

Diabetes and Insulin Signaling

by
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Part I – Research Orientation

“Hi, Dr. Kim. My name is Mia. I’m the undergraduate from the Research Experience for Undergraduates program. I’ve been assigned to work with you this summer.”

“It’s great to meet you Mia. I think this REU program is an excellent way to get research experience and plus it increases your competitive edge for professional and graduate school applications. Are you planning on applying to medical school?”

“Actually, I’m not sure yet. I kind of hoped that this summer would help me figure that out.”

“That’s a great reason to be here. Why did you want to be in this lab?”

“Because you’re working on type-2 diabetes. I’m interested because I have a family history of diabetes. It said on your website that you are trying to understand the role of insulin and cellular signaling in diabetes. I remember cellular signaling was mentioned in my cell biology class, but what exactly does it have to do with diabetes?”

“Well, cellular signaling controls our response to the environment, like changes in the temperature or responses to eating. It helps our bodies maintain homeostasis. Many medications alter cellular signaling in order to treat diseases like cancer, allergies, and diabetes.”

“So what you’re saying is that understanding cellular signaling can help us understand how diabetes occurs and how to treat it?”

“Yes that’s exactly right. The problem with cellular signaling is that each pathway involves many proteins, so the way our cells carry a message can be very complicated. Before we talk about your project and diabetes in particular, you need to understand cellular signaling in general. This afternoon I’m going to have you read some book excerpts from the lab orientation material. If you have any questions, please ask.”

