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Investigating Effects and Relative Power of Variables in the Epinephrine Signal Transduction Pathway

Varenyam Malhotra

North Carolina School of Science and Mathematics

ABSTRACT

The epinephrine signal transduction pathway is one of the most crucial signaling pathways in the human body. It controls the flight-or-fight response, which enables humans to handle dangerous and often life-threatening situations. This paper seeks to establish which of the pathway's independent variables are the most powerful, and which are the best for increasing and decreasing specific dependent variables. The chosen computational approach was testing increases in each independent variable and observing the effects on dependent variables using a STELLA Architect model created by Mr. Jon Darkow. The analysis of each test's graphical results led to conclusions regarding the relative power and effects of several variables in the pathway. I concluded that the most powerful independent variable was the Beta Blocker inhibitor, followed by the KT 5720 inhibitor. Increasing initial ATP amounts was the best solution to increasing cyclic AMP, PKA, and phosphorylase kinase, while increasing the inactive G protein was the preferred means to increase the active G protein and adenylyl cyclase. Finally, the best way to increase glucose and carbon dioxide was to increase initial glycogen amounts. The results of this paper can be applied in a medical context to assist patients with deficiencies or excessive amounts of specific variables involved in this pathway. Furthermore, the analysis and figures provide insight into the effects that increasing an independent variable has on the entire model; this insight renders useful when doctors are ensuring that an increase of one variable will not place another variable beyond its safe bodily limits.

Introduction

The Epinephrine Signal Transduction Pathway

The epinephrine signal transduction pathway is the basis of the fight-or-flight response that humans experience in frightening, stressful, and often even life-threatening situations. In the fight-or-flight response, the epinephrine signal transduction pathway is triggered when the adrenal glands above the kidneys secrete the hormone epinephrine. The secreted epinephrine then travels to liver and muscle cells, which detect the epinephrine via specific receptor proteins on their cell membranes. This detection of epinephrine sparks the signaling pathway to begin. The pathway's result is a release of glucose into the bloodstream from the cells. These glucose molecules, acting as the necessary emergency energy fuel, can then be distributed through the bloodstream to muscles and appendages so the body can prepare an appropriate response to the situation-whether the final decision is to fight, or to flee. This crucial bodily response all hinges on the ability of the liver and muscle cells to process epinephrine and release glucose molecules-the job of the epinephrine signal transduction pathway.

The epinephrine signal transduction pathway begins with the recognition of epinephrine, a ligand, by the Beta-2 adrenergic receptor on a G-protein on the cell membrane of a liver cell. After the recognition of epinephrine by the Beta-2 adrenergic receptor, epinephrine docks with the G protein on the cell membrane of the liver cells. The G protein has 3 subunits: the alpha subunit, beta subunit, and gamma subunit. When the epinephrine ligand binds with the G protein, it activates the previously inactive G protein, and causes a conformational change in the protein. This

conformational change causes the alpha subunit of the G protein to be released from the protein. The alpha subunit then travels to and docks with adenylyl cyclase, a different protein in the cell membrane, thus activating the previously inactive adenylyl cyclase protein. The newly activated adenylyl cyclase now begins its job of converting ATP molecules into cyclic AMP, otherwise known as cAMP molecules. This process occurs by removing two phosphates of the ATP and creating a cyclic portion of the sugar of the ATP (cAMP stands for cyclic adenosine monophosphate). cAMP molecules are called the secondary messengers of the epinephrine signaling pathway because they relay and amplify the signal in different parts of the cell. The cAMP molecules target the specific protein kinases of this pathway, phosphorylase kinases, which have two catalytic subunits and two regulatory subunits. The cAMP molecules bind to the regulatory subunits and thus release the catalytic subunits. The release of these catalytic subunits causes a cascade of energy. The released catalytic subunits are then phosphorylated as they pick up energy from ATP and then become activated. These newly phosphorylated catalytic subunits can act on enzymes within the cell. They drop off a phosphate to phosphorylase, which activates phosphorylase to release glucose and glycogen from within the cell and into the bloodstream. This glucose (and glycogen, which can be converted to usable glucose as well) can travel through the bloodstream to muscles so that the body is equipped with energy and ready to handle the fight-or-flight situation.

As you can see, there is a lot that goes on in the epinephrine signal transduction pathway. This, however, also means that there is a lot that can go wrong. Throughout the pathway, there are also several rates and conversions involved, such as the rates of hydrolysis, reception, catabolism, and more. There are also several inhibitors that can decrease the efficacy of the pathway. Two inhibitors demonstrated in the model I used are the Beta Blocker, which prevents the stimulation of the Beta-2 Adrenergic receptor, and KT 5720, which is an inhibitor of phosphorylase kinase, the protein kinase in this signaling pathway.

Medical Applications

One medical disorder relating to the epinephrine signal transduction pathway is that of patients having deficiencies or excessive amounts of certain variables in the pathway. Doctors can address these issues by administering specific independent variables to increase the lacking variable or quell the excessive variable. In line with this topic, I utilized an interactive, dynamic model of the epinephrine signal transduction pathway and tested the effects of 7 different independent variables on the model by increasing each of them and evaluating their effects on 7 different dependent variables. This exercise allowed me to examine the power of each independent variable. I obtained solutions regarding the relative power of each independent variable, and regarding which independent variables are best suited for increasing and decreasing specific dependent variables.

The collected results can assist doctors in understanding how to increase or decrease specific dependent variables and if necessary, alter the amount of those specific variables in patients. This way, patients with specific deficiencies or excess amounts of variables involved in the pathway can receive treatments. This would enable their epinephrine signal transduction pathways to function properly and to deal with fight-or-flight situations if they unfortunately come across one.

Computational Approach

Computational Tools

This research project utilizes a STELLA Architect model on the epinephrine signal transduction pathway. STELLA Architect, commonly known as STELLA, is a platform that allows users to create, test, iterate, and run intricate models. Under the hood, STELLA is based on ordinary differential equations, which allow the user to create models involving multiple different rates and values. These models can depict many different pathways using a combination of different tools and objects that are specific to STELLA, such as stocks, flows, connectors, convertors, and more.

STELLA models are dynamic and easily customizable due to the ability to enter formulas in stocks, convertors, and flows. Many different variables can be interconnected via the use of connectors and by entering mathematical formulas that involve multiple variables. Graphs can also be produced, allowing quantitative data to be displayed. STELLA models are extremely useful for analyzing, testing, and obtaining results for scientific pathways and phenomena that involve mathematical equations, which is why I used STELLA as the computational tool for my project.

Independent Variables

The STELLA Architect model that I used is a biological model addressing the epinephrine signal transduction pathway from start to finish, all while taking rates, values, enzymes, and inhibitors into account. As I wanted to optimize the amounts of products obtained from the pathway and understand the effects of each of the independent variables, I decided to run tests by manually increasing one independent variable at a time, leaving all other variables at their original control values. The independent variables that I manually altered and tested were epinephrine, initial ATP, glycogen synthase, the inactive G protein, the Beta Blocker inhibitor, the KT 5720 inhibitor, and the initial glycogen. As mentioned earlier, I altered one of these independent variables while leaving all the others on their control values. On each run, I measured important products, proteins, and compounds within the pathway as the dependent variables.

