SimHeart

Physiological and pharmacological experiments on Isolated Heart Muscle in a virtual laboratory

Table of Contents

1. The Virtual Laboratory 2
1.1. Entry Screen Display 2
1.2 Preparation of the active substances ("Drugs") 3
1.3 Experiments on Langendorff heart preparation ("Experiment") 5
1.4 The Data Analysis ("Analysis") 6

2. Laboratory experiments 9
2.1 Preparation of the diluted drug solutions ("**Drugs**") 9
2.2 Recordings of cardiac contractions ("**Experiments**") 11
2.2.1 Cardiac contractions in response to adrenaline and acetylcholine 11
2.2.2 The dose (concentration)-response curve of epinephrine 11
2.2.3 Effect of the β-adrenergic receptor blocker, propanolol, on the dose-response curve of adrenaline (competitive inhibition) 11
2.2.4 Effect of the Ca^{++} blocker, verapamil, on the dose-response curve of adrenaline (non-competitive inhibition) 11
2.2.5 The effect of the cardiac glycoside, g-strophantin (ouabain) 11

3. Physiological and pharmacological basis 12
3.1 Automaticity of the heart 13
3.2 Influencing cardiac activity 13
3.3 Role of the autonomic nervous system; functional antagonism 13
3.4 Receptor agonists and antagonists: competitive inhibition 14
3.5 Calcium channel-blockers: non-competitive inhibition 15
3.6 Cardiac glycosides 16

1. The Virtual Laboratory

SimHeart is a close-to-reality simulation program for the study of the activity of the heart under the influence of physiological neurotransmitters and specific cardioactive drugs. The program offers students of medicine, biology, zoology or other related fields, the possibility, without having to use an animal preparation (such as, the usually rat or guinea pig model), to perform classical experiments on the isolated perfused heart. This virtual preparation simulates the classic, so-called Langendorff model and allows experiments to be performed that aim to deepen theoretical knowledge of this very important medical field.

In the main part of the program, you can apply, by addition to the perfusion solution, various cardioactive substances and make recordings of the changes in cardiac contractions. During the experiments, you can switch to the "Analysis" and select recordings from specific sections, save them as jpg files onto your hard disk, before transferring them directly into your experimental protocol.

In this version of the program, the test substances are provided at appropriate dilution for performing the experiments. In addition, there is a chemistry lab ("Drugs"), which should be made use of in order to prepare the required dilutions from virtual drug ampoules that resemble the commercially available drug products.

1.1 Entry Screen Display

When you start the program, you come to the display shown in Figure 1. There, you can choose whether to go first to the chemistry lab (“Drugs”) in order to prepare the drug dilutions or to go the experimental animal laboratory in order to start experimenting on the isolated heart (“Experiment”).

Please note that, in the current version of the program, these two sections are not yet linked. Before your switch from one section to another, you will need to store your data, which will otherwise be lost. You will see a warning message.

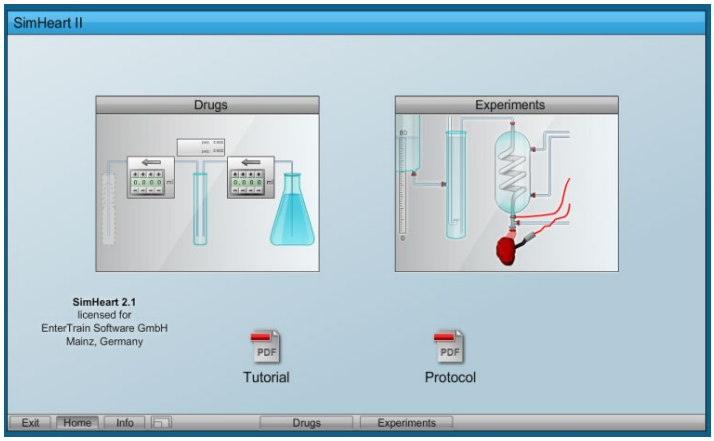

Fig 1: Entry Screen, from where you can go to the “Drug” or “Experiments” Laboratory or first open and check the tutorial and/or protocol form

Here, you can also open PDF files (or DOC versions) from the experimental tutorial guide (“**Tutorials**”) or from the protocol specifications (“**Protocol**”); these files are on the hard drive in the sub-folder "Files" of the "SimHeart" folder. In the "Videos" folder you will find a video of the preparation with a German and English commentary.

Bottom left of the displayed page, there is a superimposed text showing the program **license**. Here, it will indicate that the program is either a non-licensed "demo" version or is registered by the named license-holding Institute. In the example presented here, it is the name of the production company. This license text will appear again in every section of the program, at differing positions on the display.