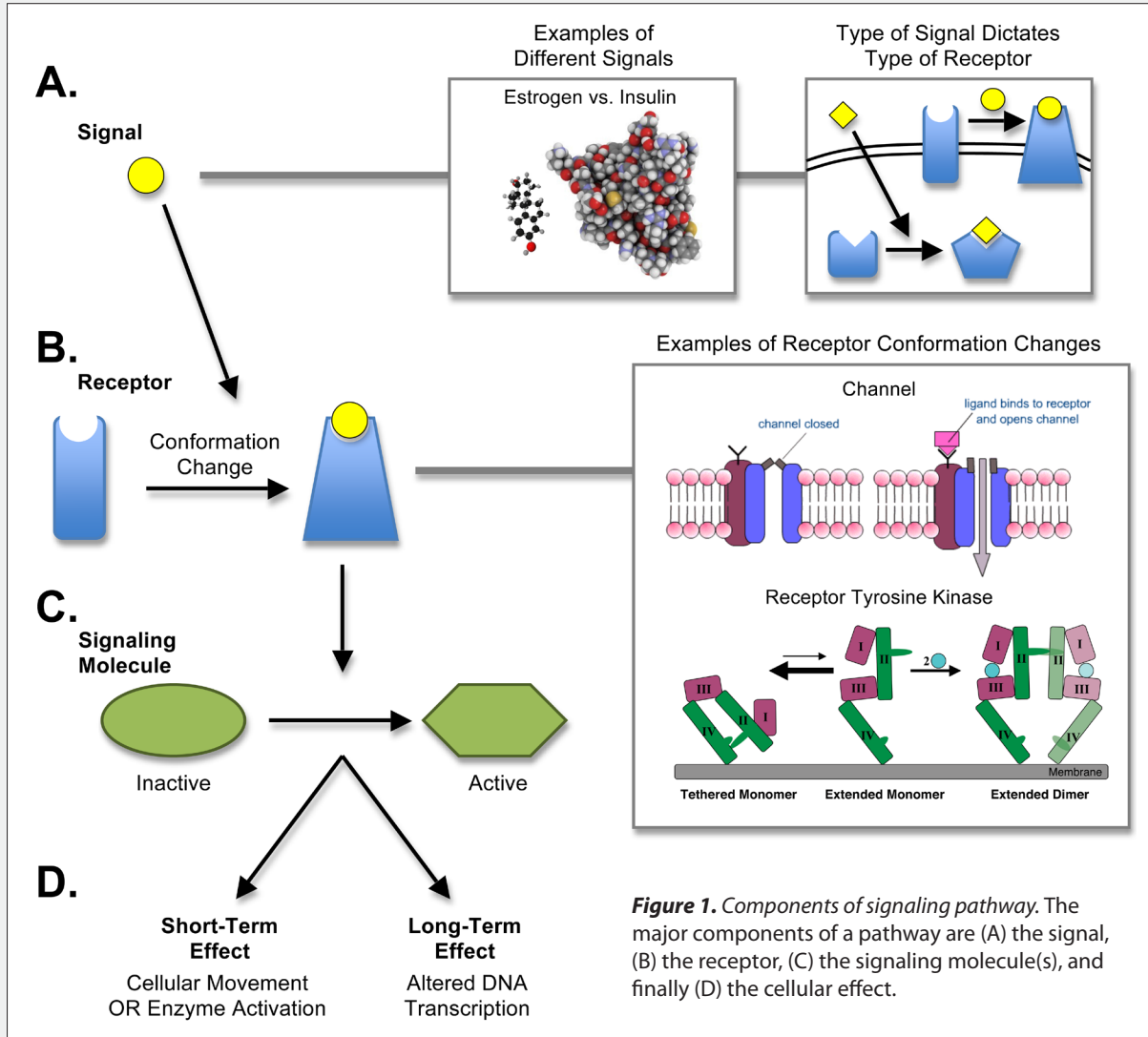
Excerpt 1

Signaling Overview

A signaling pathway has four essential components: (1) the initial signal, (2) the receptor that binds the signal, (3) the signaling molecule or molecules that transmit the message, and (4) the effector or effectors that result in a short-term or long-term cellular change. The initial signal can range in size and composition from a small molecule like nitric oxide (NO), a hormone like estrogen, or a protein like insulin (Figure 1A). The type of signal determines if the receptor signal-binding domain can be intracellular or extracellular. For example, estrogen is hydrophobic and can readily pass through the plasma membrane, so its receptor is intracellular. Other signaling molecules like the protein insulin are both too hydrophilic and too large to pass through the plasma membrane. The insulin receptor is an integral membrane protein with an extracellular signal-binding domain.

Once the signal binds to the receptor, the receptor changes its shape or conformation. This conformation change might include the opening of an ion channel allowing ions to travel into the cell, or it might include changing the organization of domains like the extracellular domain of a receptor tyrosine kinase, a receptor class to be discussed later (Figure 1B). A receptor conformation

change causes the associated signaling molecule(s) to transition from inactive to active (Figure 1C). The signaling molecule(s) can carry the message through many different mechanisms. These mechanisms will be covered in the section about signaling mechanisms. The activated signaling molecule then influences the effector(s) that cause the short-term or long-term cellular change (Figure 1D). A short-term change can be stimulating cellular movement or changing the activation state of an enzyme going from inactive to active or active to inactive. This happens for instance when activating an enzyme to increase sugar metabolism. Long-term cellular changes are generally the result of changes in DNA transcription. For example, a protein could be made to begin cellular replication by activating the cell cycle.



Signaling Mechanisms

One mechanism for multi-protein signal transduction is the protein kinase cascade like the one illustrated in Figure 2A. A protein kinase is an enzyme that uses ATP to add a phosphate group to serine, threonine, or tyrosine residues of proteins. The determination of where a kinase will phosphorylate another protein is based upon the amino acids at the site of phosphorylation surrounding the amino acid to be phosphorylated. The amino acid sequence creates a specific binding site for the kinase so one kinase can only phosphorylate very specific proteins at specific sites. The addition of phosphate groups to these amino acids can alter the behavior of these proteins by changing their enzymatic activity (turning on or off), changing their association with other proteins, or changing their localization in the cell. Each protein kinase may have multiple amino acids that can be phosphorylated. Each phosphorylation site can change the kinase's activity. In a protein kinase cascade, a receptor activates a signaling molecule that in turn activates a protein kinase that activates another protein kinase. This forms a kinase cascade that activates the next kinase until the final signaling molecule is reached, causing a cellular response.

