Week 5: Laboratory – Guinea Pig Ileum: An Investigation of the effects of hyoscine butylbromide (Buscopan®) on intestinal motility

AIM

The aim of the laboratory is to investigate the effects of hyoscine butylbromide (Buscopan®) on intestinal motility using a classical in-vitro pharmacology preparation (the guinea pig ileum), to determine what types of receptor it acts upon and its mechanism of action.

LEARNING OUTCOMES

At the end of this laboratory, you should be able to

- Perform and interpret the results of simulated in vitro pharmacological experiments on the guinea pig ileum.
- Understand and use the dilution formula.
- Have a greater understanding of the mechanisms and neurotransmitters involved in controlling intestinal motility and the mechanism of action of hyoscine butylbromide.

RATIONALE

This laboratory consolidates the learning outcomes of the following lectures and workshops: (NFGI Motility in the gut lecture and Preparation for lab work workshop). It demonstrates the pharmacological methods used to determine how a drug acts on the motility of the GI tract, the receptors it affects and provides practical experience in the use of dilution calculations. By using a simulation for this lab it means that no animals will be sacrificed ad you can repeat the experiments much more readily than what would be possible in an actual 'wet lab' setting. Since the effects of drugs and the receptor mechanisms activated are similar in many animal species to those in humans, the knowledge gained from this simulation is readily transferable.

PREPARATION

Before you attend this laboratory session you must:

- Watch the GP ileum video posted on the MP220 MyPlace page to consolidate your understanding of how this type of experiment is performed in the laboratory.
- Read the relevant safety documents. You will be required to sign these before the lab.

For this laboratory you will need:

White coat, safety goggles.

ESSENTIAL READING

The following must be reviewed before you come to the lab.

- The laboratory notes (1 – 3) on organ bath experiments, dilutions and the Chart recorder program associated with this simulated laboratory.
- Your dilution calculations and literature search results on hyoscine butylbromide produced during the preparatory workshop that preceded this laboratory.
- Chapter 15. The Digestion and Absorption of Food, Human Physiology (Vander, Sherman & Luciano)
- Chapter 1. Rang and Dale, Pharmacology.

Experimental Protocol

Using the GP ileum simulation, record and measure the effect of electrical stimulation of the enteric nervous system on the contractility of the guinea pig ileum.

Protocol

a. Open the GP simulation program (link found in MP220 MyPlace page) and click on new experiment. **See notes below on how to save your data.**
b. Start recording and switch on the nerve stimulation.
c. Record until contractions are stable (~1 minute).

Results

a) Paste a copy of the recording here.

b) Measure the peak amplitude of the contraction (if any) and note it here.

Record and measure the effect of addition of acetylcholine on the contractility of the guinea pig ileum.

Protocol

a. Start recording and wait 1 minute.
b. Add the low dose ($1x10^{-8}$M FBC) of acetylcholine (select from the list of drugs available) to the organ bath and leave until stable (~30 seconds).
c. Flush the bath with solution from the reservoir. Click the 'Flush to Reservoir to Bath' button.
d. Wait 2 minutes.
e. Add the medium dose ($1x10^{-7}$M FBC) of acetylcholine to the organ bath.
f. After 30 seconds, flush the bath with solution from the reservoir.
g. Wait 2 minutes.
h. Add the high dose ($1x10^{-6}$M FBC) of acetylcholine to the organ bath.
i. After 30 seconds , flush the bath with solution from the reservoir.

Results

a) Paste a copy of the recordings here.

b) Measure the peak amplitude of the contractions (if any) and add to your results spreadsheet.

Record and measure the effect of hyoscine on nerve- and acetylcholine-evoked contractions of the guinea pig ileum.