At the very bottom of the display, there is a bar of **control switches**, which is present in all sections of the program - in "**Experiment**" there is an additional switch to "**Analysis**". The control bar contains switches to the program sections, "**Drugs**" and "**Experiment**".

At the left of the bar, the switch "**Exit**" allows you to close the "SimHeart" program. Next to this switch is the switch "**Home**" that allows you to return to this page of the program. A click on the "**Info**" switch will display some additional background information on the program and its developers.

The program opens in full-screen display mode but with a resolution that has been optimized for a flicker-free display of the figures presented in "Experiment". This means that, depending on screen resolution settings, the SimHeart window may open without filling the screen and there would, then, be a blue background. The double rectangles to the right in control bar are a switch to the **screen settings**, which can be adjusted. A click on the **screen settings** switch allows selection of the full-screen setting and the SimHeart window will display as full-screen. Here, it is possible also return to the initial display setting. Here, as is usual, the **Esc** key, also allows you to leave the full screen mode and switch to a classic window display.

1.2 Preparation of the active substances ("Drugs")

One of the most important steps in preparing for the experiment is the preparation of the required diluted solutions of test drug.

In the program section (or Laboratory) "Drugs", you can try to prepare your own test drug solutions. There, you will find test tube racks containing test-tubes that are already labelled, each with the desired drug concentration (from 10^{-2} to 10^{-5} mol/mL) for each test tube (see figure 2). The number of diluted solutions required for each drug varies and, in some cases, is a single dilution.

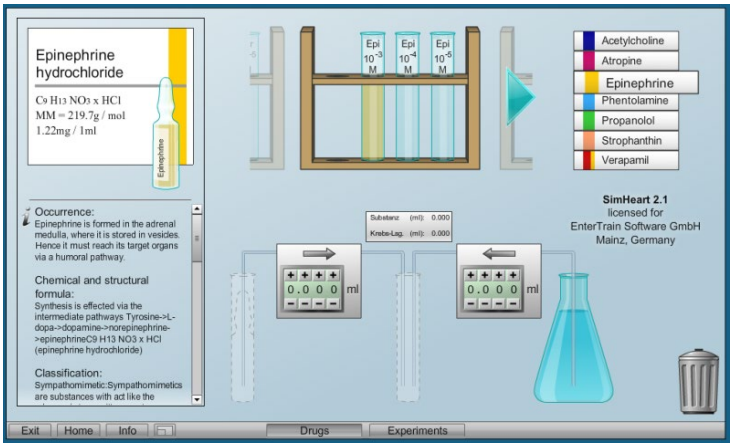

Fig 2: Preparation of the diluted drug solutions in the "Drug" laboratory.

The test drug can be selected (by mouse-click) from the list to the right of the test-tube rack (indicated in figure 2 is epinephrine which is referred to elsewhere as adrenaline). Alternatively, you can scroll

through the test tube racks with mouse click on the left or right (arrow). Once a certain substance is selected, it also will appear in form of a commercially available vial and package on the left of the screen. The package will be labelled with the substance name, its molecular weight and the concentration of the solution. This is the information you need for starting the dilution and preparing the drug as a series of dilutions. Additional information about the drug can be found in the text below.

To dilute the "stock" solution in the package ampoule to the required concentration, Krebs solution (physiological saline) is used. For diluting the solution, you click on and drag the correspondingly labelled test-tube down from the test-tube rack to the marked position between the two selection panels that are to be used to select the amount of ampoule solution (left) and Krebs solution (right) to be added to the test tube If you click on the ampoule of drug "stock" solution, you can drag it to the marked position to the left of the selection panels. To the right of these panels, you will see the flask containing a store of Krebs solution.

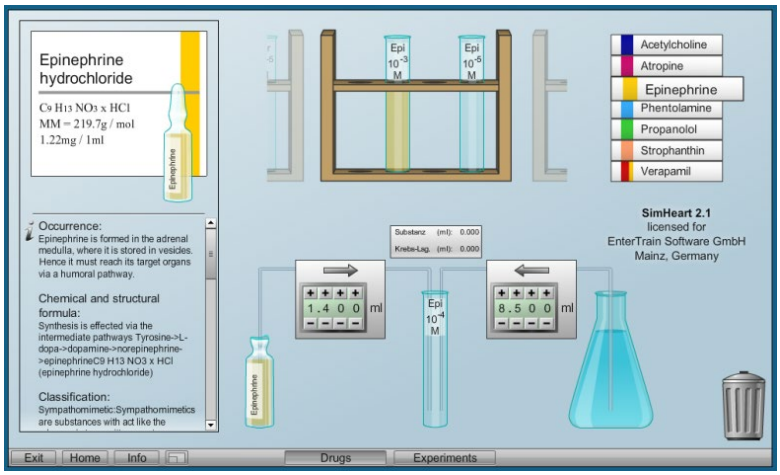

Fig 3: Dilution of the ampoule solution with Krebs ringer solution in the "Drugs" laboratory.

