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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 1** | **The small intestinal smooth muscle contraction experiment -** **Pharmacodynamic Dose-Response Curve** |

**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).

**PRINCIPLE**

A segment of the intestinal smooth muscle is suspended in an organ bath containing ringer solution with **carbogen** added. The lower end of the intestinal segment is fixed to the bottom of organ bath, while the upper end is attached to a force transducer, which transmits the tension of the intestinal segment to be recorded on the displaying monitor in grams. By selecting a range of doses of the applied agonist to the organ bath, a corresponding range of small intestine “contraction” responses can be drawn to obtain a dose-response curve. The same steps of sequential dose application of the same agonist will be then repeated in the presence of the pre-added antagonist.

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

In this computerised simulation:

Aims: to demonstrate the effect of sequential increment in the concentration of a receptor agonist on the isolated smooth muscle with or without the presence of different types of antagonists.

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

1. To draw a dose-response curve representing histamine action alone on its receptors on the ileum smooth muscles.
2. To compare the above dose-response curve with the curve in the presence of a reversible histamine receptor antagonist.
3. To compare the above dose-response curve with the curve in the presence of an irreversible histamine receptor antagonist.
4. To be able to give other examples of agonists and antagonists that may show similar actions.

**HARD- AND/ SOFTWARE**

* A computer system/software

**PROCEDURE**

Isolated organ baths are used to study the effect of drug/chemical substances on isolated tissues in vitro. The isolated 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, as shown in Figure 1.



Figure 1 showing the organ bath in the middle, the dose/concentration setting option (left) and a digital recording screen for the response (contraction in grams).

**STUDENT’S TASK**

Your task is to plot **THREE (3)** dose-response curves (preferably) on a graph paper:

1. Agonist (A) alone without any pre-added antagonists.
2. Agonist “after” antagonist ((X)).
3. Agonist (A) “after” antagonist ((Y)).

**Measuring tissue responses and converting it into a dose-response curve:**

To plot **the first dose-response curves** of tissue contractions for **Agonist (A) alone** on a graph paper:

1. Choose the lowest concentration of A to be added to the isolated ileum in the organ bath then click on “add agonist”.
2. Wait until the recording screen records maximum force of contraction (in grams) in response to that concentration.
3. Plot the point of intersection of that dose (x-axis) with the resulting contraction force (y-axis).
4. Remove the agonist **completely** from the organ bath by clicking “remove agonist” (to clear the tissue receptors from any previous binding with the agonist) till current reading returns to near “zero” grams. The significance of this step is to avoid any misinterpretation of contraction force resulting from adding (cumulatively) the new concentration of the agonist with the incompletely removed previous concentration.
5. Repeat steps (a and b) this time using a higher concentration of A, and then plotting the new point of intersection between the dose (x-axis) and the contraction force (y-axis).
6. Connect between the two resulting points on the graph. Repeat step d.
7. Repeat the whole procedure till you reach the maximum level of contraction.

In order to plot **the second and third dose-response curves** of tissue contractions for **Agonist (A) in the presence of antagonist (Y) or (X), respectively** on a graph paper:

1. First you tick “add the antagonist (Y)” to the fresh organ bath and observe the reading of the smooth muscle contraction.
2. With the antagonist remaining in the organ bath, its effect blocking the receptors is considered constant as we add/ remove the agonist (A).
3. Repeat the steps (a-e) for adding agonist A with serial concentrations and plot the corresponding intersection points.
4. Remove the antagonist (Y) and tick on “add antagonist (X)”

Then you should interpret and compare the **THREE (3)** plotted curves in terms of:

1. Maximal efficacy.
2. Shifting to the side.
3. Type of drug-receptor bond.
4. Examples for each of the **THREE (3)** drugs used in the organ bath.

**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 small intestinal smooth muscle contraction 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.