Dependent Variables

While the final product of the epinephrine signal transduction pathway is generally viewed as glucose, the product of this model is carbon dioxide, as glucose is converted to carbon dioxide through cellular respiration. Other than glucose and carbon dioxide, I also measured the following dependent variables: the active G protein, adenylyl cyclase, cyclic AMP, PKA, and phosphorylase kinase. These are all vital substances necessary for the epinephrine signal transduction pathway to effectively function.

Numerical Information

Once I had my list of independent variables to control and dependent variables to measure with each run, I decided that I would increase each independent variable by a factor of 100 to obtain an observable change in the dependent variables' values. This worked for the 5 independent variables that I hypothesized would increase the pathway's dependent variables, but I had to alter my plan for the two inhibiting independent variables: the Beta Blocker and KT 5720. This is because an increase from 0 to 100 shut down the entire pathway progress, as the inhibition was so strong for both inhibitors that there was no observable increase in any dependent variable. Thus, to be able to still compare the Beta Blocker and KT 5720, I had to alter my plan to ensure that there was still some measurable pathway progress after the controlled increase in inhibition. In the end, I increased both inhibitors from 0 to 0.1, by a factor of 0.1, which is a testament to how powerful both the inhibitors are.

Documentation

I set up a table on Microsoft Excel to document my findings, which were my observations of the graphical results from the STELLA model. With this table, I documented the changes in all 7 dependent variables, for each increase in the 7 independent variables. Figure 1 is the STELLA Model, composed by Mr. Jon Darkow.

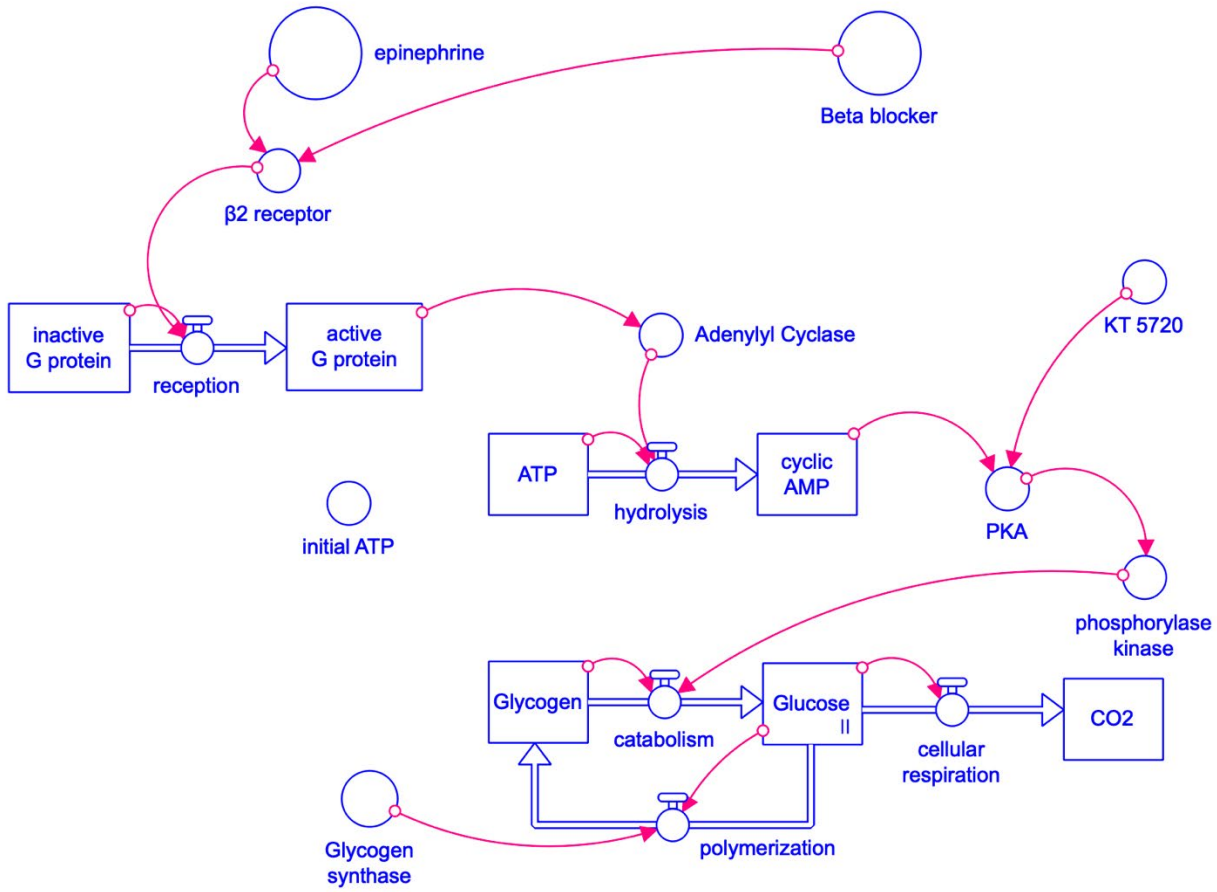

Figure 1: Mr. Jon Darkow's STELLA Model on the Epinephrine Signal Transduction Pathway

The figure at the top of the following page is the Microsoft Excel spreadsheet I used to document my observations of the graphical results I obtained through the several tests that I ran. This table can be viewed properly when zoomed in on +500%.