Using the pre-selection buttons in the panel, you can set the volumes (mL) of the contents of the ampoule and of the Krebs solution that are to be transferred to the test tube in order to obtain the required concentration, as indicated on the test-tube label. The transfer of the selected solution volumes to the test-tube is achieved by clicking on the respective arrows above the selection panels. As the test-tube fills, the volumes of the drug and Krebs solution that are now present in the test-tube are displayed. When the transfers of the solutions are complete, click and drag the test-tube back to the rack. If the solution is not correct you will receive a fail signal. Then, place the test-tube in the waste bin (below right). You get another chance as a new test-tube appears to replace the one that you had to dispose of.

It is recommended to start with the test-tube that is to take the highest concentration of drug. This test-tube with its diluted contents can then be used to start a series of step-wise dilutions, whereby this tube replaces the ampoule of "stock" solution and is itself used to provide a selected volume for dilution with Krebs solution and so generate the next in the series of dilutions.

Additional notes how the correct dilution can be obtained, i.e. how to calculate in which relation the stock solution and Krebs solution need to be transferred to the test tube, can be found in part 2 ("Laboratory Experiments") of this tutorial in chapter 2.1 Preparation of the diluted drug solutions ("Drugs").

1.3 Experiments on Langendorff heart preparation ("Experiments")

In the so-called Langendorff set-up (Fig. 4), the isolated heart with the aorta is perfused, whereby the coronary vessels are continuously flushed in a retrograde manner (thus, with closed aortic valves) with oxygenated and temperature-controlled Krebs solution that washes out from the severed vessels of the venous system.

Through a heat exchanger that is controlled by a thermostat (the device on the left of the shelf), the temperature of the perfusion solution is maintained at 37°C. With an average flow of **10 mL/min**, which is monitored by a flow meter, the hydrostatic pressure of the reservoir (left of the screen display) that supplies the perfusion system is set. The isovolumetric pressure changes in the left ventricle are measured using a pulmonary artery balloon catheter (introduced via the venus pulmonalis), a mechano-electric transducer and Statham amplifier (with a fixed setting of **1 mV/2 mmHg)**, and they are recorded on the chart recorder (on the bottom).