Protocol

a. Start recording and switch on the nerve stimulation.
b. Add $1x10^{-6}$M (FBC) hyoscine to the reservoir. Select hyoscine (concentration and volume). Select **Reservoir** from the Add To list and click the **Add To** button.
c. Flush the bath with solution from the reservoir (**Flush Reservoir to Tissue Bath**)
d. Wait 2 minutes then stop stimulating.
e. Wait 1 minute.
f. Add the low dose ($1x10^{-8}$M) of acetylcholine to the organ bath
g. After 30 seconds, flush the bath with solution from the reservoir.
h. Wait 2minutes.
i. Add the medium dose ($1x10^{-7}$M) of acetylcholine to the organ bath.
j. After 30 seconds, flush the bath with solution from the reservoir.
k. Wait 2minutes.
l. Add the high dose ($1x10^{-6}$M) of acetylcholine to the organ bath.
m. After 30 seconds, flush the bath with solution from the reservoir.

Results

a) Paste a copy of the recording here.

b) Measure the peak amplitude of the contractions (if any) and add to your results spreadsheet (downloadable from Myplace).

c) Paste the graph from the results spreadsheet here.

Complete the table below with the measurements you have taken. These will be used in next week's workshop.

	Peak amplitude of contraction (g)	
	Control	With 10^{-6} M hyoscine
Nerve-stimulation		
10^{-8}M acetylcholine		
10^{-7}M acetylcholine		
10^{-6}M acetylcholine		

This next section is for discussion in next week's workshop. Before the workshop reflect on the results that you have obtained and answer the following questions. These will be used as the basis for discussions with colleagues.

Discussion

a. What effect does hyoscine have on nerve-evoked and acetylcholine-evoked guinea pig ileum contractions?

b. Is acetylcholine an agonist or an antagonist and what types of receptors does it act upon?

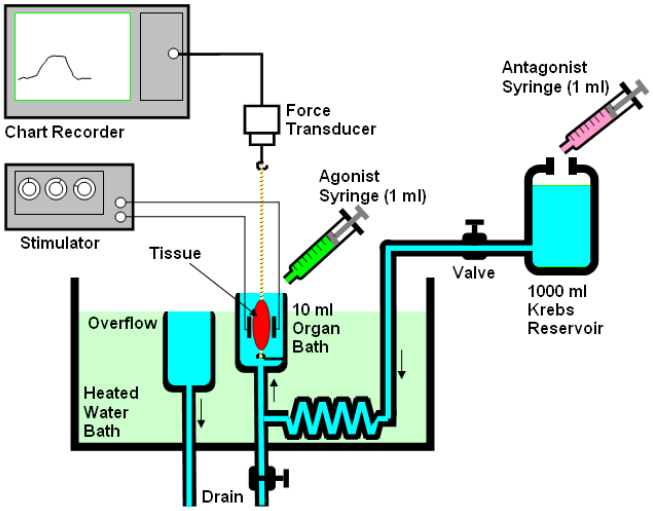
c. Is hyoscine an agonist or an antagonist and what types of receptors does it act upon?

d. Based upon the evidence in this experiment, what type of neurotransmitter is being released by nerve stimulation?

e. Using the evidence from this experiment, explain why Buscopan® is used to treat IBS and intestinal cramps.

Note 1 – The Guinea Pig Ileum Preparation – experimental set up
(N.B. watch video posted on MP220 Myplace page)

The tissue being studied (guinea pig ileum) is attached to a **force transducer** which generates an electrical signal proportional to the contractile force generated by the tissue when a drug is applied. The force transducer is connected to an **amplifier** that boosts the small voltages produced by the transducer to a level suitable for measurement by the computer. The amplified signal is then fed into an **analogue-to-digital (A/D) converter** card inside the computer which digitises the signal and stores it on computer disk, under the control of the **CHART** digital chart recorder computer program.