		Independent Variables						
		Epinephrine (Control: 1)	Initial ATP (Control: 10,000)	Glycogen Synthase (Control: 0)	Inactive G Protein (Control: 100)	Beta Blocker (Control: 0)	KT 5720 (Control: 0)	Glycogen (Control: 100,000,000)
Dependent Variables	Active G Protein	Increase rapid linear to 100 within 2 seconds then level off at 100	Increase slowly (logarithmic-like) immediately, approaches 100 after 20 seconds	Increase slowly (logarithmic-like) immediately, approaches 100 after 20 seconds	Increase super super quickly, whether exponential or logarithmic-like. Passes 100 within 1 second and keeps increasing super super super rapidly	Very very slight linear increase throughout. Only approaches about 15-20 after 20 seconds.	Did not affect too much, still had a logarithmic-like increase throughout, starting immediately, and approached 100 (almost leveling out) by 20 seconds.	Did not affect too much, still had a logarithmic increase throughout, started increasing immediately, and approached 100 at an increasingly slower rate, by 20 seconds.
	Adenylyl Cyclase	Increase rapid linear to 100 within 2 seconds then level off at 100	Increase slowly (logarithmic-like) immediately, approaches 100 after 20 seconds	Increase slowly (logarithmic-like) immediately, approaches 100 after 20 seconds	Increase super super quickly, whether exponential or logarithmic-like. Passes 100 within 1 second and keeps increasing super super super rapidly	Very very slight linear increase throughout. Only approaches about 15-20 after 20 seconds.	Did not affect too much, still had a logarithmic-like increase throughout, starting immediately, and approached 100 (almost leveling out) by 20 seconds.	Did not affect too much, still had a logarithmic-like increase throughout, starting immediately, and approached 100 (almost leveling out) by 20 seconds.
	Cyclic AMP	Starts increasing slowly (logarithmic-like) at 2 seconds, approaches 10k by 20 seconds	Starts increasing rapid linear at 2 seconds and passes 10K within 3 seconds	Starts increasing somewhat exponential at 4 seconds, inflection point at 13 seconds, slows down, approaches 7K by 20 seconds	Starts increasing after 2 seconds and then increases linearly super rapidly by reaching 10K within 2.5 seconds and then leveling off and staying constant at 10K for the rest of the domain.	Very very slight exponential increase throughout. Only approaches about 1K-2K after 20 seconds.	Not much affect by the KT 5720 as the graph remained somewhat exponential, starting to increase after 3-4 seconds, reaching an inflection point at 11-13 seconds, and reaching around 7K after 20 seconds.	Starts increasing somewhat exponential at 4 seconds, inflection point at 13 seconds, slows down, approaches 7K by 20 seconds
	PKA	Starts increasing slowly (logarithmic-like) at 2 seconds, approaches 10k by 20 seconds	Starts increasing rapid linear at 2 seconds and passes 10K within 3 seconds	Starts increasing somewhat exponential at 4 seconds, inflection point at 13 seconds, slows down, approaches 7K by 20 seconds	Starts increasing after 2 seconds and then increases linearly super rapidly by reaching 10K within 2.5 seconds and then leveling off and staying constant at 10K for the rest of the domain.	Very very slight exponential increase throughout. Only approaches about 1K-2K after 20 seconds.	Very interesting effect on the PKA by the KT 5720. The graph starts off at -5K and then starts increasing from -5K after 5-6 seconds very slowly, somewhat exponentially but quickly reaching an inflection point by 11-12 seconds, so possibly linearly. By 20 seconds the graph has only reached about 2.5K	Starts increasing exponentially at 4 seconds, has an inflection point at 13 seconds, slows down after inflection point, approaches 7K by 20 seconds
	Glucose	Starts increasing exponentially at 4 seconds, inflection point at 13 seconds, 60M at 20 seconds	Very interesting, starts increasing only after 3 seconds, increases rapid exponentially until maxes out at 100M after 7 seconds. Then actually starts slightly decreasing linear for the rest of the time. Looks like 100M is max as graph starts decreasing after hitting 100M	Starts increasing exponential at 4 seconds, inflection point at 12-13 seconds, still increases pretty fast, passes 6M by 20 seconds	Starts increasing only after 3 seconds and then increases slowly in a logarithmic-like shape. Continues to have a decreasing rate of change and a concave down shape, approaches 80M by 20 seconds and almost levels out (increases very very slowly) by the end of the domain.	Still has a notable exponential increase. A notable exponential, increasing shape which starts increasing at about 8-9 seconds and keeps increasing exponentially throughout the domain. Passes 9M by 20 seconds.	There is also a notable effect on glucose as the glucose only starts increasing very very very late, after around 16-17 seconds, but then increases rapid exponential to reach about 3M by 20 seconds.	Starts increasing rapidly and exponentially at 7 seconds. The scale of this graph is gargantuan. By 20 seconds the glucose levels have passed 4 Billion and are continuing to increase exponentially and rapidly.
	Phosphorylase Kinase	Starts increasing slowly (logarithmic-like) at 2 seconds, approaches 100k by 20 seconds	Starts increasing rapid linear at 2 seconds and passes 100K within 3 seconds	Starts increasing somewhat exponential at 4 seconds, inflection point at 13 seconds, slows down, approaches 70K by 20 seconds	Starts increasing after 2 seconds and then increases linearly super rapidly by reaching 100K within 2.5 seconds and then leveling off and staying constant at 100K for the rest of the domain.	Very, very slight linear increase throughout. Only approaches about 15K-20K after 20 seconds.	Only increases after about 6 seconds, somewhat exponentially but then quickly reaches inflection point by around 11-13 seconds so could be simply linearly. This is also very interesting as it starts from -50K and only reaches about 25K by 20 seconds, similar to the PKA graph.	Starts increasing somewhat exponential at 4 seconds, inflection point at 13 seconds, slows down, approaches 70K by 20 seconds. This is the same as the increase when the Glycogen Synthase was increased from 0 to 100.
	Carbon Dioxide	Increases exponentially but takes more time than glucose to start increasing	Increases slowly linear and takes more time than glucose to start increasing, may even start decreasing eventually as well	Stays flat at 0, no increase, graph is always flat at 0 at least for this domain	Starts increasing exponentially after approximately 5 seconds, continues with a normal exponential, concave up graph	Still has a notable exponential increase. Starts increasing later than glucose does but increases exponentially throughout the domain.	Starts increasing very very late, as late or later than glucose does, and then, very similarly to glucose, starts increasing rapidly exponentially.	Carbon Dioxide is very similar to glucose in a lot of these models. It, however, is consistently starting later, as it does here. Carbon Dioxide's graph is also exponential and increases exponentially when it starts but it starts later than glucose.
		Increase to 100	Increase to 1,000,000	Increase to 100	Increase to 10,000	Increase to 0.1	Increase to 0.1	Increase to 10,000,000,000
		Value Changes (every other independent variable remains at control)						

Figure 2: The Microsoft Excel table used to document my observations and analyses of my graphical results.

Results

Control Graph

To understand the results of this project, it is important to first view the control graph, which is Figure 1, located below. The control graph is the graph of the STELLA model run with all independent variables are at their original (control) values. The control values of each independent variable are as follows: Epinephrine: 1; Initial ATP: 10,000; Glycogen Synthase: 0; Inactive G Protein: 100; Beta Blocker: 0; KT 5720: 0; Glycogen: 100,000,000.

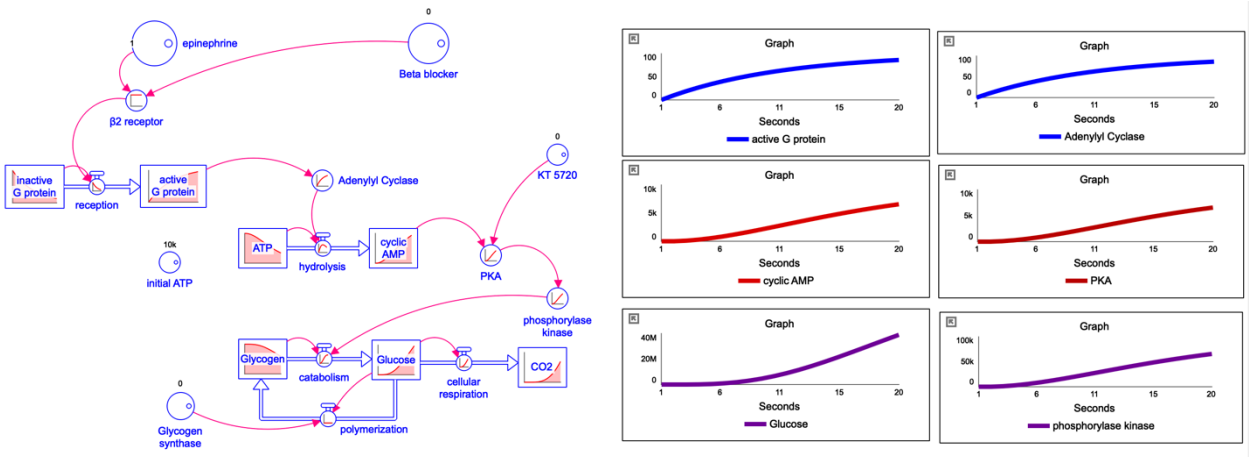

Figure 3: Control Graph

After obtaining the control graph as my baseline for my data, I ran specific tests for each independent variable.