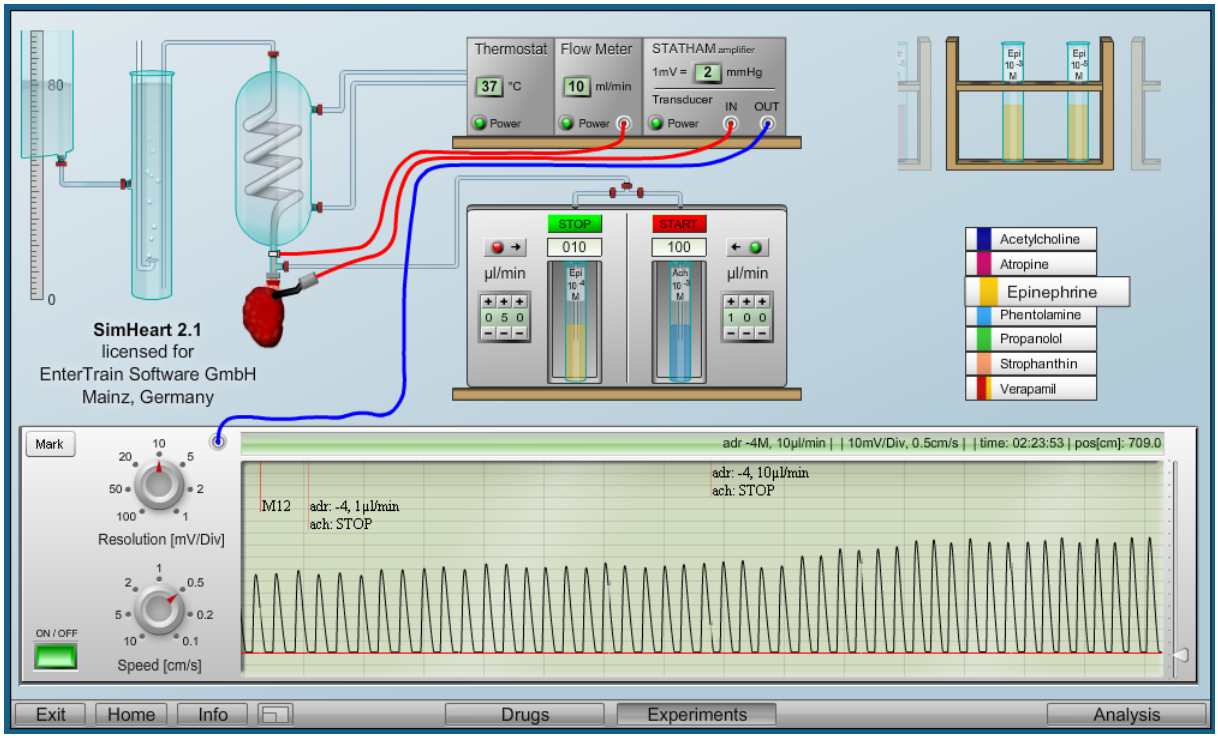

Fig 4: The experimental Langendorff heart preparation in laboratory, "Experimentation".

For **<u>adding the test drug to the perfusion solution</u>**, you click and drag the test tubes in the test-tube holder to one of the two **injection pumps** that are positioned above the chart recorder. Selecting and switching between the different test-tube holders is done in the same way as in the "Drug" Lab when preparing the dilutions; you click on the substance list or scroll along the test-tube holder. Two injection pumps are available for simultaneously application of two different substances. For changing the applied drug solution, first click and drag away the test-tube that is attached to the perfusion system and replace it with next test-tube of solution to be applied.

The selection switch is used to set flow rate for delivery of **the desired amount of drug solution**. The value is accepted only by pressing the button with the arrow. This will prevent any change in the selection switch being effective immediately. It allows to set the values during an ongoing experiment and then activate the arrow button .

The drug solution can only be supplied to the perfusion solution by pressing the red "START" button, which then becomes green and shows "STOP". To stop the supply, press this green button.

Please note that the rate of supply of drug solution is set in µL/min while the flow of perfusion solution is 10 mL/min.

On **the recorder**, the knob switches, "**Resolution**" and "**Speed**", can be used to set the resolution of the tracing and the chart speed, respectively, with the units indicating the subdivisions (Div) on the chart paper in the vertical direction (as seen on the screen display) (the resolution) and the cm intervals marked on chart paper in the horizontal direction (as seen on the screen display) (the chart speed). The recorder can be temporarily stopped and restarted using the **on/off** switch, in order to prevent that, during an hour-long experiment with data analysis in between, a lot of useless tracings are made. By clicking the "**Mark**" button, a mark is made on the chart paper and may be made use of in some of the analyses (see below). The cursor to the right of the chart can be used to adjust the **baseline** of the recording.

Every experimental manoeuvre or change in the settings is **automatically recorded** as text in the **documentation bar** (above the chart in the screen display). This includes not only the details of the applied drug and chart settings but also the time of the start of the experiment, the time that has elapsed during the experiment and the current position (cms) of the chart pen in the ongoing experiment.

When calculating the concentration of the applied drug within the heart, it is important to note that on the chart, the concentration of the applied drug is recorded in terms of its molar concentration in the test tube. The concentration of the applied drug within the heart is then calculated by applying a dilution factor that is derived from the relative flow rates of the drug and perfusion solutions. For example (Fig. 4), 10 µL/min of drug from a test-tube containing 10^{-3} mol/L adrenaline and flowing in to the perfusion solution, which has a flow rate of 10 mL/min, gives a dilution factor of 10^{3} (10 µl/min / 10 ml/min) and a concentration of adrenaline in the heart tissue of 10^{-6} mol/L.

1.4 The Data Analysis ("Analysis")

By clicking on the "Analysis" button, you will open the evaluation part of the "Physiology" laboratory from where you can return by clicking the "Experiment" button. In this way, you can switch freely between the experiment and the analysis, while the experiment continues to run uninterrupted. When the evaluation section is open, a part of the recording remains visible, so that you can still follow the current changes in cardiac contractions.

In the lower half of the display, you will see the **chart paper** and to the right a section of the current recording. On switching to “Analysis”, you always come to the end of chart paper and, from there using the cursor arrow, you can flick through the chart recordings, or you can move the chart by holding the mouse cursor over it.

By clicking on the "Mark" button at the top left border, you can switch to markings you made in “Experiments” or the chart positions at which you interrupted the recordings. In the bar above the chart paper, you will find all the necessary information on chart speed, resolution and the applied drug(s) that are relevant to the displayed section of recording. Thus, here and by using the chart markings, you can access the complete documentation of your recordings.