The sites phosphorylated by the previous kinase activate the next kinase, but another site of phosphorylation on the same kinase could turn it off. The activity of each kinase in the cascade can be regulated in this manner. One common mode of regulation is called feed-back inhibition (Figure 2B). This occurs when some downstream effector (or result of the cellular response) inhibits an earlier step in signal transduction. Thus, the dynamics of speed and magnitude of response can be fine-tuned or stopped entirely. This negative regulation is reversible. In the example in Figure 2B, another enzyme called a phosphatase could remove the phosphate group from the kinase, allowing it to be activated again.

Another common mechanism for multi-protein signal transduction is the activation of a second messenger (Figure 3). A second messenger is generally a small molecule that can travel freely through the cytoplasm or the membrane. Some examples of second messengers are cyclic-AMP, Ca^{2+} ions, phosphoinositides (PIP3, PIP2, etc.), and diacylglycerol (DAG). These second messengers are either released from intracellular stores (like Ca^{2+} ions) or created through enzymatic action (like cyclic-AMP). Once released, second messengers can interact with many targets throughout the cell simultaneously. Thus, second messengers lead to signal amplification and increased speed in signal transduction.

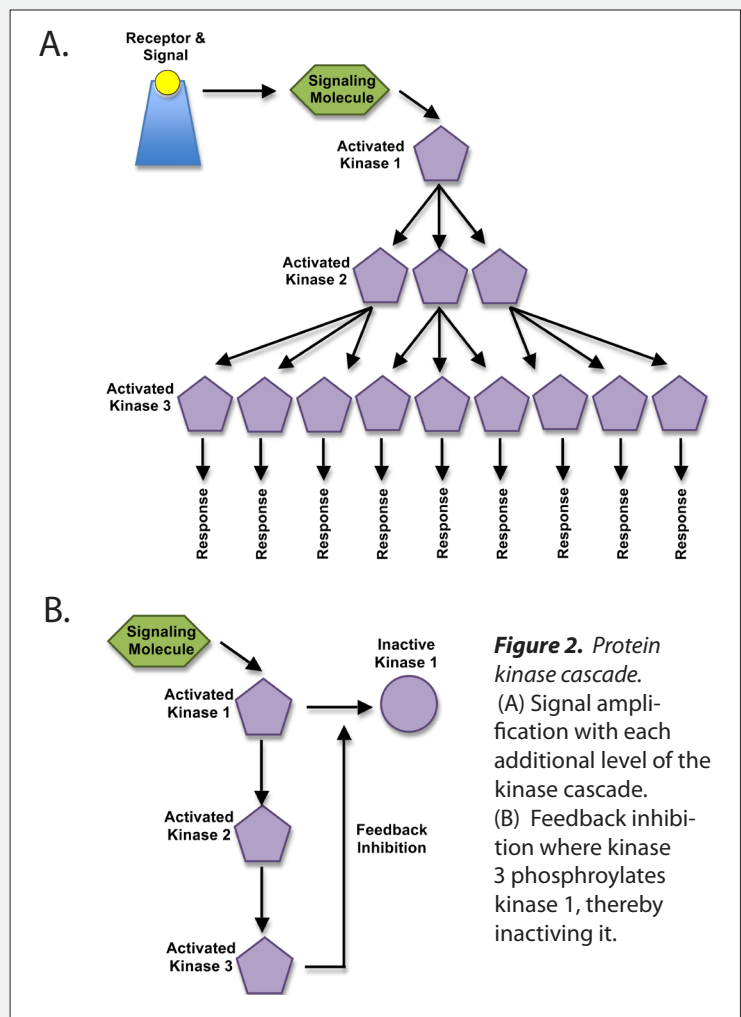


Figure 2. Protein kinase cascade. (A) Signal amplification with each additional level of the kinase cascade. (B) Feedback inhibition where kinase 3 phosphorylates kinase 1, thereby inactivating it.

