LABORATORIES/TUTORIALS

CAL LABORATORY

COMPUTER SIMULATION OF THE CARDIOVASCULAR SYSTEM OF AN ANAESTHETISED RAT

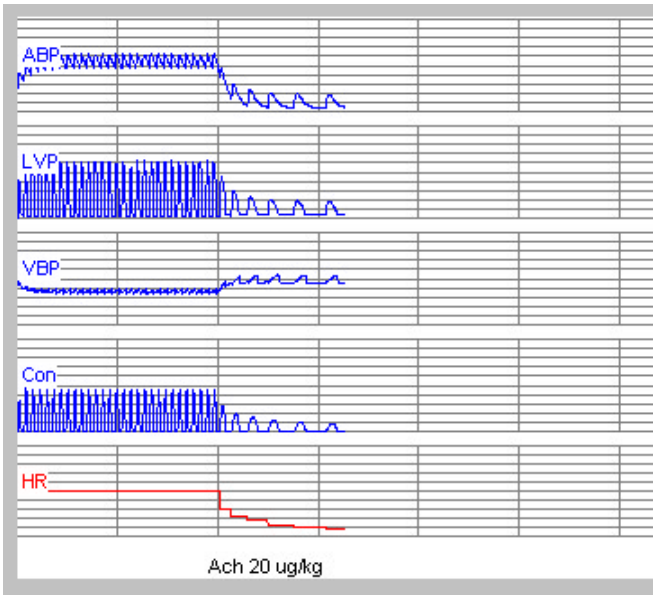
Objectives

- To reinforce the content of the lectures on the cardiovascular system.
- To demonstrate the effects of a range of drugs on different haemodynamic parameters in a pithed rat computer simulation.
- To allow students to elucidate the mechanism of action of drugs.
- To allow comparisons of drugs belonging to either the same or different pharmacological groups.

Introduction to the computer simulation of a pithed rat

The aim of these workshops is to provide an opportunity to observe the effects of a range of drugs on the cardiovascular system. The aneasthetised rat (or alternatively the pithed rat) is a standard preparation to determine the actions of drugs on the cardiovascular system in pharmacological research. By inserting a catheter into a main artery, heart rate and arterial blood pressure can be measured directly. A further catheter inserted into the lumen of the left ventricle allows indirect measurement of the force of contraction of the heart. Finally, a catheter in a vein allows both measurement of central venous pressure and administration of drugs.

In order to reduce to a minimum the number of animals used in teaching, a computer simulation of this experiment will be used. This programme can simulate the effects of drugs on arterial and central venous blood pressures, heart rate and force of contraction of the ventricles. Drugs, administered intravenously, can be studied over a wide concentration range. Measurements of systolic and diastolic arterial blood pressure (mmHg), heart force, central venous pressure (mmHg) and heart rate (beats min^{-1}), are shown. The trace below gives an example of what you will see on your computer screen.



ABP = Arterial blood pressure

LVP = Left ventricular pressure

VBP = Venous blood pressure

Con = Force of contraction of heart

HR = Heart rate

The pithed rat. This kind of experiment would be performed using a rat which has been anaesthetised prior to destruction of the brain and spinal cord (pithed). Despite the loss of the central

nervous system, by placing the animal on a respirator the heart will continue to beat. The lack of CNS means that any drug effects observed will not be complicated by baroreceptor reflex-induced changes. In a pithed rat, the blood pressure is highly dependent upon the amount of the vasoconstrictor angiotensin II (AII) circulating in the plasma. AII is converted from its inactive pre-cursor, AI, by angiotensin converting enzyme (ACE). AI is in turn, generated from angiotensininogen by the action of renin, which is released from the kidney.

Cardiovascular problems

During the CAL session you will be required to work your way through the following problems. You should measure the appropriate haemodynamic variables (using the measurement cursor) and enter the results in the table to allow you to reach a final conclusion. At the end of the session the tutor will go over the main points with the class.

First cal lab – complete problem 1 and start problem 2
Complete problem 2 in your own time
Second cal lab - problems 3 and 4

PROBLEM 1: a and b-adrenoceptors

Select "Pithed". Record the effect of the following on arterial blood pressure and heart rate:

(i) Intravenous injections (select a mid-range concentration from the menu) of

- noradrenaline (NA) and
- isoprenaline (ISOP)

(ii) Stimulation of the sympathetic nerves to the heart (select from nerve stim pull-down menu)

(iii) Inject 0.5 mg kg^{-1} prazosin.