Format

For all the following tests, the graphical results will be displayed first, and a table of my documented observations of the results will be exhibited below the graph. To reiterate once again, when one independent variable is being increased, all other independent variables are left at their control values-only the targeted independent variable is increased from its control value.

Results of Variables Predicted to Increase Pathway Progress

For the first 4 independent variables that I tested, I predicted that an increase in the independent variables would cause an increase in the pathway progress, resulting in increases for all 7 dependent variables. I predicted this because more initial value would increase the amount of initial value converted to products, and the products were the dependent variables being measured.

Epinephrine Increase 1 to 100

My first test was the epinephrine, which I increased from its control value of 1 to 100.

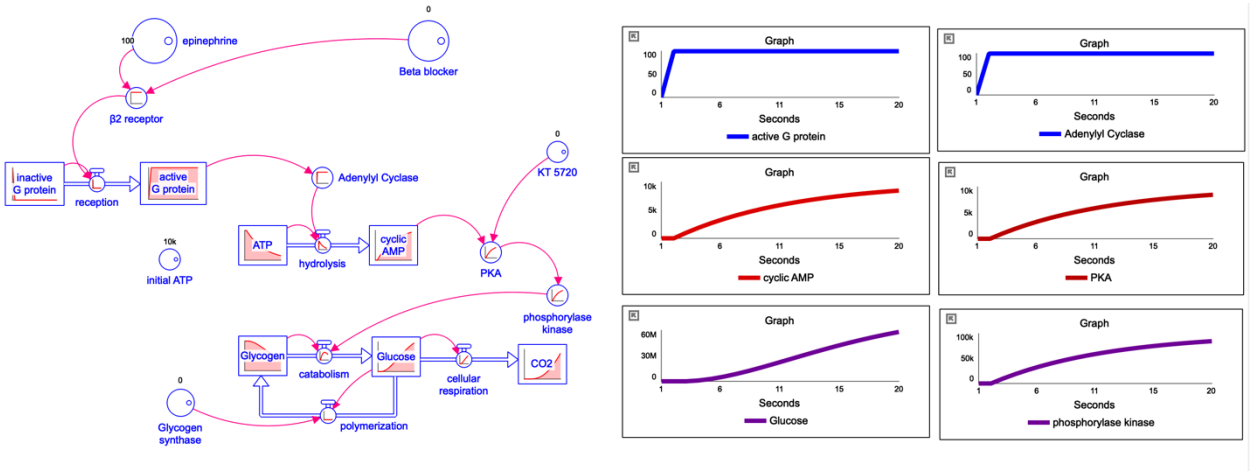

Figure 4: Graph when Epinephrine Increased from 1 to 100

Table 1: Graphical Observations

Dependent Variable	Effect when Epinephrine is Increased
Active G Protein	Increase rapid linear to 100 within 2 seconds then levels off at 100
Adenylyl Cyclase	Increase rapid linear to 100 within 2 seconds then levels off at 100
Cyclic AMP	Starts increasing slowly (logarithmic-like) at 2 seconds, approaches 10k by 20
PKA	Starts increasing slowly (logarithmic-like) at 2 seconds, approaches 10k by 20
Glucose	Starts increasing exponentially at 4 seconds, inflection point at 13, 60M at 20
Phosphorylase Kinase	Starts increasing slowly (logarithmic-like) at 2 seconds, approaches 100k by 20
Carbon Dioxide	Increases exponentially but takes more time than glucose to start increasing

I then changed epinephrine back to 1 and progressed to testing the effects and strength of initial ATP, which I changed from 10,000 to 1,000,000.

Initial ATP Increase 10,000 to 1,000,000

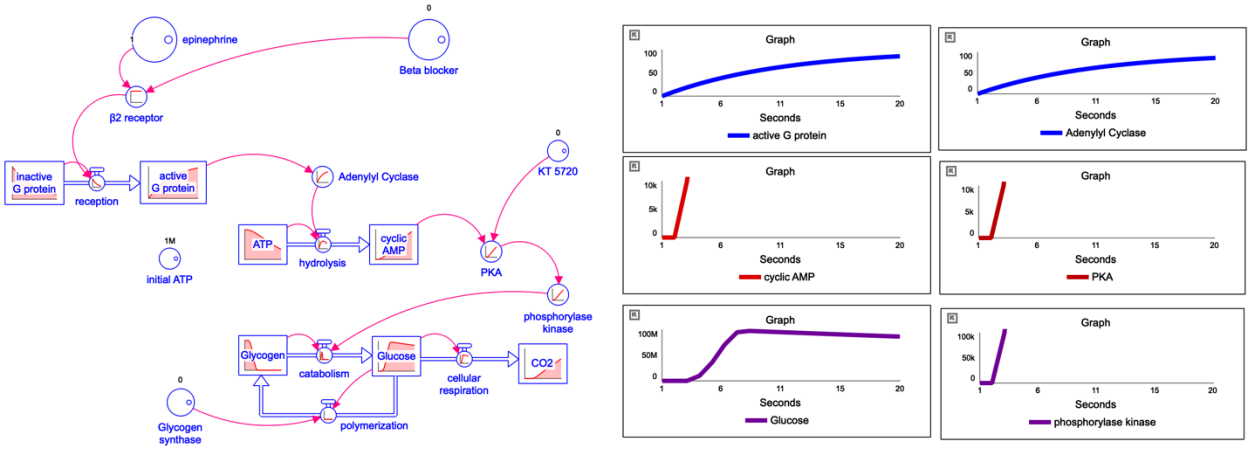

Figure 5: Graph with Initial ATP Increase from 10,000 to 1,000,000

The increase in initial ATP values created a dramatic increase in the rate of change and in the levels of most variables being measured. I documented my observations of my results in the table 2.