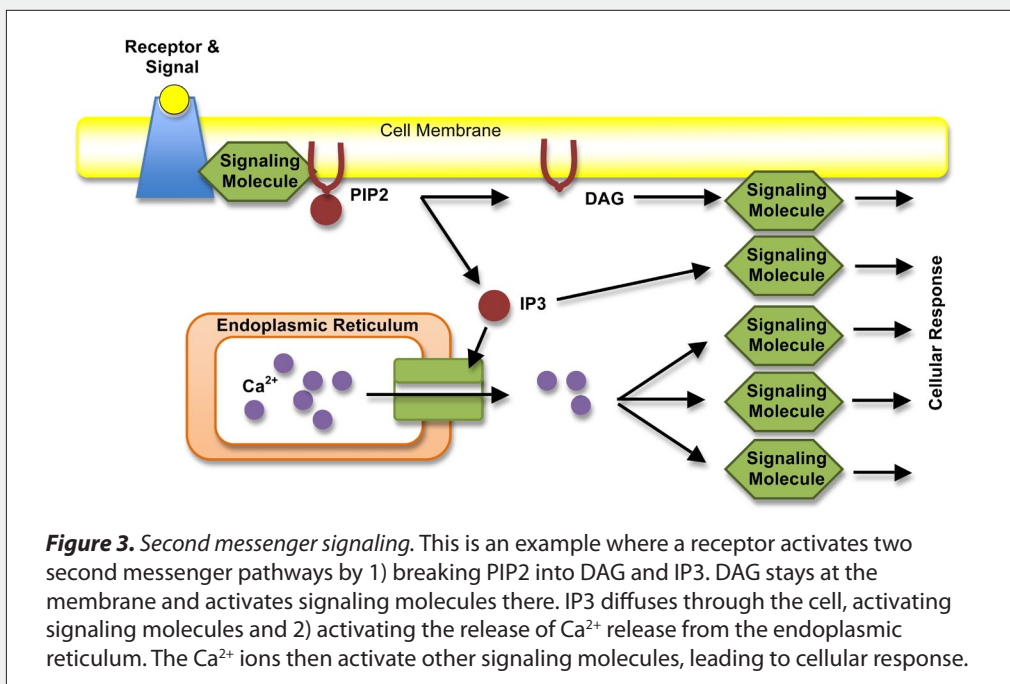


Figure 3. Second messenger signaling. This is an example where a receptor activates two second messenger pathways by 1) breaking PIP2 into DAG and IP3. DAG stays at the membrane and activates signaling molecules there. IP3 diffuses through the cell, activating signaling molecules and 2) activating the release of Ca^{2+} release from the endoplasmic reticulum. The Ca^{2+} ions then activate other signaling molecules, leading to cellular response.

“Dr. Kim, I have a few questions about the excerpts that you had me read.”

“Ok, Mia what didn’t you understand?”

“So in the first part, the general signaling pathway, the signaling molecule step is represented as one protein. Yet in the mechanisms part it talks about the kinase cascade and second messenger signaling where lots of proteins are activated. Doesn’t it take a lot of energy to make all those extra proteins? Why couldn’t the signal be transmitted with just one signaling molecule?”

“Mia, that is a great question. What you’re asking is, ‘what are the advantages to multiple protein methods like the kinase cascade and the second messenger pathway.’ I want you to look at Figure 2A and Figure 3 in the excerpts. You’ll notice with the kinase cascade that with each additional kinase activated, more of the next kinase is activated.”

“Yes. It shows something like an exponential growth.”

“Well, having more kinases activated leads to signal amplification. Signal amplification can lead to greater cellular changes, and it also speeds up the cellular response. It works the same with second messenger pathways too, with the small molecules activating lots of signaling proteins.”

“Ok, I can see that now with all of the extra arrows leading to cellular response.”

“Another reason is the possibility of improved regulation. Each new signaling molecule provides another opportunity for the body to regulate the signaling. The example in Figure 2B is a feedback inhibition, but that’s not the only way it could be regulated. Signaling could be regulated through the stimulating of signaling, not the inhibition. Regulation could also involve responding to another environmental signal so that the body’s response can be coordinated.”

Questions

1. What are the essential parts of a signaling pathway?
2. How could activating a transcription factor cause long-term cellular changes?
3. What roles can phosphorylation play in protein function?
4. What is the enzymatic activity of a kinase and of a phosphatase?
5. What determines where a protein kinase or protein phosphatase will perform its enzymatic activity?
6. Why would a signaling pathway need to be regulated?
7. Hypothesize some situations where it would be necessary for signal transduction to happen very rapidly, as happens after the activation of either a kinase cascade or a second-messenger pathway.