(iv) Repeat the intravenous injections of noradrenaline and isoprenaline. Repeat the nerve stimulation. How have these been affected by prazosin ?

Answer:

(v) Inject 5 mg kg^{-1} propranolol.

(vi) Repeat the intavenous injections of noradrenaline and isoprenaline. Repeat the nerve stimulation. How have these been affected by propranolol ?

Answer:

(vii) Select a new rat and obtain control responses to sympathetic nerve (heart only) stimulation.

(viii) Administer 1 mg kg^{-1} phentolamine

(ix) Repeat the nerve stimulation. How has this been affected by phentolamine ?

Answer:

(x) Inject 10 mg kg^{-1} atenolol.

(xi) Repeat the nerve stimulation. How has this been affected by atenolol ?

Answer:

Conclusion: What can you conclude about the location of α and β-adrenoceptors in blood vessels, the heart and the sympathetic nerves ?

RESULTS TABLE - pithed rat simulation

Intervention	Systolic ABP mmHg	Diastolic ABP mmHg	Heart rate Beats/min
Control – before drug			
After noradrenaline			
Change due to NA			
Control – before drug			
After isoprenaline			
Change due to ISOP			
Control- before nerve st			
After nerve stimulation			
Change due to nerve st			
Control			
After NA + prazosin			
Change due to NA + Pra			
Control			
After ISOP + prazosin			
Change due to ISOP + Pra			
Control			
Nerve stim + prazosin			
Change due to NS + Pra			
Control			
After NA + propranolol			
Change due to NA + Pro			
Control			
After ISOP + propranolol			
Change due to ISOP + Pro			
Control			
Nerve stim + propranolol			
Change due to NS + Pro			
Control- before nerve st			
After nerve stimulation			
Change due to nerve st			
Control			
Nerve stim + phentolam			
Change due to NS + Phe			
Control			
Nerve stim + atenolol			
Change due to NS + Aten			

PROBLEM 2: organic nitrates and endothelium

Select “Pithed”. Record the effect of the following on arterial blood pressure and heart rate:

(i) Intravenous injection (select mid-range concentrations from the menu) of:

- acetylcholine (Ach)
- glyceryl trinitrate (GTN)

(ii) Inject 50 mg kg^{-1} of the NO synthase inhibitor L-NOArg.

(iii) Repeat the injections of ACh and GTN. How have their responses been changed by L-NOArg ?

Answer: Ach

GTN

Select “new rat”.

(iv) Repeat control responses to acetylcholine and GTN.

(v) Inject 5 mg kg^{-1} atropine. Does this have any effect on heart rate and blood pressure?

(vi) Repeat the injections of ACh and GTN. What are their effects now?

Answer:

Conclusion: What can you conclude about the mechanisms by which ACh and GTN produce their haemodynamic effects?

RESULTS TABLE - pithed rat simulation

Intervention	Systolic ABP mmHg	Diastolic ABP mmHg	Heart rate Beats/min
Control – before drug			
After acetylcholine			
Change due to ACh			
Control – before drug			
After glyceryl trinitrate			
Change due to GTN			
Control- before L-NOArg			
After L-NOArg			
Change due to L-NOArg			
Control			
After Ach + L-NOArg			
Change due to Ach + L-NOArg			
Control			
After GTN + L-NOArg			
Change due to GTN+L-NOArg			
Control			
After ACh			
Change due to ACh			
Control			
After GTN			
Change due to GTN			
Control			
After atropine			
Change due to atropine			
Control			
After Ach + atropine			
Change due to Ach + atropine			
Control			
After GTN + atropine			
Change due to GTN + atropine			

PROBLEM 3: isoprenaline, digoxin and milrinone

Select “Pithed”. Record the effect of the following on left ventricular pressure, venous blood pressure, force of contraction of the heart and heart rate:

(i) Intravenous injection (select mid-range concentrations from the menu) of:

- isoprenaline
- digoxin
- milrinone (You may need to select a new rat **before** administration of this drug. Why ?)

ii) Select “new rat”. Inject propranolol (5 mg/kg).

iii) Repeat the intravenous injections of isoprenaline, digoxin and milrinone. How have these been affected by propranolol ?