Table 2: Graphical Observations

Dependent Variable	Effect when Initial ATP is Increased
Active G Protein	Increase slowly (logarithmic-like) immediately, approaches 100 after 20 seconds
Adenylyl Cyclase	Increase slowly (logarithmic-like) immediately, approaches 100 after 20 seconds
Cyclic AMP	Starts increasing rapid linear at 2 seconds, passes 10K within 3 seconds
PKA	Starts increasing rapid linear at 2 seconds, passes 10K within 3 seconds
Glucose	Very interesting, starts increasing only after 3 seconds, then increases rapid exponentially until maxes out at 100M after 7 seconds. Then actually starts slightly decreasing linear for the rest of the time. Looks like 100M is max as graph starts decreasing after hitting 100M
Phosphorylase Kinase	Starts increasing rapid linear at 2 seconds, passes 100K within 3 seconds
Carbon Dioxide	Increases slow, linear, takes more time than glucose to start increasing, may even start decreasing eventually as well Increase to 1,000,000

Then, after changing Initial ATP back to 10,000, I altered Glycogen Synthase from 0 to 100.

Glycogen Synthase Increase 0 to 100

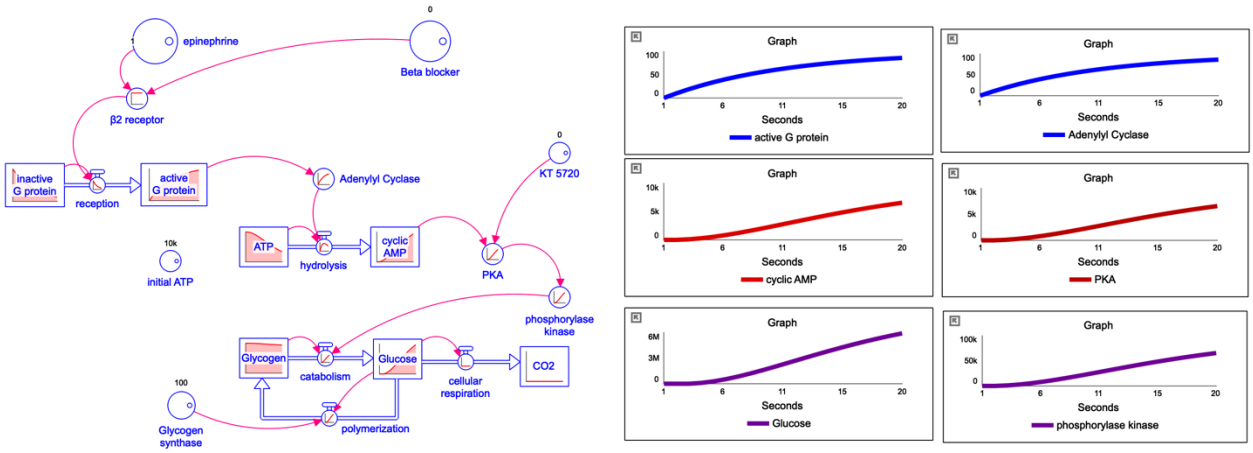

Figure 6: Graph with Glycogen Synthase Increase from 0 to 100

Table 3: Graphical Observations

Dependent Variable	Effect when Glycogen Synthase is Increased
Active G Protein	Increase slowly (logarithmic-like) immediately, approaches 100 after 20 seconds
Adenylyl Cyclase	Increase slowly (logarithmic-like) immediately, approaches 100 after 20 seconds
Cyclic AMP	Starts increasing somewhat exponential at 4 seconds, reaches inflection point at 13 seconds, slows down, approaches 7K by 20 seconds
PKA	Starts increasing somewhat exponential at 4 seconds, reaches inflection point at 13 seconds, slows down, approaches 7K by 20 seconds
Glucose	Starts increasing exponential at 4 seconds, inflection point at 12-13 seconds but still increases quite fast, passes 6M by 20 seconds
Phosphorylase Kinase	Starts increasing somewhat exponential at 4 seconds, inflection point at 13 seconds, slows down, approaches 70K by 20 seconds
Carbon Dioxide	Stays flat at 0, no increase, graph is always flat at 0 at least for this domain Increase to 100

And finally, with the last of the independent variables that I hypothesized would increase the pathway progress, I increased the Inactive G Protein from 100 to 10,000 after resetting Glycogen Synthase to 0.

Inactive G Protein Increase 100 to 10,000

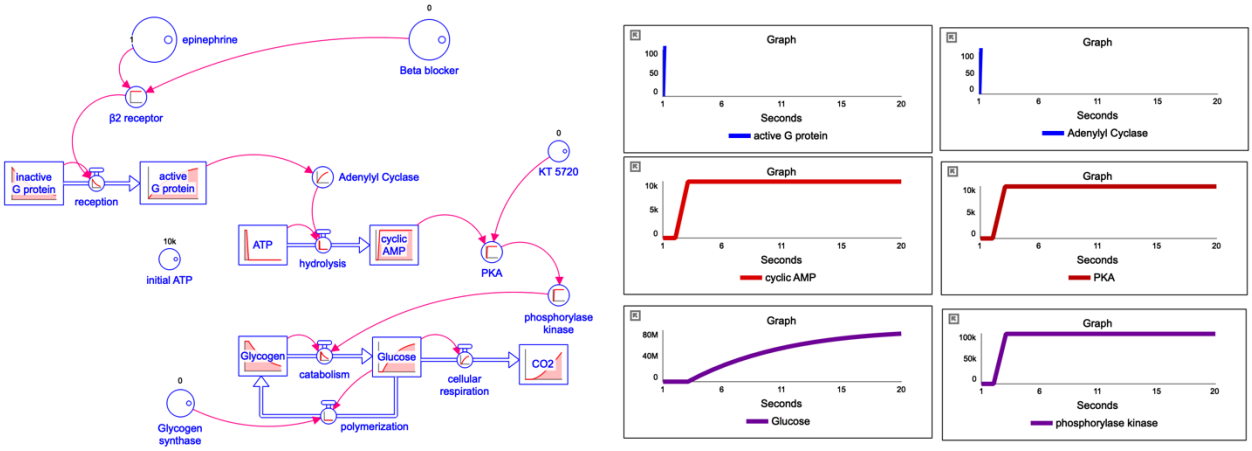

Figure 7: Graph with Inactive G Protein Increase from 100 to 10,000

Table 4: Graphical Observations

Dependent Variable	**Effect when Inactive G Protein is Increased**
Active G Protein	Increases extremely quickly. Passes 100 within 1 second and keeps increasing at an amazingly rapid pace.
Adenylyl Cyclase	Increases extremely quickly. Passes 100 within 1 second and keeps increasing at an amazingly rapid pace.
Cyclic AMP	Starts increasing after 2 seconds and then increases linearly, very rapidly, reaching 10K within 2.5 seconds, then levels off and stays constant at 10K for the rest of the domain.
PKA	Starts increasing after 2 seconds and then increases linearly, very rapidly, reaching 10K within 2.5 seconds, then levels off and stays constant at 10K for the rest of the domain.
Glucose	Starts increasing only after 3 seconds and then increases slowly in a logarithmic shape. Continues to have a decreasing rate of change and a concave down shape, approaches 80M by 20 seconds and almost levels out asymptotically by the end of the domain.
Phosphorylase Kinase	Starts increasing after 2 seconds and then increases linearly, very rapidly, reaching 10K within 2.5 seconds, then levels off and stays constant at 10K for the rest of the domain.
Carbon Dioxide	Starts increasing exponentially after approximately 5 seconds, then continues for the rest of the domain with a normal exponential, concave up graph

Results of Variables Predicted to Decrease Pathway Progress

Now we will move on to the inhibitors, which are the Beta Blocker inhibitor and KT 5720 inhibitor. I predicted that an increase in the inhibitors, as alluded to by their name, will inhibit the pathway progress and reduce the amounts-as well as rates of appearance-of the dependent variables. As with the independent variables that I predicted would cause an increase in pathway progress and dependent variables, I attempted to increase these inhibitors by a factor of 100. However, as explained earlier, this was too much inhibition for the pathway to function at all, as it shut down completely and there were no observable amounts of products formed. Because of this, I had to alter my factors of increase for the inhibitors. I reduced the factor of 100 to a factor of 0.1, and increased both the Beta Blocker and KT 5720 from 0 to 0.1

Beta Blocker Increase 0 to 0.1

The graph of the pathway with the Beta Blocker changed to 0.1 is below, and the associated documentation table, as always, is below the graph.