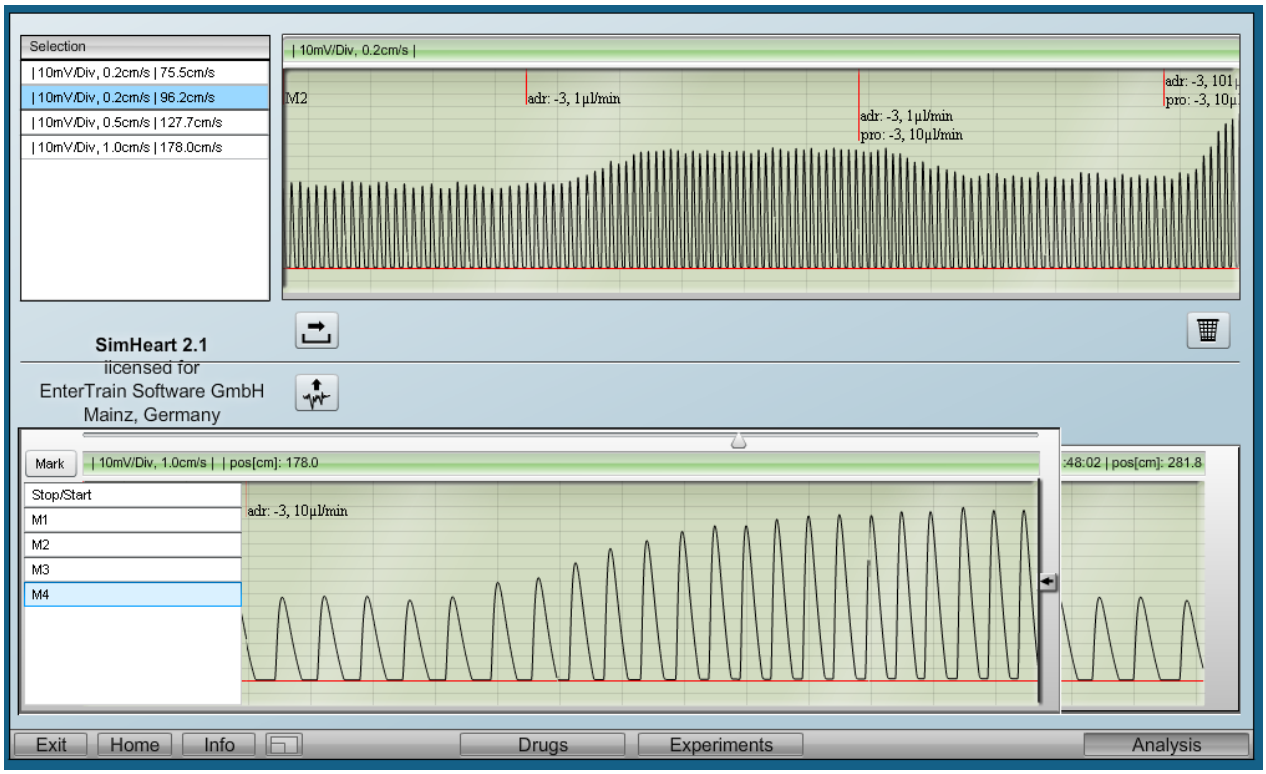

Fig 5: Reviewing and storing the recordings in the program section “Analysis”.

You can place selected pieces of the recordings in a **Temporary Store** by clicking on the button with the upwardly-pointing arrow. If you do not want the complete displayed recording, you can use the cursor on the right to mark the limits of the section of recording of interest.

You can inspect the collected sections of recording in the temporary store and delete any by clicking on the waste-bin button. Using the right-pointing arrow you can move sections of recording to your hard drive where they will be stored as jpg files that can be used in your practical report.

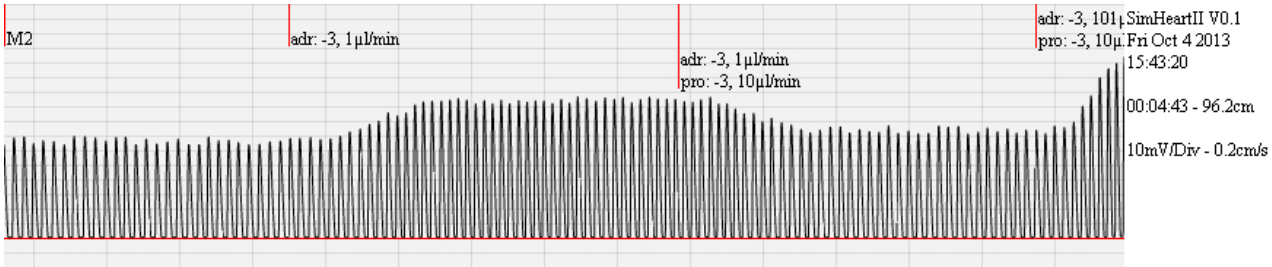

Fig 6: An example of a graphic of a selected section of chart recording with the relevant experimental documentation.