Answer:

iii) Deduce which of the drugs (isoprenaline, digoxin or milrinone) would be of use in the treatment of heart failure.

Answer:

PROBLEM 4: Renin-angiotensin system

Select “Pithed”. Record the effect of the following on arterial blood pressure and heart rate:

(i) Intravenous injection of:

- losartan 10 mg/kg
- captopril 10 mg/kg

(ii) Select “new rat”. Record the effect of the following on arterial blood pressure and heart rate (for angiotensin II and phenylephrine choose a mid-range concentration from the menu):

- renal nerve stimulation
- angiotensin II
- phenylephrine (PE)

(ii) Inject captopril 5 mg/kg. Repeat the injections of angiotensin II and phenylephrine. Repeat renal nerve stimulation. How are the effects of angiotensin, phenylephrine and renal nerve stimulation modified by captopril ?

Answer:

(iv) Inject losartan 10 mg/kg. Repeat the injections of angiotensin II and phenylephrine. Repeat renal nerve stimulation. How are the effects of angiotensin, phenylephrine and renal nerve stimulation modified by losartan ?

Answer:

(v) Inject phentolamine 1 mg/kg. Repeat the injections of angiotensin II and phenylephrine. Repeat renal nerve stimulation. How are the effects of angiotensin, phenylephrine and renal nerve stimulation modified by phentolamine ?

Answer:

RESULTS TABLE - pithed rat simulation

Intervention	Left Ventricular pressure mmHg	Force of contraction of the heart	Heart rate Beats/min
Control – before drug			
After isoprenaline			
Change due to isoprenal			
Control – before drug			
After digoxin			
Change due to digoxin			
Control- before drug			
After milrinone			
Change due to milrinone			
Control			
After ISOP + propranolol			
Change due to ISOP + propranolol			
Control			
After digoxin + propranolol			
Change due to digoxin + propranolol			
Control			
After milrinone + propranolol			
Change due to milrinone + propranolol			

RESULTS TABLE - pithed rat simulation

Intervention	Systolic ABP mmHg	Diastolic ABP mmHg	Heart rate Beats/min
Control – before drug			
After captopril			
Change due to captopril			
Control – before drug			
After losartan			
Change due to losartan			
Control- before nerve st			
After renal nrve stim			
Change due to renal n s			
Control			
After angiotensin II			
Change due to ang II			
Control			
After phenylephrine			
Change due to phenylep			
Control			
After angioten + captopr			
Change due to AII + cap			
Control			
After PE + cap			
Change due to PE + cap			
Control			
After nerve st + captopr			
Change due to ns + cap			
Control			
After AII + losartan			
Change due to AII + los			
Control			
After PE + losartan			
Change due to PE + los			
control			
After nerve st + Losartan			
Change due to ns + los			
control			
After AII + phentolamine			
Change due to AII + Phent			

control			
After PE + phentolamine			
Change due to PE + phent			
control			
After renal nerve stim + phent			
Change due to renal n s + phe			

Cardiovascular workshop

The aim of this workshop is to help students to develop their problem-solving and interpretation skills. Students must prepare thoroughly for the workshop by:

- reading the attached problem-solving exercise
- revising the actions of the drugs mentioned and the relevant cardiovascular physiology
- preparing an answer to the problem solving exercise using the attached guidelines on problem solving exercises and essay writing. This must be completed before the workshop.

To answer the question set students should:

- read through the question (i.e. paragraph 1) and make sure you understand what you are being asked to do
- read through the experimental data section from start to finish
- read through experimental data section a second time, making notes of how each point is relevant to answering the questions
- your written answer should address each point of data and explain how the evidence shows (a) the mechanism underlying each action and (b) which mechanism(s) can be excluded
- your written answer should also include a conclusion that brings this evidence together. There may be different mechanisms in different vascular beds

During the workshop there will be an opportunity to discuss your answer with other students and to seek advice from a member of staff about any aspect that you do not understand. The member of staff will also provide verbal feedback on the written answers.