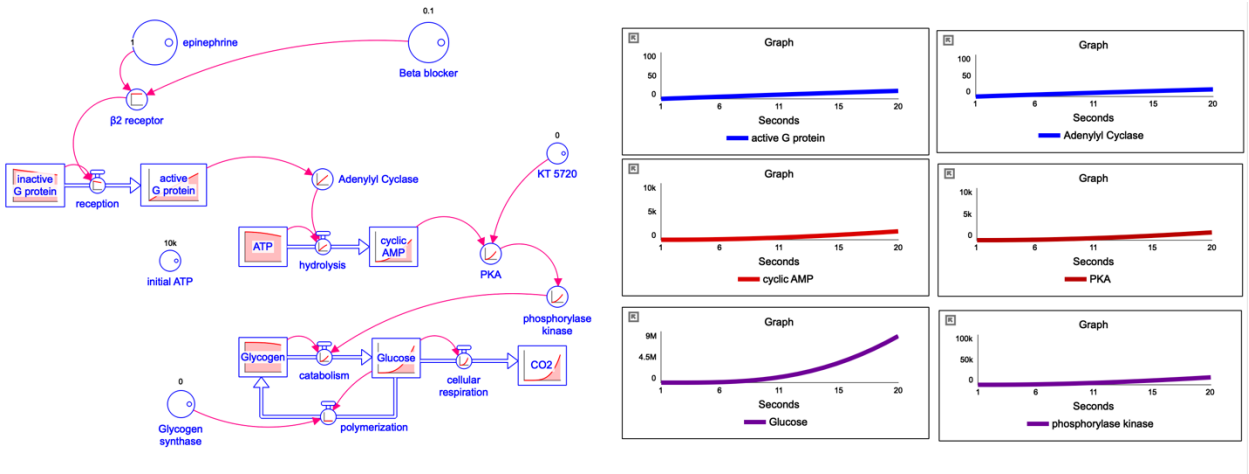

Figure 8: Graph with Beta Blocker Increase from 0 to 0.1

Table 5: Graphical Observations

Dependent Variable	Effect when Beta Blocker is Increased
Active G Protein	Very, very slight linear increase throughout. Only approaches about 15-20 after 20 seconds.
Adenylyl Cyclase	Very, very slight linear increase throughout. Only approaches about 15-20 after 20 seconds.
Cyclic AMP	Very, very slight exponential increase throughout. Only approaches about 1K-2K after 20 seconds.
PKA	Very, very slight exponential increase throughout. Only approaches about 1K-2K after 20 seconds.
Glucose	Still has a notable exponential increase and a notable exponential, increasing shape which starts increasing at about 8-9 seconds and keeps increasing exponentially throughout the domain. Passes 9M by 20 seconds.
Phosphorylase Kinase	Very, very slight linear increase throughout. Only approaches about 15K-20K after 20 seconds.
Carbon Dioxide	Still has a notable exponential increase. Starts increasing later than glucose does but increases exponentially throughout the domain.

Afterwards, I set the Beta Blocker back to 0 and moved on to the KT 5720 inhibitor, increasing it from 0 to 0.1.

KT 5720 Increase 0 to 0.1

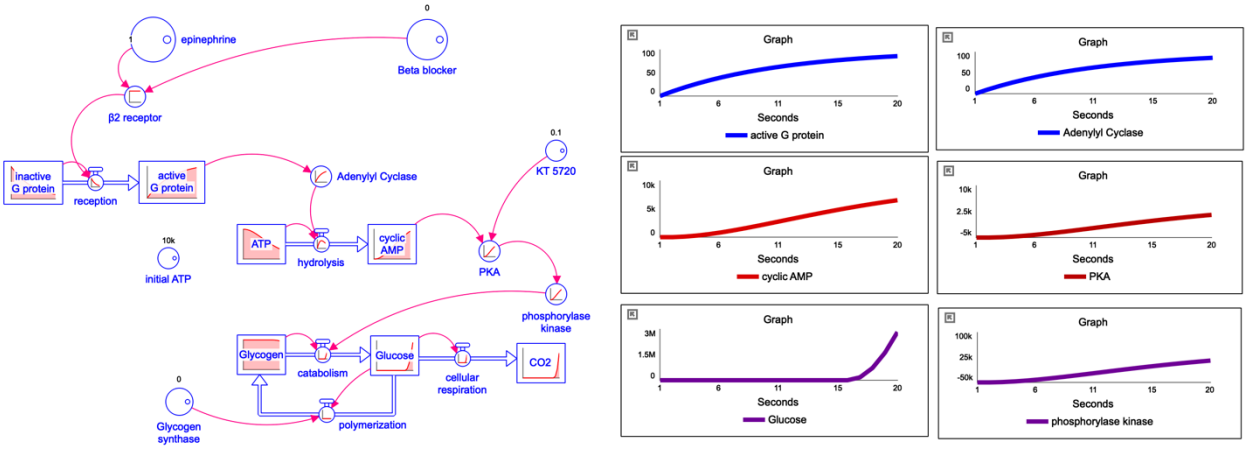

Figure 9: Graph with KT 5720 Increase from 0 to 0.1

Table 6: Graphical Observations

Dependent Variable	**Effect when KT 5720 is Increased**
Active G Protein	Did not affect too much, still had a logarithmic increase throughout, started immediately, and approached 100 by 20 seconds.
Adenylyl Cyclase	Did not affect too much, still had a logarithmic increase throughout, started immediately, and approached 100 by 20 seconds.
Cyclic AMP	Not much affect by the KT 5720 as the graph remained increasing exponentially. It started to increase after 3-4 seconds, reached an inflection point after 11-13 seconds, and passed approximately 7K after 20 seconds.
PKA	KT 5720 had a very interesting effect on the PKA. The graph starts off negative, at -5K, and then starts increasing exponentially, but very slowly, from -5K after 5-6 seconds. The graph quickly reaches an inflection point by about 11-12 seconds. By 20 seconds the graph has only reached about 2.5K
Glucose	There is also a notable effect by the KT 5720 on glucose as the glucose only starts increasing very, very late, after around 16-17 seconds, but then increases rapidly, and exponentially. The graph reaches about 3M by 20 seconds.
Phosphorylase Kinase	Only starts increasing after about 6 seconds. Increases exponentially but quickly reaches an inflection point within 11-13 seconds. Phosphorylase Kinase also starts off negative at -50K, and only reaches 25K by 20 seconds, and is overall very similar to the PKA graph.
Carbon Dioxide	Starts increasing very, very late, as late or later than glucose does, and then, very similarly to glucose, starts increasing rapidly and exponentially.

