**Group member first names:**

**Introduction to Part 1:**

*Acetylcholine* is a key neurotransmitter, which means it is a molecule used to send signals between nerve cells or from a nerve cell to a muscle. Nerve cells must respond very quickly to neurotransmitters, which also means that there can be problems if there is too much neurotransmitter around and signaling lasts too long. Acetylcholinesterase (AChE) is an important enzyme near the surface of nerve cells that destroys acetylcholine and therefore stops the signal. In this module, we will examine how acetylcholinesterase acts.

Acetylcholinesterase catalyzes a hydrolysis reaction that converts acetylcholine into acetate and choline:



We will analyze data from cell culture experiments that measured how initial reaction rates change by increasing the available amounts of the substrate, acetylcholine. In the experiment, cells expressed high levels of the enzyme, the substrate [S] was added to the enzyme, and then the products were measured with a fluorescent label to determine initial reaction rates (Vi). Keep in mind that this reaction does not occur spontaneously at a measurable rate.

**Students: To receive credit for this exercise, provide short answers and supporting calculations for all questions below. You are encouraged to work in groups.**

**Part 1 uninhibited enzyme kinetics (3 questions)**

Data is provided in the Excel Sheet **NIQB Cell Biology-Enzyme Kinetics Dataset** (note there are 2 tabs/sheets- start with the Uninhibited data).

1. What are the units of initial velocity and substrate concentration?

2. Determine the **maximum velocity (Vmax)** of the reaction by estimating the asymptote from the trendline on the blue graph. Describe this graph in terms of the relationships between the variables and write your answer for Km here. Include units!

Michaelis and Menten developed some useful methods to describe enzyme kinetics. They defined the **Michaelis constant, Km**, as the substrate concentration [S] at which a reaction is occurring at half of its maximum rate. They presumed that the concentration of Enzyme was always much higher than needed, so they did not consider this value. After this, they noted the following mathematical relationship:

**V=(Vmax \*[S]) /([S]+Km)**

Lineweaver and Burk realized that the enzyme kinetic graphs would be easier to work with and interpret if they were linear. By taking the reciprocals of the [S] and V, they could generate a linear graph (a Lineweaver-Burk plot). This is shown below the first graph.

Key parameters to describe an enzyme’s activity are clear from this plot:

The slope of the line is = Km/Vmax

So,

The y-intercept is = 1/Vmax

And the x-intercept is = -1/Km

3. Use the graph and information above to determine the Km and Vmax of acetylcholinesterase. Write your answers here. Include units for Km and Vmax!

**Introduction to Part 2, inhibited enzyme kinetics:**

We will now examine the impacts of two different inhibitors on the enzyme kinetics of acetylcholine with acetylcholinesterase. For each inhibitor, you will create a Lineweaver-Burk plot and use the KM and Vmax values to determine whether the inhibitor is competitive or non-competitive.

Acetylcholinesterase has numerous known inhibitors that can interact with it reversibly, semi-irreversibly, or irreversibly. Some of these inhibitors are allosteric and bind to other regions on the enzyme to inhibit its activity, while many others bind directly to the catalytic domain active site, blocking acetylcholine from binding. Both types of inhibitors will prevent acetylcholine from being hydrolyzed into choline and acetate. When acetylcholine is not hydrolyzed, it remains in the synaptic cleft between the two neurons or the neuron and muscle tissue for a longer period of time and will continue to relay signals between the cells.

Environmental exposure to acetylcholinesterase inhibitors has been extensively studied. Various pesticides in the organophosphate and carbamate families as well as heavy metals like lead and mercury are known to act as acetylcholinesterase inhibitors. Other types of inhibitors are being examined for their medical use in the treatment of degenerative neurological diseases like Alzheimer's and Parkinson’s disease. This module explores two specific biologically relevant acetylcholinesterase inhibitors: caffeine and galantamine.

Most of us are well aware of the physiological effects of drinking substances containing caffeine. We have probably all experienced an increase in alertness from the caffeine within our daily cups of coffee, tea, soda, or energy drinks. What you may not be familiar with is how that daily dose of caffeine interacts with the nervous system in your body. Caffeine acts as a central nervous system stimulant and affects several key neurotransmitter systems including inhibiting the activity of acetylcholinesterase. Due to its potential for inhibiting acetylcholinesterase, there have numerous animal model and human epidemiology studies examining the benefits of caffeine in protecting against and treating neurodegenerative diseases like Alzheimer’s disease. In fact, many pharmaceutical drugs produced to treat Alzheimer’s disease act as acetylcholinesterase inhibitors. We will be examining the effects of one of these drugs, galantamine, in this module.

**Part 2 - inhibited enzyme kinetics (3 questions)**

**Activity:**

4. Prior to examining the effect of each inhibitor, each group should draw/sketch a graph with your predictions of the expected outcome for a competitive inhibitor and a non-competitive inhibitor of acetylcholine for acetylcholinesterase. Try sketching out both a standard graph and a Lineweaver Burk plot. (You can do this on paper, you do not need to do it digitally.) Write down whether you expect Vmax to increase, decrease, or stay the same in the presence of each type of inhibitor. Write down whether you expect Km to increase, decrease, or stay the same in the presence of each type of inhibitor.

 **Note: that the competitive inhibitor has the same Vmax and non-comp. have the same Km as the uninhibited reaction**

Each group will now open Excel **Sheet2** labeled “Inhibitor data” and analyze the Lineweaver-Burk plot of the two inhibited acetylcholine reactions.

5. Using the linear regression equation (to the right of the graph) from each inhibited reaction, calculate the KM and Vmax for each inhibitor. Determine which intercepts (x or y) changed for each inhibitor compared to uninhibited reaction. Write your answers in the chart below.

|  |  |  |  |
| --- | --- | --- | --- |
| Type of Inhibitor | KM (mM) | Vmax (mM/min) | Changed intercept(x or y) |
| Caffeine  |  |  |  |
| Galantamine  |  |  |  |
| Uninhibited reaction (from above, for reference) |  |  |  |

Expected changes to KM and Vmax for each inhibitor:

**Competitive inhibitor:** KM will increase (change in x-intercept) and Vmax will stay the same

**Non-competitive inhibitor:** KM will stay the same and Vmax will decrease (change in y-intercept).

6. Using the values of Vmax and KM of the inhibited enzymatic reaction and the expected changes to each shown above, explain in a complete sentence for each inhibitor whether that inhibitor binds competitively or non-competitively to acetylcholine.

**Challenge/Sponge Questions (optional):**

7. Explain why competitive inhibitors make effective drugs for medical treatments.

8. Explain why certain inhibitors might be more commonly found naturally within organisms.

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