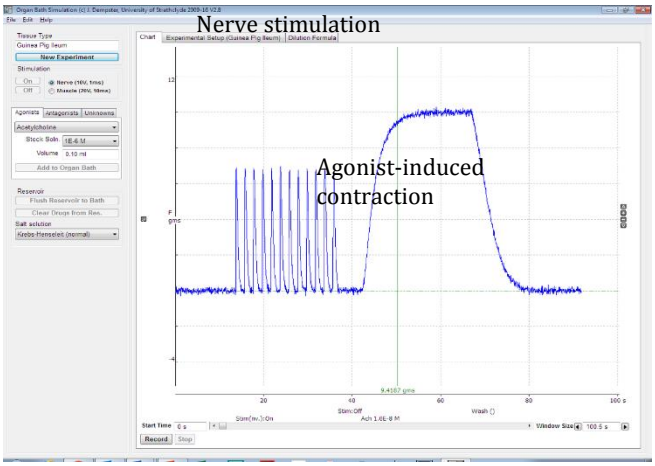
The tissue is immersed in a small **10 ml** volume organ bath containing a physiological salt solution, Krebs-Henseleit (K-H), which approximates the extracellular fluids normally bathing the tissue *in vivo*. The organ bath is contained within a Perspex bath contains tap water maintained at a temperature close to the normal body temperature of the guinea pig (37°C) by a heater and thermostat. The organ bath is connected to a reservoir containing K-H solution. Opening the reservoir tap allows physiological solution to flow through the warming coil into the organ bath. A mixture of oxygen (95%) and carbon dioxide (5%) is bubbled into both the reservoir and organ bath to provide oxygen and maintain the pH of the tissue.

Drugs can be applied to the tissue by pipetting small volumes of drug-containing solution directly into the the bath and removed by flushing the organ bath with fresh solution from the reservoir. The tissue can also be stimulated electrically with 50V electrical pulses using a stimulator attached to a pair of electrodes placed on either side of the tissue within the organ bath.

Note 2: Using the Guinea Pig Ileum Simulation for studying the effects of hyoscine on nerve-mediated and acetylcholine-mediated contractions.

The Organ Bath simulation program allows you to perform simulated *in vitro* experiments using a computer model of the guinea pig ileum preparation.

To start the **Organ Bath** program, click on its icon in the Lab section of the MP220 BAP1 Myplace page.



Starting an experiment

To begin a simulated experiment:

a) Select **Guinea pig ileum** as the type of tissue you intend to experiment on from the **Tissue Type** list.

b) Click the **New Experiment** button to clear the chart display and select a new piece of guinea-pig ileum tissue.

c) Click the **Record** button to start recording the tissue contractile force in the chart recorder window.

d) If using agonist-induced contractions, follow instructions below for adding relevant agonist(s) to the organ bath to induce contractions.

e) To start nerve-mediated contractions, make sure the nerve stimulation is checked and click the on button. To test agonist effects against nerve-mediated contractions, follow instructions below for adding relevant agonists to the organ bath to induce contractions.

You can also stop and re-start recording at any time using the **Stop / Record** buttons.

***Note**. Each time a new piece of tissue is selected its response characteristics change. To avoid large variability in your concentration-response curves, you should be avoid changing the tissue during an experiment.*

Applying agonists

Agonists are injected directly in to the organ bath, **which has a volume of 10 ml.**

To apply an agonist:

a) Select the type of agonist to apply from the **Agonist** list.

b) Select the concentration of the agonist stock solution to be added from the Agonists **Stock Soln**. list.

c) Enter the volume of stock solution to be added into the **Volume** box. *(The maximum volume that can be added is **1 ml**.*

d) Click the **Add to Organ Bath** button.

e) Wait till the tissue response reaches a maximum.

f) Click the **Flush Reservoir to Bath** button to remove the agonist by flushing the bath with solution from the reservoir.

g) When investigating the actions of an unknown drug in the current lab, please follow the instructions for applying an agonist.

