SimHeart

Experiments with an isolated heart in a virtual laboratory

– Protocol

Preparation steps

1. Creation of a dilution (see Experimental Protocol)

Calculate how to perform the dilutions in order to prepare the required solution concentrations, according to the values specified in the protocol. In the "Biochemistry Laboratory - Drugs" section, you can set up the solutions and check whether your calculations were correct. A filled test tube standing placed in test-tube rack will be proof to the supervisor that you have succeeded in preparing the solution.

For your calculations:

2. Experiments in the physiology laboratory

The calculations and reporting should be performed in parallel to the recordings, so that any corrections to the experiments can still be made!

In this protocol, placeholders are provided for your recordings. At the end of this protocol, there is a table for collecting the values so that dose-response curves can be generated.

2.1 Cardiac contractions in response to adrenaline and acetylcholine

Record the heart contractions starting from control baseline conditions and then after addition of adrenalin and acetylcholine (suggested concentrations for a clearly detectable effect are $5x10^{-7}$ M and $5x10^{-6}$ M, respectively).

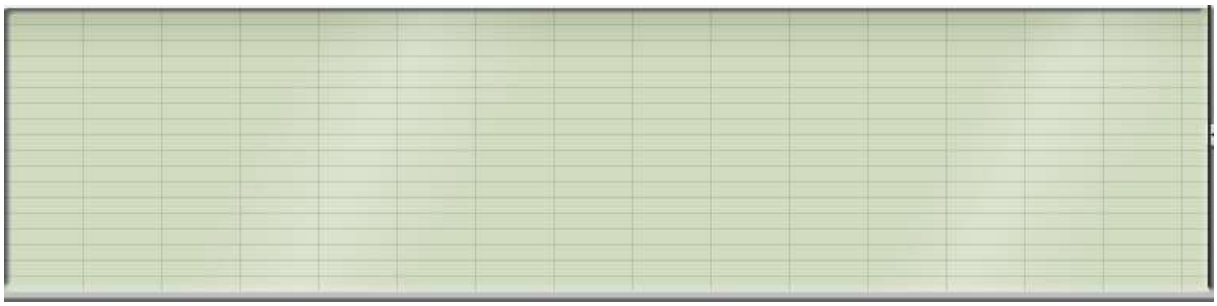
Set the recording speed so that not only the pressure changes but the changes in frequency are measurable.

Record the data in your protocol and determine the values for parameters (a) and (b) in the following:

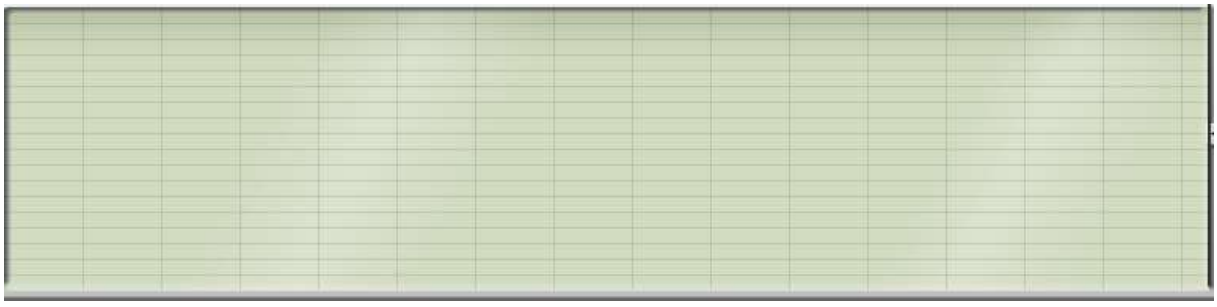
parameter		control	$5x10^{-7}$ M adrenalin	$5x10^{-6}$ M acetylcholine
(a)	Heart rate	_____	_____	_____
(b)	Pressure amplitude	_____	_____	_____

How can the different effects of adrenaline and acetylcholine be explained?

Effects of adrenaline



Effects of acetylcholine



2.2 The dose-response curve of adrenaline

With the heart muscle at baseline conditions, start applying adrenaline at increasing concentrations (doses), waiting for the maximal response to be reached between successive concentrations. The suggested starting concentrations is 10^{-9} M with increases of a single order of magnitude (x10) with successive test concentrations until no further increase in response is observed (expected to be at 10^{-5}M).

Measure the **absolute maximum pressures (P)** (relative to zero) and the **changes in the maximum pressure (ΔP)** (relative to the baseline value). You should notice that at high non-physiological doses, the heart was not completely relaxed, while the **pressure amplitude** (not the maximal pressure) even decreases again. Stop adding adrenalin, after these recordings, so that control conditions are re-established.

