest permeability in the resting state. The potential might change a few millivolts more negative, since the equilibrium potential for K⁺ is about -90 mV.

Since toxins that affect sodium channels are so much more effective, evolution of toxins would tend to target the sodium channel for interference.

8. *Caffeine inhibits the phosphodiesterase that breaks down cyclic AMP. Can you explain how caffeine can induce a “high” energy state?*

The level of cyclic AMP in the cell is the result of the balance between formation by adenylate cyclase and destruction by phosphodiesterase. Caffeine inhibits the destruction side leading to build up and persistence of cyclic AMP in the cell. This induces liver to release glucose into the blood, preparing muscles (and brain) for immediate action.

How can consuming coffee and a donut induce the hypoglycemic state sometimes called caffeine “crash”?

The donut is mostly starch, which is broken down to glucose in the digestive system. Elevated blood glucose levels in blood stimulates insulin release, which stimulates glucose uptake.

Meanwhile, caffeine causes cyclic AMP levels to rise, activating protein kinase A.

A downstream effect of the protein kinase results in phosphorylation of GPCR for glucagon and epinephrine, allowing the complex with β-arrestin to form, and the GPCR are withdrawn from the plasma membrane.

Sometimes glucose uptake proceeds too rapidly, causing blood glucose levels to drop.

Normally, glucagon release would compensate, but if too many GPCR are hidden inside the cell, the response to glucagon is weak and the individual goes into a mild hypoglycemic state with trembling and shakiness.