2. Laboratory experiments

2.1 Preparation of the diluted drug solutions ("Drugs")

To prepare the drug dilutions given in the Laboratory "Drugs", you need to determine the relationship between the vial concentration of the drug and the mixing of this solution with Krebs perfusion solution. All the information required for this are on the ampoule boxes in the laboratory. They are also listed in the following table, so that you can make the necessary calculations before you start the experimental part of the practical tutorial.

Enter your calculation in the place provided in the Protocol (the first part). Whether your calculation is correct will be checked in the Drug laboratory at the beginning of the practical class.

Information on the drugs contained the ampoules

Drug	**Structural formula**	**Molecular weight (g/mol)**	**Cravimetric concentration (mg/mL)**	**Required Molarity M (mol/L)**
Acetycholine (chloride)	$C_7H_{16}NO_2.Cl$	181.7	10	10^{-2} to 10^{-5} M
Atropine (sulphate)	$(C_{17}H_{23}NO_3)_2.H_2SO_4$	676.8	0.5	10^{-4} and 10^{-5} M
Adrenaline (hydrochloride)	$C_9H_{13}NO_3.HCl$	219.7	1.22	10^{-3} to 10^{-5} M
Phentolamine (methane-sulfonate)	$C_{17}H_{19}N_3O.CH_3HSO_3$	377.5	10.0	10^{-3} M
Propranolol (hydrochloride)	$C_{16}H_{21}NO_2.HCl$	295.8	1.0	10^{-3} M
Verapamil- (hydrochloride)	$C_{27}H_{38}N_2O_4.HCl$	491.1	2.5	10^{-3} M
g-Strophantin (Ouabain)	$C_{29}H_{44}O_{12}$	584.7	2.5	10^{-5}

Calculation instructions for preparing the required substance dilutions

On the ampoule packages the **gravimetric concentration** is given while the effects of a certain substance depend on the number of molecules that are available. This can be quite different, at the same gravimetric concentration, because of different **molecular weight** (g/mol). As a measure that considers the molecular weight, mostly the **molecular concentration** (g/mol) is used, in brief also referred to as **molarity** (M) as on the tubes.

To prepare a solution with a certain substance molarity it is necessary first to know the molarity in the ampoule and then to calculate the dilution factor.

In the following, the calculation of the diluted solutions of drug substances is presented with acetylcholine, as the example.

1. Calculate the molar concentration (mol/L), i.e. the molarity (M), of the ampoule solution dividing the gravimetric concentration (g/L) by the molecular weight (g/mol), both listed in the Table as well as on the substance packages n the "Drugs" laboratory.

$$\frac{\text{gravimetric concentration (mg/mL = g/L)}}{\text{molecular weight (g/mol)}} = \text{molar concentration (mol/L)}$$

which gives, for Acetylcholine:

$$\frac{10\ \text{g/L}}{181.7\ \text{g/mol}} = 5.5 \times 10^{-2}\ \text{mol/L}$$

2. To know to which amounts such ampoule solution needs to be diluted in Krebs solution to achieve the requested molarity first calculate the dilution factor in dividing the requested molarity by the ampoule molarity:

$$\frac{\text{requested molarity (mol/l)}}{\text{ampoule molarity (mol/l)}} = \text{dilution factor}$$

which gives, for 10^{-2} M acetylcholinechloride:

$$\frac{10^{-2}\ \text{mol/L}}{5.5 \times 10^{-2}\ \text{mol/L}} = 0.182 \quad \Rightarrow \quad \frac{0.182}{1 - 0.182} = \frac{0.182}{0.818}$$

Hence, to obtain a concentration of acetylcholine of 10^{-2} M, the ampoule solution must be diluted by a factor of 0.182 which means that 0.182 parts of the ampoule solution have to diluted in 1 – 0.181 = 0.818 parts of Krebs solution. If the required final volume of diluted solution, for example, is 5 mL, 5 x 0.182 = 0.91 mL of the ampoule solution shall be added to 5 x 0.818 = 4.09 mL Krebs solution providing 0.91 + 4.09 = 5 mL of 10^{-2} M acetylcholine solution.

In case that a series of solutions differing in molar concentration by one order of magnitude have to be prepared, as for acetylecholine, it is recommended first to prepare the solution of highest concentration. For the next lower solutions you just can dilute, step by step, 1 volume of the previous solution in 9 volumes of Krebs solutions (1:10 dilutions).

2.2 Recordings of cardiac contractions ("Experiments")

2.2.1 Cardiac contractions in response to adrenaline and acetylcholine

You should familiarize yourself with the experiments in Langendorff laboratory, especially with regard to substance administration and the recorder settings. Then, you can start making the recordings and calculations for measuring the effect on the strength of cardiac contractions and on heart rate in response to applying adrenaline and acetylcholine (each at two different concentrations) (see protocol for more details).

