## Practical schedule and peer assessment schedule

## Year 1 Pharmacology Students

### SCHEDULE 2 : AGONISTS AND ANTAGONISTS ON INTESTINAL MUSCLE.

**Objectives**.
1. To obtain dose-response data for agonists and calculate a potency ratio.
2. To observe the effects of an antagonist.

The pharmacological effect of a drug can often be readily studied by using an isolated tissue preparation. In this way a number of the problems which arise when examining the effect of the drug on a whole animal can be circumvented. For instance, when a drug is administered orally its absorption from the gut, and its distribution throughout the body may complicate the interpretation of the observations. These factors do not have to be considered when the response of a piece of isolated tissue is examined.

This simulation mimics a segment (about 2 cm long) of guinea-pig ileum set up in freshly prepared, oxygenated Tyrode's solution such that changes in the length of the piece of tissue (i.e. contractions or relaxations) may be recorded by means of a lever system attached to an isotonic transducer (i.e. an instrument which converts changes in length into electrical current) which can then be used to drive a pen recorder. This is the record you see on the computer screen. The temperature of the Tyrode's solution should be maintained at 32ºC by means of a thermostatted bath.

Compounds which produce a response when administered to a tissue are often termed AGONISTS. The size of response produced by an agonist acting on a given tissue will vary according to the dose administered. All drugs have a THRESHOLD DOSE below which no response can be detected. As the dose of drug administered is increased above the threshold the response evoked is also increased until it reaches a MAXIMUM VALUE. Regardless of how much the dose is then increased, the response will not increase. While the term DOSE can be use correctly to describe the amount of drug added to the tissue bath it is much better to use (and to think in terms of) **concentration**. This makes much easier comparisons between experiments where different sized organ baths have been used (therefore needing different doses to achieve the same concentration).

Each tissue from an animal may respond to a number of different drugs and each drug may evoke a response from a number of different tissues. Furthermore, the response evoked from a tissue (such as the guinea-pig ileum) by a fixed concentration of a given drug will not always be of exactly the same magnitude. It will be subject to BIOLOGICAL VARIATION.

Procedure.

1. Expose the tissue to increasing concentrations of carbachol starting with a concentration which produces no effect and ending when a 2-fold increase in the

concentration produces no further increase in the size of the response. Washout the tissue after each concentration is applied.

2. Repeat but use pilocarpine instead of carbachol.

3. Select a concentration of carbachol which produces about 80% of the maximal response and administer it 5 times, washing out between each administration. Add atropine ($5x10^{-8}$M) to the bath and, without washing out repeat the concentration of carbachol.

Treatment of data.

1. The size of the responses to carbachol in mm (y) should be plotted against the concentrations of carbachol (x) in the bath. Why is a maximal response reached? Would the maximal response be the same for all agonists?

2. Plot a second graph in which response in mm (y) is plotted against the logarithm (base 10) of the concentration of carbachol (x).

3. Plot the size of the responses to pilocarpine against the log concentration of pilocarpine on the same sheet of graph paper as used to plot the carbachol data. Calculate $EC_{50}$ and $pD_2$ values and a relative potency for the 2 drugs. Note the relative potency of two agonists CANNOT be estimated from the ratio of responses produced by identical concentrations of each drug. Rather, THE POTENCY RATIO is estimated from the dose or concentration of one drug required to produce the same response as a given dose or concentration of the other. The concentration chosen for comparison should be one which produces a response on the proportional region (straight line part) of the log concentration response curve. In order that the potency of two drugs may be compared in this way two criteria must be fulfilled:
-the drugs must produce the same type of effect on the biological system;
-they must produce parallel $\log_{10}$ concentration-response curves.

Questions

Would the relative potency of these drugs determined on one piece of isolated guinea pig ileum be expected to be significantly different from that found:

(a) on another piece of isolated guinea pig ileum 3 times as long?
(b) on a piece of elephant ileum?
(c) on a piece of isolated heart muscle?
(d) on a piece of skeletal muscle?

What change has the presence of atropine produced in the response to carbachol? Would atropine have the same effect on (a) pilocarpine and (b) histamine?

Have you observed any (simulated) "biological" variation?

Explain and comment on your answers.

**PEER ASSESSMENT SCHEDULE FOR SCHEDULE 2 : AGONISTS AND ANTAGONISTS ON INTESTINAL SMOOTH MUSCLE.**

Why are we doing this? You are expected to get several things out of this method of assessment which may be new to many of you: **1.** It is an open marking system; therefore you can see what was required and how to improve your work. **2.** You see mistakes others make and therefore can avoid them; you also see the standard achieved by others and can set your own work in the spectrum of marks. **3.** You get an explanation of the practical and how you should have processed the data and done the discussion. Therefore your information and understanding is improved. **4.** You get practise in assessing others and their work. You will need this skill quite early in a career and you will need to come to terms with the problem of bias; someone who is a good friend may have done poor work; it can be disturbing to have to give them a poor mark. **5.** In assessing others you should acquire the ability to stand back from your own work and assess that as well. This is an essential ability in a scientist; an unbiased and objective assessment of the standards you have achieved in your own work. Once you are away from the teacher/pupil relationship (i.e. leave University) you will be the person who decides if a piece of work is good enough to be considered as finished and passed on to your boss. **The method of marking adopted in this exercise is designed with the above factors in mind.**

NOTE: the numbers in parentheses are the marks available. You may award all or part of the available marks depending on how completely the point has been covered. For each point write the mark given and draw a circle round it on the write-up next to the item assessed. At the end add up the marks (don't forget presentation) and write the total mark and your name (legibly) in a circle at the top of the first page.
**Up to (20) marks are available for presentation; assess neatness, clarity, legibility etc as you go through.**

1. Is it dated. All work should be. (1)

2. Is it named. (1)

3. It should have a title (1) and a heading of introduction.(1)
Does the introduction state the objectives;
- to experience the use of an isolated tissue preparation
- to obtain dose-response data for an agonist and to calculate a potency ratio
- to observe the effects of an antagonist (5).

