LAB 8: ENZYMES AS DRUG TARGETS.

Objectives

- To review an appreciation of enzyme structure and function
- To categorise and define the major types of enzyme inhibitors
- To develop an understanding of the consequences of inhibition of enzymes by drugs

Open the ***Enzymes as Drug Targets*** link, and click on the intro page to commence. Click on the protein icon to enter the tutorial. Make notes as necessary according to the guide below.

1/ Enzymes as catalysts.

Degradation

Rearrangement

Synthesis

2/ Location of enzymes

Acetylcholine esterase ……

Alcohol dehydrogenase ……..

Mixed function oxidase ……..

Pepsin ……

DNA polymerase ……..

Pyruvate dehydrogenase ……

3/ Structure of enzymes.

(A) Primary structure

Secondary structure

Tertiary structure

Quaternary structure

(B) Activity centre

4/ Catalysis and activation energy

(A) What happens to the enzyme and substrate following binding?

Draw the graph showing lowering difference in activation energy for non-enzyme and enzyme-catalysed reactions

(B) Give an example of stereoselectivity with respect to enzyme catalysis:

5/ Acceleration of equilibrium

(A) Enzymes and equilibrium – turnover number

What is the turnover number per active site of acetylcholine esterase?

(B) Enzyme kinetics (draw the graph and label it)

BACKGROUND: A number of very important drugs act by inhibiting enzymes. Drugs vary how they achieve enzyme inhibition. The rate of an enzyme reaction (V) varies with substrate (S) concentration. Increasing S increased V until Vmax is reached. K_m, concentration of substrate is the concentration at which reaction rate is half maximal., represents a measure of how tightly the substrate is bound. i.e. the affinity of the substrate for the enzyme. A large K_m represents low affinity/low binding and vice versa.

Vmax = ……..

Km = ……..

(C) Lineweaver Burke plot (draw graph)

I/Vmax =

-1/Km =

6/ Isoenzymes

(A) An example of an enzyme isoform:

(B) Ignore

7/ Enzyme inhibitors

A) Competitive reversible inhibitors

Example. ACE inhibitor ……..

C) Other examples of competitive inhibitors. Match the following drug with their target enzyme:

allopurinol	xanthine oxidase
edrophronium	monoamine oxidase
ibuprofen	bacterial dihydrofolate reductase
moclobemide	cyclooxygenase
trimethoprim	acetylcholinesterase

D) Enzyme kinetics

The Lineweaver Burk plot (draw)

Definitions:

Km = ……

V = ……..

S = ……..

What is the effect of a **competitive reversible inhibitor** on

V_{max}	increase, decrease, or no change?
K_m	increase, decrease, or no change?

8/ Competitive irreversible inhibitors

Come to equilibrium – time dependent

Two groups: Active site inhibitors (covalent binding)

Mechanism-based irreversible inhibitors (suicide substrates)

9/ Active site inhibitors

A) *Example (drug and enzyme substrate)……*

B) *COX actions ……..*

C) *Further examples (match):*

Dyflos	acetylcholinesterase
Benzylpenicillin	DNA polymerase (viral)
Acyclovir	glycopeptide transpeptidase
Methotrexate	Dihydrofolate reductase (human)

D) *Kinetics* *Lineweaver Burk plot (draw)*

What effect do non-competitive reversible inhibitors have on

V_{max} Increase, decrease, or no change?

K_m Increase, decrease, or no change?

10/ Mechanism-based suicide inhibitors

(A) Examples……

Mechanism of action?

……

(B) Clavulanic acid

……..

11/ Non-competitive reversible inhibitors (a.k.a. allosteric inhibitors)

A) *Example……...*

B) *Eicosanoid synthesis and actions*

C) *Lineweaver Burk plot (draw)*

What effect do non-competitive reversible inhibitors have on

V_{max} Increase, decrease, or no change?

K_m Increase, decrease, or no change?

D) Allosteric activators

A few drugs work by enhancing enzyme activity. These are called allosteric activators

Example……

Mechanism of action?

12/ Consequences of enzyme inhibition

(A) Inhibition of an enzyme could prevent:

A fall in substrate concentration? (see B)

A rise in substrate concentration?

A fall in concentration of products?

A rise in concentration of products? (see C)

B) Neostigmine

Mechanism of action?

C) Thromboxane A_2 (TXA_2)

Actions in the vasculature?

Aspirin. Mechanism of action?

13/ Duration of action

Reversible, e.g. captopril

Irreversible, e.g. aspirin

RETURN TO MAIN MENU AND DO THE MCQ TEST TO CHECK YOUR NOTES AND KNOWLEDGE

EXIT PROGRAMME