Part II – Diabetes and Insulin Signaling

“Mia, now that you understand the basics of signaling and the mechanisms it uses, I want to talk about why this lab studies cellular signaling. You said that you had a family member with type-2 diabetes, right?”

“Yeah, my grandpa.”

“So how does diabetes affect his life?”

“Well, he is really careful what he eats and he goes for walks most days. He also has to check his blood glucose level all the time and he gives himself injections before most meals. And for some reason my mom has to remind him everyday to check his feet, but I never understood why.”

“Your grandpa’s goal is to keep his glucose levels balanced; it can’t be too high or too low. He checks his blood glucose to see if he needs to do something to change it. For instance, after people eat their blood glucose generally goes up. This causes the pancreas to release a signal known as insulin into the blood stream. In diabetics, the cellular signaling is messed up so it doesn’t work as well. So your grandpa is probably injecting himself with insulin or an insulin analogue.”

“So what you’re saying is that my grandpa controls his diabetes by controlling cellular signaling.”

“Yes, the effects of diabetes are varied and can be devastating. By changing signaling, they can be managed. You can see a list of resulting symptoms in the next excerpt (Excerpt 2). The symptoms occur for two reasons: 1) there are high glucose concentrations in the blood and 2) very little of the glucose is getting into the cell. Since glucose is an energy source and it needs to get into the cell to be used, the cells need to use something else for energy. What other things can the cell use for energy?”

“Well, I guess proteins or fats.”

“That’s right. The cells start using proteins, which leads to a buildup of ketoacids. As acids, what would they do to the pH in the blood?”

“The pH of the blood would decrease. Isn’t that bad? Don’t we want our pH to be around 7?”

“Yes, this lower pH is bad and it can damage lots of tissues, causing the symptoms listed in the next excerpt (Excerpt 2). This brings me to why your grandpa has to check his feet. One problem with diabetics is that they lose feeling in their feet, so if they get a blister on their foot they may not feel it. Then it may get infected because diabetics have poor wound healing, and if the infection isn’t noticed it may lead to amputation of the foot or leg.”

“I guess I’ll help my mom bother my grandpa to check his feet.”

Excerpt 2

Diabetes affects 25.8 million people in the US. The number of young people that are annually diagnosed with diabetes is on the rise, with 15,600 diagnosed with type-1 diabetes and 3,600 diagnosed with type-2 diabetes. In 2007, the estimated annual cost of diabetes was \$174 billion dollars. Average medical expenditures of individuals with diabetes are 2.3 times higher than people without diabetes. The major complications with diabetes are heart disease, stroke, high blood pressure, blindness, kidney disease, nervous system damage, and amputation.

Type-2 diabetes results from target cells that don’t respond as well to insulin. If untreated, type-2 diabetes leads to excess glucose in the blood. If cells cannot use glucose metabolism for energy, they can start breaking down protein. Excess protein metabolism can lead to a buildup of byproducts known as ketoacids. These ketoacids in combination with excess blood glucose can lead to a host of physiological problems including:

- Blurred vision → blindness
- Fatigue
- Kidney problems (frequent urination, dehydration) → dialysis
- Loss of sensation in limbs (especially feet)
- Poor wound healing- with foot lesions → amputation
- Weak immune system
- Impaired cognitive function
- Irregular heartbeat → heart attack
- Coma/death

“So now I want you to connect these ideas to diabetes and insulin signaling specifically.”

“Ok, what do you want me to do?”