Initial Glycogen Increase from 100,000,000 to 10,000,000,000

The one independent variable left to test is the amount of initial glycogen.

Separation from Other Independent Variables

I separated the increase in initial Glycogen from the other 6 independent variables because of Glycogen's special status as an independent variable despite still receiving a rate of polymerization. I chose to still count Glycogen as an independent variable because the inflowing rate of polymerization is fueled by glycogen itself. What is flowing into glycogen is glucose that was catabolized from glycogen in the first place. This special status as an independent variable that is still receiving an inflowing rate is the reason I separated glycogen from the other independent variables.

Hypotheses for Initial Glycogen Increase

I still expected an increase in the dependent variables that glycogen eventually flowed into. Similar to the first 4 independent variables, I increased glycogen by a factor of 100, from 100,000,000 to 10,000,000,000. The graph of the STELLA model after the change in glycogen is shown below, with its associated table below the graph.

Initial Glycogen Increase 100,000,000 to 10,000,000,000

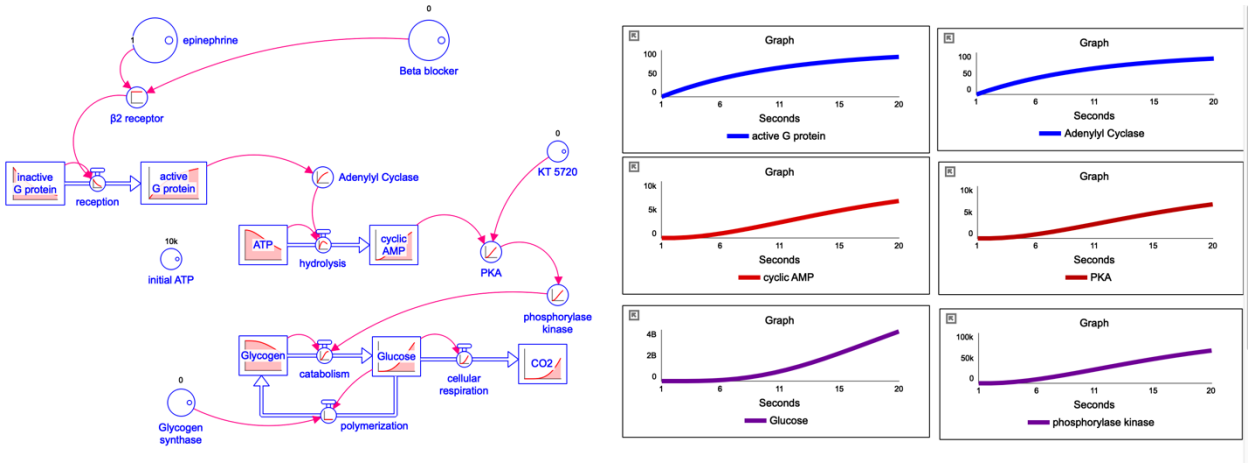

Figure 10: Graph with Initial Glycogen Increase from 100,000,000 to 10,000,000,000

Table 7: Graphical Observations

Dependent Variable	Effect when Initial Glycogen is Increased
Active G Protein	Did not affect too much, still had a logarithmic increase throughout, started increasing immediately, and approached 100 by 20 seconds at an increasingly slower rate.
Adenylyl Cyclase	Did not affect too much, still had a logarithmic increase throughout, started increasing immediately, and approached 100 by 20 seconds at an increasingly slower rate.
Cyclic AMP	Starts increasing exponentially at 4 seconds, has an inflection point at 13 seconds, slows down after inflection point, approaches 7K by 20 seconds
PKA	Starts increasing exponentially at 4 seconds, has an inflection point at 13 seconds, slows down after inflection point, approaches 7K by 20 seconds
Glucose	Starts increasing rapidly and exponentially at 7 seconds. The scale of this graph is gargantuan. By 20 seconds the glucose levels have passed 4 billion continue to increase exponentially and rapidly
Phosphorylase Kinase	Starts increasing exponentially at 4 seconds, has an inflection point at 13 seconds, slows down after inflection point, approaches 70K by 20 seconds.
Carbon Dioxide	As in many of the tests I ran, Carbon Dioxide is very similar to glucose, as in this situation it increases exponentially throughout the domain, but it starts increasing later than glucose does.

Analysis of Results

Analysis of Hypotheses

I concluded from the observations that my hypotheses on how increasing independent variables would affect dependent variables were correct. Increasing the initial values of epinephrine, ATP, glycogen synthase, the inactive G protein, and glycogen all caused the dependent variables to increase in both the rate and amount of appearance, as I hypothesized. Likewise, an increase in the inhibitors (the Beta Blocker and KT 5720) led to a decrease in the rate and amount of appearance of the dependent variables. While my predictions were correct, there were also some intriguing, unexpected results that arose.

Analysis of Unexpected & Notable Results

Going through the unexpected results, we start with the active G protein and adenylyl cyclase when epinephrine was increased from 1 to 100. The active G protein and adenylyl cyclase, after increasing through a steep linear slope,

leveled off at 100 and became horizontal lines. This was not expected and led me to believe that 100 must be the maximum value limit, in a medical context, of both the active G protein and adenylyl cyclase.

However, my prediction that 100 would be the upper bound of the active G protein and adenylyl cyclase was proven wrong when I increased the initial inactive G protein from 100 to 10,000. When I increased the inactive G protein, I found that both the active G protein and adenylyl cyclase soared past 100 within 2 seconds. This means that it was the epinephrine itself and the epinephrine's own medical limits that were preventing the active G protein and adenylyl cyclase from increasing past 100 earlier. This must have been specific to the epinephrine, as in all the other tests that I ran, the values for the active G protein and adenylyl cyclase did not level off horizontally but did so logarithmically.

I noticed that, except when the inactive G protein or Beta Blocker inhibitor are increased, the active G protein and adenylyl cyclase graphs were the exact same in every test, for each and every independent variable increase. This meant that all the independent variables, including the KT 5720 inhibitor, except for the Inactive G Protein and Beta Blocker inhibitor, had the exact same effect on the Active G Protein and Adenylyl Cyclase. Looking back at the STELLA model to explain this, I realized that the reason for this effect is because only the Inactive G Protein and Beta Blocker inhibitor directly affect the active G protein and adenylyl cyclase or variables that end up affecting the active G protein or adenylyl cyclase. All the other independent variables have no effect on the active G protein and adenylyl cyclase or other variables that end up affecting the active G protein or adenylyl cyclase. A simplified way to think about this is that the Beta Blocker and Inactive G Protein come before the Active G Protein and adenylyl cyclase in the flow of the model, so they affect them. The other independent variables come after the Active G Protein and adenylyl cyclase, so they do not affect them. The way to think about what comes before or after a variable is to follow the path of the model, which also represents the actual path of the epinephrine signal transduction pathway. The way to read the pathway is to follow the flows and connectors from top to bottom, starting with epinephrine and the Beta Blocker, and ending with carbon dioxide.