Applying antagonists

If application of antagonists is required, these should be added to the **reservoir** (which has a volume of 1000 ml) and applied to the tissue in the organ bath by flushing from the reservoir. This allows agonists to be added and washed out, while maintaining a constant concentration of antagonist in the bath.
To apply an antagonist:

a) Select the type of antagonist to apply from the **Antagonists** list.

b) Select the concentration of the antagonist stock solution to be added from the Antagonists **Stock Soln**. list.

c) Enter the volume of stock solution to be added into the **Volume** box. . *(The maximum volume you can add is **1 ml**.)*

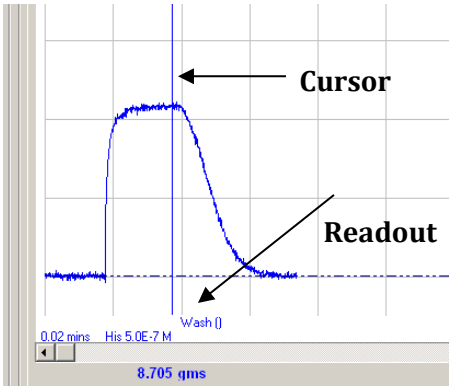
d) Select **Reservoir** from the Add To list and click the **Add To** button.

e) Click the **Flush Reservoir to Tissue Bath** button to add the antagonist to the bath.

You only need to add the antagonist once as it remains in the reservoir throughout the experiment. If you want to change the antagonist concentration, click the **Fresh Reservoir** button and add a new concentration, as above.
Notes *Remember to take account of the dilution that will occur in the 1000ml reservoir when calculating the final bath concentration.*
After adding an antagonist, it is useful to add approximately the EC_{50} concentration of agonist to determine by how much the antagonist has shifted the agonist concentration response curve. Remember when constructing concentration-response curves also that you will have to use higher concentrations of agonist than you used in the control regime because of the effects of the antagonist, and will need to continue adding agonist until the maximum response is again achieved.

Making Measurements

The force of the tissue contraction can be measured (in gms) by using the mouse to drag the blue vertical measurement cursor to the point on the trace to be measured and reading off the contractile force at the point at the bottom of the display.



Printing/Copying Traces

If you wish to print out the trace displayed on the screen, select **Print** from the **File** menu.
To copy an image of the trace to the Windows clipboard for pasting into Word or PowerPoint, select **Copy Image** from the **Edit** menu.

Saving/Loading experiments

You can save the recorded traces from an experiment to a file by selecting **Save Experiment** from the **File** menu. A saved file can be re-loaded by selecting **Load Experiment**.

Note 3. Calculating the Final Bath Concentration of a Drug

The response of a tissue to the application of a drug is proportional to the **concentration** (number of drug molecules per unit volume of solution, in units of Moles per Litre (M)) of the drug in the solution surrounding the tissue. In an organ bath experiment this is the concentration of drug **in the organ bath**.

In this experiment the drugs to be used (acetylcholine and hyoscine) are supplied in the form of stock solutions of fixed concentration – 10^{-4} M, 10^{-5}M. This allows us to add the drug to the organ bath by injecting a small volume of the stock solution.
However, when a volume of stock solution is added to the 10 ml organ bath it will be diluted. In order to obtain a specific concentration of drug in the organ bath we need to calculate the volume of stock solution to be added which will produce that concentration, after dilution. This is known as the **final bath concentration**.

This can be done using the dilution formula which relates the drug concentration (C_1) and volume (V_1) of a stock solution of a drug with the resulting concentration (C_2) after it is diluted by the additional of solution to make it up to the new volume V_2.

$$C_1V_1 = C_2V_2$$

When any 3 of the variables C_1, V_1, C_2, V_2 are known the 4th one can be calculated.

In the case of the organ bath experiment, the diluted volume, V_2, is the volume of the organ bath =10 ml. The stock solution concentration, C_1, is written on the stock solution bottle ($1x10^{-4}$M). If we want to determine the volume, V_1, to be added to achieve a final bath concentration of C_2=$1x10^{-6}$M

$$1 \times 10^{-4} \times V_1 = 1 \times 10^{-6} \times 10\ ml$$

$$V_1 = \frac{1 \times 10^{-6} \times 10}{1 \times 10^{-4}} = 0.1\ ml$$

So we need to add 0.1 ml of the $1x10^{-4}$M stock solution to the organ bath to achieve a $1x10^{-6}$M final bath concentration.