2.2.2 The dose (concentration)-response curve of epinephrine

Now, based on the systematic, quantitative measurements of the power of cardiac contractions (maximum pressure) at different concentrations of adrenaline you can create the **dose-response curve** of adrenaline. In its usual form, the increase in cardiac force is plotted against adrenaline concentration, applied in steps of one order of magnitude.

2.2.3 Effect of the β-adrenergic receptor blocker, propanolol, on the dose-response curve of adrenaline (competitive inhibition)

In order to see the characteristics of competitive inhibition, you can first apply the "β-blocker", propanolol, and then repeat your recordings of the effect of adding increasing doses of adrenaline. By entering the values from this experiment on to the same graphical axes presenting the first dose-response curve of adrenaline, you can directly compare the two curves and should be able to identify the **effects of competitive inhibition on the adrenalin dose-response curve caused by the β-blocker**.

2.2.3 Effect of the Ca^{++} blocker, verapamil, on the dose-response curve of adrenaline (non-competitive inhibition)

In order to see the characteristics of non-competitive inhibition, the Ca^{++} channel blocker is applied to the preparation under control conditions and again before applying the highest concentration of adrenaline used in the previous experiments. Again, by entering the response values on to the same graphical axes presenting the first dose-response curve of adrenaline, you can directly see **the differences between the effects of competitive and non-competitive inhibition**.

2.2.4 The effect of the cardiac glycoside, g-strophantin (ouabain), on cardiac muscle

The **increased contractile strength of cardiac muscle** caused by g-strophantin (ouabain) can be demonstrated in the next experiment. This effect is best seen after the heart preparation has been treated with verapamil. There can also be **arrythmia** in response to g-strophantin and this can be seen without verapamil and when the heart is beating normally or when it is more rapidly beating, such as after applying adrenaline.

3. Physiological and pharmacological basis

Learning Objectives: Through their own experiments in a virtual lab, you will learn how to change the activity of the heart by application of physiologically-important signal substances and how their activity can, in turn, be altered pharmacologically. You should be able to explain the effects that you have observed in the virtual experiments, on the basis of cellular processes (the activation or blocking/inhibition of membrane receptors, ion channels, ion pumps and processes of ion exchange across membranes).

Clinical Relevance: The treatment of heart disease is one of the most common activities in daily medical practice. Cardiac diseases differ a lot in their clinical characteristics and have different causes. To understand the mode of action of cardiac medications, you need to know about the physiological processes that control cardiac activity and how these processes are affected by different drugs. You will work with the physiologically-important transmitters and test the effects of drug substances that are widely-used as heart medications. The virtual laboratory used here replicates the classic experimental heart model, the so-called "Langendorff heart". This model and the type of experiments that you will be performing represent the standard procedure that continues to be used for screening for cardioactive drugs in the development of new heart medications.

Required background knowledge (in brief)**:** the automaticity of the heart and the control of the heart by the autonomic nervous system; the automatic activation pattern of the heart; impulse transmission in the heart; the intrinsic cardiac impulse conduction system; regulation of heart rate and cardiac force (Ca^{++} effects); cardiac effects of (i) the sympathetic and parasympathetic nervous systems, (ii) adrenaline, (iii) noradrenaline, (iv) adrenergic receptors, (v) cholinergic receptors, (vi) β- and α-adrenergic receptors, (vii) acetylcholine, (viii) receptor agonists and antagonists, (ix) Ca^{++}-channel blockers, (x) cardiac glycosides, (xii) functional antagonism, competitive and non-competitive inhibition.

Questions to address in preparation:

1. Draw the form of an action potential of the ventricular myocardium, the associated changes in the cytosolic Ca^{++} concentration and the contraction curve (the time-course of the contraction-induced pressure change with approximate numerical values).
2. Through what processes can the cytosolic Ca^{++} concentration in the myocardium be increased or decreased?
3. How do the sympathetic and parasympathetic nervous systems affect the heart?
4. Through which transmitter is cardiac activity controlled?
5. Which membrane receptors, ion channels and second messengers are involved in regulating cardiac muscle activity?
6. Through what mechanisms do the substances used in this experiment work?
7. Are there any differences in the way cardioactive substances affect the heart in vivo and the isolated heart?
8. What form does a typical dose-response curve have and how does that arise?
9. What is meant by the term "competitive inhibition"? Give an example.