Another unexpected result from my tests came when I increased the initial ATP amounts from 10,000 to 1,000,000. When this happened, every dependent variable (other than the Active G Protein and Adenylyl Cyclase) increased rapidly, but glucose had a very interesting graph. The glucose levels increased exponentially and rapidly, reaching 100M after 7 seconds. However, 100M seemed to be the maximum amount of glucose permitted, and the glucose levels, very surprisingly, started slightly decreasing linearly after hitting 100M, for the rest of the domain.

More unexpected results ensued from my tests with the inactive G protein. I increased the inactive G protein from 100 to 10,000, and that dramatically changed the graphical results. First, the rate of increase that this change brought to the active G protein and adenylyl cyclase was unprecedented, as both variables crossed 100 within a second. Then, both the cyclic AMP and PKA started, after 2 seconds, increasing linearly and rapidly, but then leveled out and stopped horizontally at 10K. The same type of increase occurred with Phosphorylase Kinase, which leveled out horizontally at 100K. Glucose started increasing logarithmically after 3 seconds and remained concave down throughout the graph's domain. Surprisingly (and quite rarely in this model), carbon dioxide had a different type of increase than Glucose, as it increased exponentially, and is concave up throughout its domain.

Another notable result is the mammoth power of inhibitors, especially the Beta Blocker inhibitor. I already mentioned that I had to increase the Beta Blocker and KT 5720 inhibitors only by a factor of 0.1 because increasing by a factor of 100, 10, 5, 1, and even 0.5 was too much for the pathway to progress. When I increased the inhibitors, most notably the Beta Blocker, by factors of 0.5 or greater, the entire pathway was shut down as all of the dependent variables showed no notable increase and were constant at 0 for their entire domains.

When I increased the Beta Blocker by 0.1, all the dependent variables had very, very slight increases at very slow rates. The only dependent variables which still had decent rates of increase were glucose and carbon dioxide, and they also only started increasing quite late in their domain (glucose only started increasing after 7 seconds).

When I increased the KT 5720 inhibitor to 0.1, the results were not as dramatically decreased as they were for the Beta Blocker inhibitor. However, one important thing to note was that, for glucose and carbon dioxide, although

the values were greater due to the lesser effect of KT 5720, the initial increases from the x-axis only started much later (at about 17 seconds) than they did for the Beta Blocker test.

The last unexpected result that I want to note is the massive increase in glucose when glycogen was increased from 100,000,000 to 10,000,000,000. When on all the other scales, glucose passes 100M at 20 seconds, on the glycogen-increased scale, glucose passes 4B at 20 seconds.

Discussion & Practical Applications of Results

These results can be used to help understand which of the variables have the greatest effect on the epinephrine signal transduction pathway. If we begin to interpret these results, we see that while results can differ in impacts on specific dependent variables, a few general results regarding the model, and thus even regarding at about 17 seconds 54 at about 17 seconds + . the epinephrine signal transduction pathway, can be made.

Evaluation of Results and Key Takeaways

We can start by evaluating which of the independent variables has the greatest effect on the pathway. I claim that it is the Beta Blocker inhibitor, followed by the KT 5720 inhibitor. The fact that I was forced to reduce the planned increase by a factor of 1000, from 100 to 0.1, shows the immense power of the two involved inhibitors. Even after the slight increase of 0.1, the Beta Blocker was so powerful that there was still almost no increase in most dependent variables. While the KT 5720 inhibitor wasn't as powerful as the Beta Blocker, it is important that we keep in mind that this was an increase of a factor of 0.1 instead of a factor of 100, like the other independent variables. Furthermore, most of the dependent variables' increase rates were quelled (except the active G protein and adenylyl cyclase) for the KT 5720 test as well.

Moving, we will have to analyze the other independent variables from a dependent variable-specific view, as there was not a single independent variable that broadly increased all dependent variables like the two inhibitors broadly decreased all dependent variables. We can start by saying that increasing the initial ATP values was the best solution and method to increase the cyclic AMP, PKA, and phosphorylase kinase levels. We can also declare that increasing the inactive G protein is the best way to increase the values of the active G protein and adenylyl cyclase. Finally, we can say that increasing the glycogen value is the best solution to increasing the values of glucose and carbon dioxide.

Practical Applications in the Medical Field

These results could be used in medical science in the situation of wanting to increase a specific protein, compound, or product which is involved in this pathway. For example, in the medical context, if a patient is producing too little of the active G protein or adenylyl cyclase, doctors could administer cells containing the inactive G protein. Another example would come if a patient is low in glucose. If this is the case, then doctors could administer glycogen supplements to the patient. (This last example is something that the body can often do on its own, where the liver could release glycogen (triggered by glucagon) if glucose is low. If this doesn't work, doctors can administer glycogen. This is what happens in diabetes.) Another example of the medical use of these results of this project would be if a patient is severely low in cyclic AMP, PKA, or phosphorylase kinase. If this is the case, doctors could administer ATP, which actually can be administered through intravenous bolus injections. Furthermore, say a patient's epinephrine signal transduction pathway is overexerting itself and producing an extremely large surplus of products. In this case, doctors could administer extremely small amounts of the Beta Blocker or KT 5720 (KT 5720 if specific variables are overproducing) to subdue the pathway so that it does not overwork itself. All of these are just some of the examples of the uses of the results from my project.

Conclusion

There are many different variables involved in the epinephrine signal transduction pathway, and my results have quantified which independent variables cause the greatest effects on specific dependent variables. I have concluded that the most powerful variables are the inhibitors: the Beta Blocker, followed by KT 5720. After these two, the strength of the independent variables is specific for increasing specific dependent variables. For increasing cyclic AMP, PKA, or phosphorylase kinase, increasing the initial ATP values is the best solution. To increase the active G protein and adenylyl cyclase, increasing the inactive G protein is the best way. Finally, for increasing glucose and carbon dioxide values, increasing glycogen is the best tactic to employ.

As mentioned previously, the results of this paper can be applied in medical contexts if doctors may need to increase or decrease a specific variable which is involved in the epinephrine signal transduction pathway. However, when targeting to increase or decrease one specific dependent variable, it is also important that doctors take into account the effects on other dependent variables when an independent variable is altered. I believe that along with my written results, medical professionals could also use the included graphs and tables to survey the effects of increasing one independent variable on different dependent variables to accurately adjust their injection plans to ensure that all involved variables are within bodily limits.

Limitations

Some possible limitations in this research include the fact that my results are based off the computational model that I used, and that there is no additional computational method of verifying these results outside of mathematical computations and using the STELLA model. However, these results can also be verified with experimental scientific procedures.

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