CARDIOVASCULAR WORKSHOP

PROBLEM SOLVING EXERCISE

Read the information that describes the cardiovascular effects of CVI99. Use all the evidence presented to suggest the mechanisms that may mediate the cardiovascular effects of CVI99 and those that are unlikely to contribute to the actions of this agent. Your answer should attempt to explain both possible vasoconstrictor and vasodilator actions of CVI99 which may be different in various regions of the vasculature.

CVI99 is a small, synthetic peptide. The cardiovascular actions of this peptide have been investigated in several experimental models and a summary of the results is presented below.

In pithed rats, intravenous injections of CVI99 induced dose-related statistically significant increases in arterial blood pressure, which attained a maximum after 2 min and returned to normal levels after approximately 30 min. Responses to noradrenaline reached a maximum within 20 sec and returned to normal levels within 5 minutes. In all cases the increase in arterial blood pressure produced by CVI99 was preceded by a transient and dose-dependent decrease. Examination of blood flow to different regions showed that CVI99 decreased blood flow in the skin, spleen, small and large intestines and skeletal muscle but increased blood flow in the liver, kidney and lung. CVI99 had no significant effects on heart rate or stroke volume.

The increases in arterial blood pressure were not modified by a) prazosin, b) yohimbine, c) phentolamine, d) captopril, e) the vasopressin antagonist SK&F 100273, f) indomethacin or g) guanethidine. However, the increases were reduced, by about 50%, by calcium channel blocking drugs verapamil, diltiazem or nifedipine. Increasing the doses of these calcium channel blockers did not produce any further antagonism of the CVI99 induced increases in arterial blood pressure. The decreases in arterial blood pressure were not modified by a) propranolol, b) indomethacin, or c) a combination of mepyramine and cimetidine.

The effects of CV199 on tension developed in isolated blood vessels was also examined. CVI99 produced concentration-dependent contractions of rat isolated mesenteric arteries (ie those supplying the gastrointestinal tract). When the endothelium was removed the EC_{50} of CVI99 decreased from 4.00 ± 0.6 nM to 0.9 ± 0.2 nM ($P < 0.05$). The contractions were reduced by 84% by the complete removal of extracellular Ca^{2+}. The contractions were reduced, but not abolished, by diltiazem, verapamil or nifedipine.

In rat isolated pulmonary arteries, (ie those that supply the lungs) which were pre-contracted with potassium chloride, CV199 produced concentration dependent relaxations. These relaxations were not modified by the nitric oxide synthase inhibitior, LNO-Arg, although LNO-Arg did prevent acetylcholine-induced relaxation in this tissue. Likewise, indomethacin did not prevent the CV199 induced relaxation. The CV199 induced relaxations in pulmonary arteries were abolished by removal of the endothelium.

Drugs and Disease I

Problem Solving Exercise

This will be based upon an exercise similar to the one that was done in the cardiovascular workshop and will be carried out under exam conditions. Notes on how to prepare a written answer of this type and the marking guide that is used are attached.

In the majority of interpretation exercises the objective will be to determine the site and/or mechanism of action of a drug. In order to do this, you will need to use both your knowledge of physiology and pharmacology and the data presented to draw logical conclusions about how the drug is acting. Before attempting to answer the questions you should:-

1. Read the exercise thoroughly at least twice

2. Go through the exercise sentence by sentence extracting all the information given.

3. Use the information to construct a diagram of the likely site and/or mechanism of action of the drug.

IMPORTANT POINTS TO BEAR IN MIND

In writing your answer to the question(s) you should bear in mind that:-

1. Often there is no single correct answer

2. Use must be made of all the evidence provided

3. Conclusions must be drawn that can be substantiated by the data given

4. No assumptions should be made. However, at times it may be necessary to use your knowledge of pharmacology to lead you to likely conclusions. E.G. if it is stated that the effects of the unknown drug are blocked by atropine, then since atropine is known to block muscarinic cholinoceptors it would be reasonable to conclude that the unknown was acting as a muscarinic agonist. However, if there is evidence in the exercise which contradicts the “assumed” action of commonly used drugs then this must not be ignored.

5. The answers should be concise. Do not rewrite all of the results presented but use them to support the conclusions drawn.