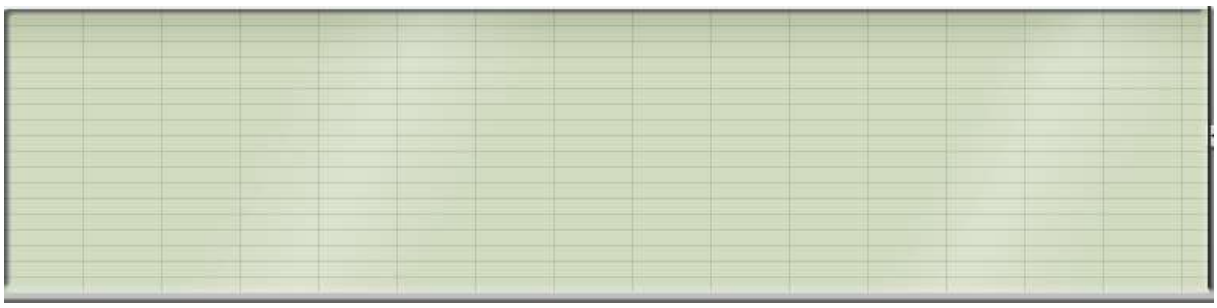
For these recordings, set tracer speed to the lowest setting. This should allow the tracing of the curve reaching close to the total width of the recorder display, before it is stored. However, with this setting, you can then no longer see the individual contractions.

You should make sure that the gain on the recorder is not too large, because considerable increases in pressure (up to 5-times the starting value) are expected in this experiment. So the setting should allow the initial response curve to reach only about one-fifth of the maximal span of the display; you probably need to switch to a resolution setting of 5 mV/division.

Determine from these recordings **P** and **ΔP** (relative to baseline) at each adrenaline concentration. Enter these values in the table, along with the corresponding adrenaline concentrations. A plot on a graph of **ΔP** against adrenaline concentration represents the **dose-response curve** of adrenaline.

If the dose-response curve cannot be drawn with enough certainty from the data points that you obtained, then make more recordings at concentrations of adrenaline that are between the test concentrations that were used.

Effect of adrenaline at increasing concentration



2.3. The effect of the β-blocker, propranolol (competitive inhibition)

2.3.1 When the heart preparation has returned to the baseline, control state, apply **propranolol** (at a proposed dose of 10^{-6}M).

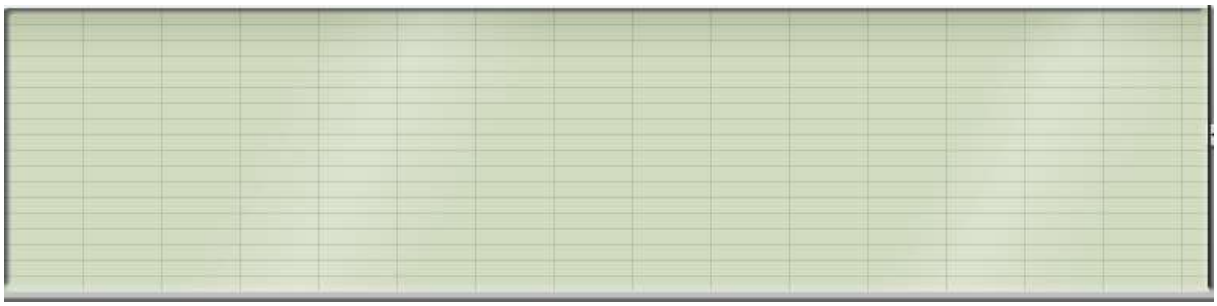
What effects do you observe?

Why would this propranolol effect be different under in vivo conditions?

2.3.2 Dose-response curve of adrenaline in presence of propranolol

After applying a single propranolol concentration, apply adrenaline at increasing concentrations, exactly as in the previous experiment.

Effect of increasing concentrations of adrenaline in the presence of propranolol



Again, measure, from these recordings, **P** and **ΔP** at each adrenaline concentration and enter into the table these values, along with the adrenaline concentration values.

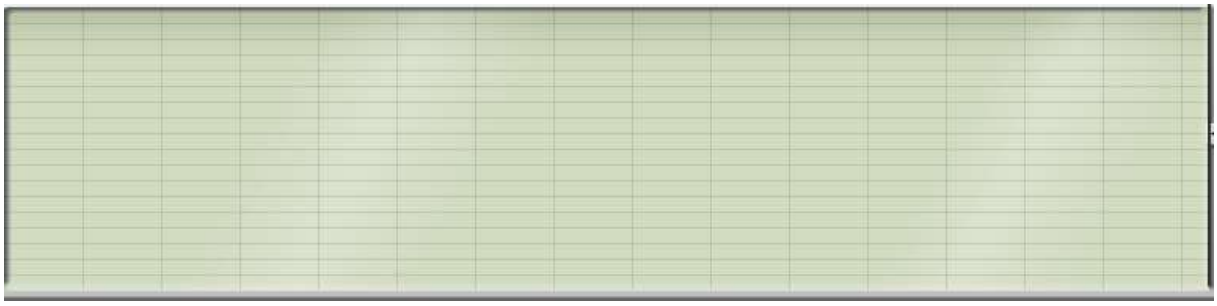
When you plot the **ΔP** values against adrenaline concentration, you should clearly see the change in the dose-response curve compared to the previous plot.