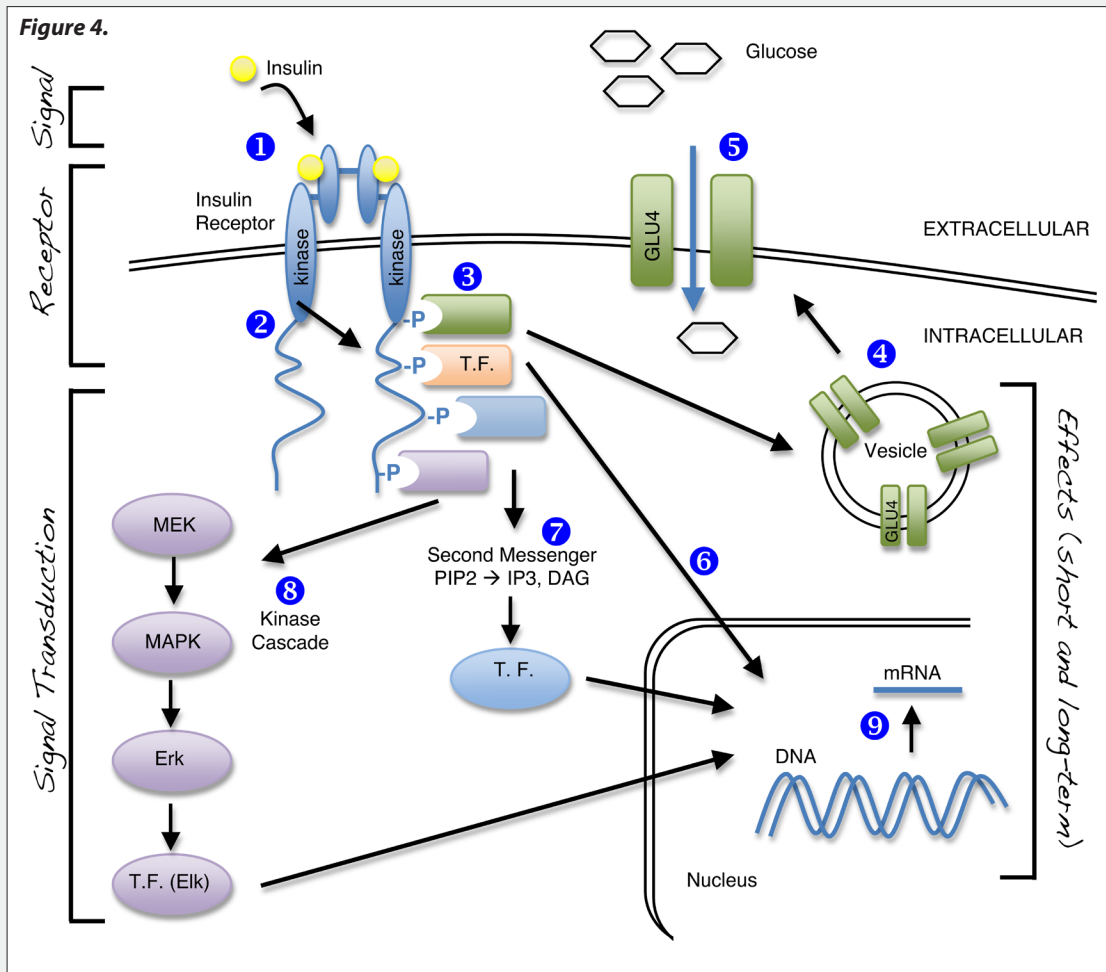
“This afternoon, I want you to label this figure on insulin signaling to identify the different parts of a signaling pathway. I want you to label the signal, the receptor, the signal transduction, and the effects. This figure is labeled with the names of some of the proteins like MEK or MAPK but it’s not as important as realizing, for instance, that they are part of a kinase cascade. The thing that I want you to focus on is the different signaling modes and how they are all connected to the insulin receptor.”

Excerpt 3

Insulin Signaling

Insulin binds to the insulin receptor (1); Receptor is activated, causing a conformation change known as dimerization (the coming together of two insulin receptors). Receptor adds a phosphate to amino acids (tyrosines) on the tail of the other insulin receptor in the pair (2). Signal transduction proteins interact with phosphate group (3). Interaction of the phosphate groups with all of the different signaling proteins occurs simultaneously, but each pathway will be discussed individually. Signaling proteins and pathways will cause the short-term and long-term changes in response to the increased glucose in the blood stream. One major short-term change is the fusion of vesicles containing glucose transporter (GLUT4) to the cell membrane (4). Once these transporters are part of the cell surface, glucose is transported into the cell (5).