ASSESSMENT

Your answers will be assessed on the basis of:-

1. The conclusions drawn with supporting experimental data

2. A logical approach

3. Clarity and conciseness

Marking guide for interpretation question

In general a written answer to an interpretation question will be expected to contain all, or at least some of the following qualities:

(a)	Shows the ability to collate information from various parts of the exercise.
(b)	Can come to an overall conclusion, which is justified on the basis of the evidence presented.
(c)	Shows logic in explaining the rationale underlying the choice of drugs to test a particular hypothesis.
(d)	Can demonstrate the use of evidence to support or reject a hypothesis.
(e)	Shows awareness that data can be interpreted in different ways.
(f)	Shows originality in approach and/or interpretation.
(g)	Is presented in a local order and ideas are expressed clearly and concisely.

Based on the above criteria, marks will be awarded to the answer according to the following scheme:

1st	**Outstanding answer which**		
	(1)	Includes a, b, c, d, e, f, g	90 - 100
	(2)	Includes a, c, d and g	76 - 89
	(3)	Includes a, c (or d), g	70 - 75
2(i)	**Very good answer which**		
	(1)	Includes c and **one** from b, d and e	66 - 69
	(2)	Includes c and **one** from b, d and e above but with a small number of mistakes	60 - 65
2(ii)	**Incomplete answer:**		
	(1)	As for 2(i) but has missed a few important interpretations	56 - 59
	(2)	As for 2(i) but has missed some interpretations and made some errors	50 - 55
3rd	**Rather weak answer**		
	(1)	Has serious omissions but some sound interpretations	46 - 49
	(2)	Has several serious omissions, poor presentation, in part illogical but evidence of some ability to interpret pharmacological data	40 - 45
Fail	**Very weak answer**		
	(1)	Has made some attempt at interpretation	35 - 39
	(2)	Is a clear failure with serious errors	25 - 34
	(3)	Is a clear failure with some hint of understanding but errors serious and daft	10 - 24
	(4)	Either failure with no hint of understanding or knowledge but containing one or two attempts to interpret the data	0 - 9

Department of Physiology & Pharmacology

GUIDELINES FOR ESSAY WRITING

There are common guidelines to which you should adhere to whether you are writing an essay under exam conditions or for an assignment.

1. Read the question carefully

If you are in an exam, jot down the main points that you think are important to cover. If you are preparing an essay for an assignment, you should devote time to collecting and organising the information before you sit down to plan the essay. It is firstly important to be aware of the Stirling marking guide in order to know how much information/detail is required for the respective classes (1, 2:1, 2:2, 3) of mark. It is perhaps important to note that information detailed in the handout is sufficient for a borderline 2:1/1st class degree mark. To obtain a good 1st class mark evidence of reading outside lecture material should be included in the essay.

2. Planning the Essay

Once you have noted/collected the information that is pertinent to the essay title, start planning the essay.
Group together similar ideas and arrange the ideas in logical succession.
Decide the importance of each idea and how many paragraphs you might devote to it.
Think about the link phrases for sequencing your ideas. You may wish to introduce headings dividing up the essay appropriately.

3. Introduction

This is important as the examiner will know from the Introduction whether the writer has grasped the gist of the question implicit in the title of the essay, or whether the essay will be pages of waffle.
In a few lines tell the readers what you are going to explain and discuss in the essay to prepare their minds for the way you will approach the subject matter.

4. The main part of the essay

Here you should present in detail the discussion and analysis needed to answer the question in the title. In this part of the essay you should use examples to support your claims and (if appropriate) offer arguments and criticism. Each paragraph should describe and discuss a particular theme or specific point relating to a theme. Diagrams can be used, if appropriate, but make sure that they are referred to and described in the text, properly headed and fully labelled. Avoid the following pitfalls: Lack of attention to planning. Repetition of information. Ambiguity - failure to make clear your views. Lack of logical ordering. Lack of relevance to the question. Contradictory statements. Lack of attention to logic, e.g. arguing in circles. Technical errors, e.g. use of undefined abbreviations.

5. Conclusion

Remind the readers of the main points you have told them. Reinforce their memory with the principal points of your arguments and how these have lead to your conclusion. Do not introduce new ideas - be concise.

6. Allocation of Time

Under exam conditions you should spend about 5-10 min **PLANNING** an essay for which 40 min is allocated.