Can you identify some of the features that are typical of competitive inhibition?

2.4. Effect of the calcium channel blocker, verapamil (non-competitive inhibition)

2.4.1 Once your heart preparation is back to the baseline, control state, apply verapamil up to a concentration that evokes a marked weakening of cardiac activity.

Effect of verapamil



Can you explain why verapamil, in contrast to propranolol, decreases the strength of the heart even under baseline control conditions?

2.4.2 Now apply the higher concentration of adrenaline that was sufficient to overcome the effect of propranolol and produced a maximal pressure that was equal to that evoked under control conditions.

Why, despite the higher adrenaline concentration, is the maximal force not reached in the presence of verapamil?

2.4.3 Enter the values into the table from the measurements made in experiments 5.1. and 5.2 and plot them on the graph (note: **ΔP** may be negative here).

2.4.4 Can you imagine the shape of the curve representing non-competitive inhibition with verapamil?

Try to draw such a curve in the diagram and test its accuracy by making measurements at 2 or more additional concentrations.

Can you describe the typical characteristics of a non- competitive inhibition?

2.5 Effect of the cardiac glycoside, ouabain

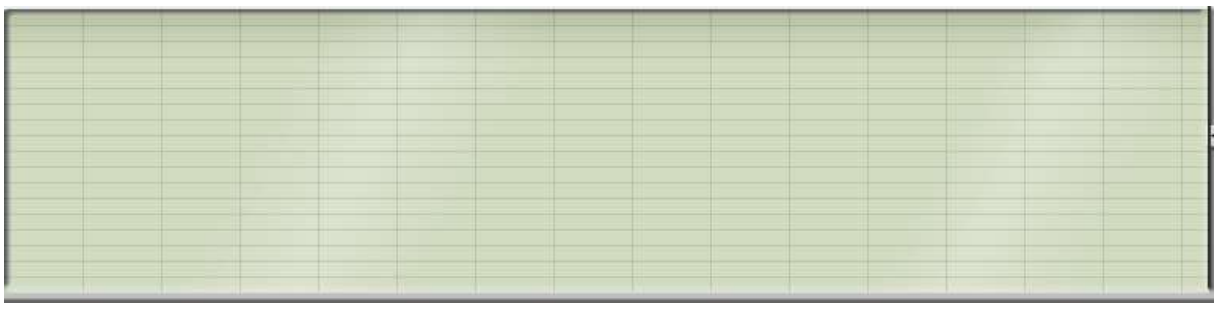
Save and draw some of the contraction series under the following experimental conditions: (i) control, (ii) with verapamil, (iii) with verapamil plus ouabain, (iv) only ouabain.

Please note: The effects of ouabain can differ greatly, depending on the initial basal state of the heart. Compare your records with those of your fellow students.

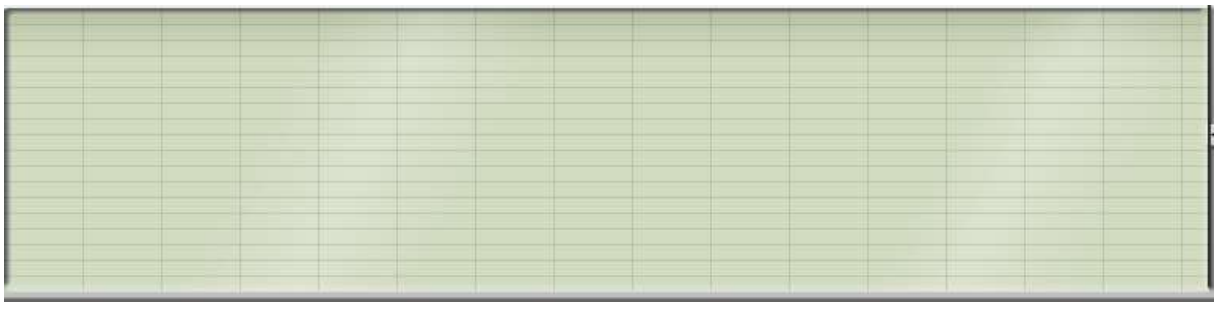
2.5.1 Apply a verapamil concentration that evokes a significant weakening of the cardiac activity and try to reverse this effect by the addition of ouabain and so revive the heart`s initial contractile strength.

2.5.2 Now stop the addition of verapamil and apply adrenalin at a concentration that increases the heart`s contractile strength. Then, re-apply ouabain at the concentration used in **2.5.1**.

The effect of ouabain on a verapamil-induced weakened heart



Effect of ouabain on an adrenaline-induced strengthened heart



Can you explain what is involved in the heart strengthening effect of ouabain?

By what is the risk of arrhythmia explained?

Dose-response curves

Data table:

Adrenalin concentration	Control		Propranolol _____M		Verapamil _____M	
	P	ΔP	P	ΔP	P	ΔP

Figure: dose response curves (do not forget to add the scales to the axes)

Can you explain the main differences between competitive and non-competitive inhibition using the curves generated in the presence of propanolol and in the presence of verapamil?