Long-term cellular changes are caused by changes in gene transcription that result in specific proteins being made or not made. These pathways utilize many different signaling patterns, such as the direct activation of a transcription factor (T.F.) (6), the release of second messenger (7), and the activation of a kinase cascade (8). All of these signaling pathways can result in the activation of transcription factors and their movement to the nucleus to activate transcription (9).



“Dr. Kim, I finished going over insulin signaling.”

“Yeah it looks like you did a great job on identifying the different components of a signaling pathway. You have both the long-term and short-term effects on one bar. Can you tell me which effect can be categorized as a long-term effect and which can be a short-term effect?”

“The long-term effect is changes in transcription by the activation of the transcription factors and the short-term effect is the movement of the glucose transporter to the cell surface.”

“Great. Oh Mia, before we get into our specific research question, there is one more thing we need to discuss. Do you think insulin causes the same long-term and short-term effects in different kinds of tissue in your body, like your muscle and liver?”

“Well, insulin is released into the blood stream so it could bind to receptors on all the different tissues. So, I would assume that insulin would have the same effect in different tissues.”

“Not quite. Insulin binding to the insulin receptor doesn’t have the same effect in the different cell types in our body. You’re right in thinking that insulin is released into the blood stream, but the amount of a receptor or really any downstream signaling effector could affect the short-term and long-term effect.”

“How can different tissues have a different amount of the receptor? All cells have the same set of DNA, right?”

“While different cells have the same set of DNA, the accessibility of that DNA is changed in different cell types. The insulin receptor DNA might not be expressed as much in different tissues because of the DNA packing or a variety of other reasons that we won’t get into now. Actually that’s one of the things we study in this lab in relation to diabetes.”

Questions

1. How could the study of insulin signaling help people with diabetes?
2. Why does it make sense that Mia’s grandfather may be more fatigued than a non-diabetic?
3. Examine the figure of insulin signaling. Why does one receptor have so many different signal transduction proteins/pathways?
4. How does a lack of insulin prevent the cell from using glucose?
5. Why is it important that specific tissues respond to insulin in different ways?
6. Hypothesize a mechanism to explain how tissues respond to insulin in different ways, even tissues that have the same amount of the receptor.

Part III – Insulin Resistance

“In this lab we are focusing on insulin resistance; the primary cause of type-2 diabetes. This means that even though insulin is present in the blood stream, the cells don’t respond as robustly. Type-2 diabetes occurs as a result of continuous insulin signaling due to genetics, poor diet, obesity, and lack of exercise. This continuous over stimulation of insulin signaling alters how the insulin receptor and its down-stream signaling pathways will respond to insulin.”

“So that is what the lab looks at...insulin resistance and cell signaling.”

“There have been lots of possible changes to insulin signaling proposed as the key mechanisms responsible for insulin resistance, but the reality is that insulin resistance isn’t understood. So here is a list of possible research topics to investigate and determine their relative role in insulin resistance.”

Excerpt 4

Some of the documented changes in insulin signaling as a result of diabetes are (numbers refer to Figure 4 in Excerpt 3):

- Decreased insulin receptor kinase activity upon binding of insulin to the receptor (2).
- Decreased binding of signaling effectors to the insulin receptor due to
 - Less phosphorylation sites on the insulin receptor (3).
 - Feedback inhibition on the signaling molecule, preventing its binding to the insulin receptor.
- Decreased signaling effector binding leads to defective downstream activation of the kinase cascade and second messenger signaling pathways (7), (8).
- Decreased GLUT4 (glucose transporter) fusion to the cell membrane and less glucose transported into the cell (4), (5).

Questions

1. What is the general purpose of feedback inhibition? What is the problem if feedback inhibition happens when it isn’t supposed to?
2. How do the effects of insulin resistance compound to decrease cellular responses to insulin?
3. Hypothesize a mechanism by which the GLUT4 transporter’s fusion to the cell membrane could be decreased.
4. Consider the possible projects that Mia could study.
 - a. Which project would you pick?
 - b. Why do you think this project is important?
 - c. What kind of experiments would you do?



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