4. Is there a heading of method (1) and is the method and any change in the published method given - e.g. see schedule -- (3)

5. is there a heading of results (1); is the trace (or a copy) included? The trace should be attached and properly annotated - with the doses or concentrations of the drug which were used - always give the crude data (10)

6. Is there a graph of response/concentration of carbachol?
a) it should be titled (2) - Carbachol concentration / response relationship - or somesuch.
b) y axis should be labelled RESPONSE (2) with units (e.g. mm; cm; inches; anything will do [even squares] but the unit must be stated) (5)
c) x axis should be labelled CONC CARBACHOL (2) with units in moles/l (M). NOT DOSE. These units should be correctly abbreviated (m; m/l; little m and big L are NOT correct abbreviations). Units in moles/ml (never abbreviate moles to m) are just acceptable but you are strongly advised NOT to use this presentation as it causes confusion (8).
d) is the scale correct? This is an arithmetic scale i.e. 0, 0.005, 0.01, 0.015, 0.020, 0.025, 0.030 ... are all equally spaced. Note that this translates in exponential format into 0, $5x10^{-3}$, $1x10^{-2}$, **$1.5x10^{-2}$, $2x10^{-2}$, $2.5x10^{-2}$, $3.0x10^{-2}$**.. etc all equally spaced. IT IS WRONG to have 0, $1x10^{-4}$, $1x10^{-3}$, $1x10^{-2}$ all equally spaced - the latter is not an arithmetic scale (10 marks).
e) the curve should then be plotted, points visible, points joined by line (2)
f) A maximum is reached because either all receptors are occupied or the physical maximum shortening has been achieved. Would not be the same for all agonists as some may be partial agonists or may act on different receptors which are able to generate less pharmacological stimulus and therefore less contraction.(5)

7. Is there a second graph with a $LOG_{10}$ concentration axis? Is it titled - $\log_{10}$ concentration / response - or somesuch.(2); y axis labelled (2) with units (response mm) (2), x axis labelled [$\log_{10}$ concentration OR concentration ($\log_{10}$ scale).]. (8). Note the difference; the first is where the numbers on the axis are logs (e.g. -5.3), the second is where the numbers are arithmetic (e.g. 1 x $10^{-5}$). The units should be specified (M)(2). The axis should run the right way i.e.(left) -6 --> -5--> -4 --> -3 (right); NOT (left)-3--> -4--> -5--> -6 (right) (5 marks). Are the data for pilocarpine and carbachol presented (5).

8. $EC_{50}$ is the concentration giving 50% of the maximal response (units should be MOLAR) (5)

9. the $pD_2$ is the $-\log_{10}$ of the $EC_{50}$ providing the units of the $EC_{50}$ are M. (5)

10. Relative potency is the ratio of $EC_{50}$s (5).

11. Has the response to carbachol in the presence of atropine been noted? (5).

QUESTIONS.
12). would the potency be the same on: (5 for each part)
a) another piece of GPI; No Significant Difference (NSD); same receptors (muscarinic) in all bits of GPI; both drugs act on the same receptors therefore differences in receptor numbers between tissues affect both drugs equally.
b) rat gut; NSD; same muscarinic receptors as in GPI.
c). isolated heart; NSD; same muscarinic receptors as in gut.
d). skeletal muscle; not the same. Significant difference. The receptors in skeletal muscle are Nicotinic. The potency ratio you have measured represents the relative ability of the

two drugs to stimulate muscarinic receptors where ever they are. The relative ability of the drugs to stimulate nicotinic receptors would be different.
Generally, when two drugs which act on the same receptor have the same potency ratio in two tissues it means both tissues contain the same type of receptor. The converse is not always true. Envisage two tissues, both contain the same type of receptors. Two drugs; both act on the same receptors; you would expect the potency ratio to be the same in the two tissues. However, suppose ONE tissue has an enzyme which quickly destroys ONE of the drugs. This will mean you need much more of that drug and the potency ratios will not be the same even though the receptors are. This is why an anticholinesterase is used when acetylcholine (destroyed by cholinesterase which is present to a variable extent in different tissues) is used in attempts to characterise receptor types in different tissues.
13. Atropine should have reduced the response to carbachol because it is a competitive antagonist and competes for the same receptors, occupies them and reduces the proportion of receptors occupied by carbachol, therefore reducing the response.(5)
a). Since carbachol and pilocarpine both cause contraction by acting on the same muscarinic receptors atropine would have the same effect on pilocarpine or on any muscarinic agonist.(5)
b). histamine acts on different (histamine) receptors to cause a contraction and therefore would not be antagonised to the same extent by atropine. Atropine does act on both types of receptor but is about 10,000 time more effective on muscarinic receptors. Except at very high concentrations therefore atropine blocks muscarinic agonists but not those agonists acting on histamine receptors.(5)
NOTE. **Potency** is the quantity of drug (dose or concentration) which is needed to produce an effect. A small dose of a high potency drug produces an action. Potency is a comparative term. One drug is more or less potent than another. Even when we talk of "a potent drug" without a specific comparison there is an implicit comparison with other drugs generally i.e. the "potent drug" is effective in a smaller dose than that at which many other drugs produce an action.
**Specificity or selectivity** expresses how much of a drug is required to produce effect {A} RELATIVE to effect {B}. Drugs can have a high potency and a low selectivity or vice versa.

14. Repeating the concentration of carbachol 5 times will not have resulted in 5 identical responses. They will be different in both size and shape. These are both examples of biological variation. (5)
Total = 161