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**PRACTICAL MANUAL**

**PHARMACOLOGY OF GIT & HEPATOBILIARY SYSTEM**

**PHM 20202**

**Bachelor of Pharmacy with Honours [B.Pharm (Hons)]**

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| **Practical 2** | **Isolated organ “intestine” experiment -** **Drugs affecting the smooth muscle contraction and relaxation of guinea pig ileum** |

**INTRODUCTION**

In vitro, pharmacological experiments on isolated organs or tissues provide a means of discovering or quantifying the effects of drugs on specific tissues before their application in humans or in living animals. The properties of most drugs currently in use were elucidated using this method and, in vitro, experiments continue to be an essential stage in the drug discovery process.

***Tissues type***

Guinea pig ileum: The guinea pig ileum is a section cut from the ileum region of a guinea pig’s gastrointestinal tract. The smooth muscle within the ileum contracts in response to the application of a variety of agonists. It also has nerves which can be electrically stimulated to produce contraction.

***Drugs to be studied***

***Agonists***are drugs which, when applied to tissue, cause a response (in our case smooth muscles of the intestinal tissue studied in organ baths) by binding to specific receptors on the surface of the cells within the tissue.

***Antagonists*** are drugs which block the actions of agonists on tissue, reducing or preventing the tissue response. Application of an antagonist will thus have no apparent effect on the tissue in the organ bath unless an agonist is present (or the tissue is being stimulated).

**AIM(S)/OBJECTIVE(S)**

In this computerised simulation:

Aims: to apply the knowledge about drugs acting on GIT receptors that affect the contractility of the intestinal wall smooth muscles, and relating this activity to bowel symptoms such as diarrhoea and constipation.

Objectives: at the end of the teaching session the student should be able to:

1. describe the organ bath constituents and how it operates.

2. apply electrical stimulation to an isolated intestinal organ to get the baseline contraction.

3. analyse the graph after administering a receptor agonist and antagonists to the organ bath.

**HARD- AND/ SOFTWARE**

* A computer system/software

**PROCEDURE**

The tissue under study 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 that contains tap water maintained at a temperature close to the normal body temperature of the animal (37°C) by a heater and thermostat.

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

The tissue 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. This 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 recorded on the computer under the control of a digital chart-recording program. Figure1 showing the organ bath in for this simulation experiment.

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Figure 1: The organ bath setting for this simulation experiment.

**Steps involved**

**Adding agonists or antagonists to the organ bath:**

To add an agonist or an antagonist drug to the organ bath or Krebs’ solution reservoir:

1. **Click** either on “agonists” or “antagonists” title heading.
2. From the drop-down list **select the agonist or antagonist** to be applied.
3. Select the **concentration** of the drug solution to be applied from the Stock Soln. list.
4. Enter the **volume** (between 0 and 1 ml) of the stock solution to be applied into the Volume box.
5. Click the Add to button to inject the volume of the selected stock solution agonist into the organ bath or reservoir.



List of agonist and antagonist are:



**Measuring tissue responses**

To measure the peak amplitude of tissue contractions:

1. Click the Stop button to stop recording.
2. Using the **scroll bar (\*)** at the bottom of the chart display, select a section of the recording containing the tissue contraction to be measured. You can also adjust the size of the display window by entering a new duration into the Window Size box, expand or contract the window using the arrow buttons.
3. Drag the **measurement cursor (#)** on the chart display to the point on the recording trace to be measured (the peak of nerve stimulated responses, or the plateau of agonist responses). The contractile force at the cursor point (in units of grams.) is displayed below the cursor.

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**STUDENT’S TASK**

Your task is to:

1. Observe and record the effect of (muscarinic, histamine and mu opioid) receptors agonists on intestinal smooth muscle contraction and relaxation.
2. Comparing potencies of agonists affecting intestinal receptors including (muscarinic, histamine and mu opioid).
3. Observe and record the effect of (muscarinic, histamine and mu opioid) receptors antagonist on intestinal smooth muscle contractions.
4. Comparing potencies of the antagonists affecting intestinal receptors including (muscarinic, histamine and mu opioid).

**REPORT**

The report shall consist of:

1. **Introduction** (explain the scientific background and rationale for the experiment)

Write a paragraph stating about agonists and antagonists and as well as the significance of in-vitro experiments involving agonists and antagonists’ properties. What do you expect to learn from this experiment? Briefly describe the techniques you will use (principle of the simulation) to understand the concept of drugs affecting the smooth muscle contraction and relaxation of guinea pig ileum in this experiment.

1. **Material and methods** (give enough details)

Outline the working of the simulation apparatus and the materials and reagents. Procedure describing step by step of the work that was undertaken.

1. **Results and Discussion** (present in a clear or concise manner)

Complete the student’s task and include in the report appropriately. Interpret the findings of the experiment. Conclusion should be based on the results of the experiment.

1. **References**

Please use relevant references to support your interpretation of the results.