3.1 Automaticity of the heart

Present in the sinus and AV nodes of the heart are special cells, which have cellular membrane potentials that are not constant. At these **centres of automaticity** (or **pace-making**), when there is a full membrane repolarization, there is a spontaneous diastolic depolarization and triggering of an action potential. This action potential is transmitted via the **cardiac conduction system**, spreads from cell to cell of the myocardium (these cells are connected by **gap junctions** and form a **functional syncytium**) and fans out over the entire myocardium to cause a synchronized contraction of the heart.

In these experiments, you will investigate how cardiac contractions are changed by specific cardioactive substances.

3.2 Influencing cardiac activity

The heart must adapt to the changing demands on the circulatory system by changing the force of contraction, such as during exercise. There are 5 characteristics of heart activity that can be modified and so allow regulation of cardiac function: the strength of the force of contraction (**inotropy**), the rate of activation potential (impulse) formation at the pacemaking centres (**chronotropy**), the impulse conduction rate in myocardium (**dromotropy**), the excitability of the cardiac muscle (**bathotropy**) and the relaxation rate of the cardiac muscle (**lusitropie**). In these experiments, you will mainly measure the inotropic and chronotropic effects of various cardioactive substances. Under physiological conditions, the activity of the heart is controlled mainly through the autonomic nervous system. When activated, the sympathetic nervous system causes a so-called ergotrophic reaction involving increased heart activity (this is part of an overall state of readiness for increased action), while activation of the parasympathetic nervous system is associated with initiation of the so-called trophotropic reaction and lowering of cardiac activity (this is a state of recovery with reduced demands on heart activity).

3.3 Role of the autonomic nervous system; functional antagonism

The heart is well supplied with sympathetic (adreneric) and parasympathetic (vagal) (cholinergic) nerves and their respective main neurotransmitters, noradrenaline (NA) and acetylcholine (Ach). In addition, an important cardioactive hormone is adrenaline, which is produced by and released from the adrenal gland (medula) and reaches the heart via the circulation. These cardioactive transmitters act by binding to membrane receptors on cardiac cells and, thereby, act to control the rate of cardiac contractions, the rate of impulse transmission in the myocardium, the force of contraction and the excitability of the heart.

As in many smooth muscle organs, there exists in the heart a functional antagonism between the adrenergic and cholinergic innervations. Thus NA (and adrenalin) and Ach exert opposite effects.

The **Ach receptors** on the heart are of the **muscarinic** type and are found mainly in the pacemaker centre of the atrium. Therefore, they are mainly involved in the control of heart rate, with an increase in parasympathetic activity leading to the lowering of the heart rate,

while an increase in the sympathetic activity, via **adrenergic receptors**, increases heart rate and has additional chronotropic effects.

The most commonly performed form of the Langendorff preparation uses the isolated heart of a rat and this is simulated in this virtual experiment. However, it is important to note that, in the rat and guinea pig, Ach causes a moderate weakening of the power of cardiac contractions, whereas in humans, no direct negative inotropic effects of muscarinic receptor stimulation is observed. Positive inotropic effects on the ventricular myocardium are exerted via activation of **β_1-adrenergic receptors**.

Within the heart, there are very specific receptors with differing functions and important differences in distribution. In particular, the different distribution and actions of α- and β-adrenergic receptors on cardiac muscle and on the blood vessel smooth muscle have significant physiological and pharmacological significance, based on a more-or-less separate and selective regulation of the heart and peripheral blood vessels.

It should be clear to you that a signal transmitter or drug may act on different receptor molecules and activate different second-messenger pathways and, thereby, have quite different biological effects. For example, the sympathic nervous system, through the action of NA, can increase the force of contraction of the heart, via activation of β_1-adrenergic receptors, and can decrease the force of contraction of intestinal smooth muscle, via activation of α_1-receptors (compare here SimHeart and SimVessel).

As you perform these experiments, you should also be aware that the heart **in vivo** is under the continual influence of the autonomic nervous system, while **in vitro** the influence of the sympathetic and parasympathetic innervation is absent.

3.4 Receptor agonists and antagonists: competitive inhibition

For receptor-mediated effects, in addition to the physiological neurotransmitter or hormonal substances, there are other substances (drugs) available for pharmacological experiments that bind to the receptors and, thereby, compete competitively with the physiological substance for binding to the receptor. The relative proportion of receptors occupied by either type of molecule is determined by the affinity of the respective molecules for the receptor. If the drug substance has the same receptor-mediated effect as the physiological substance, then it is referred to as an **agonist**. If the drug molecule only occupies the receptor, without causing the receptor-mediated effect, it is simply blocking the receptor`s availability for the physiological substance (or agonist), and is so referred to as an **antagonist**.

Agonist and antagonist drugs can often be found that are much more specific in their affinity for the different sub-types of receptors than the respective physiological agonist. For example, the **nicotinic** and **muscarinic** sub-types of Ach receptors are defined by their selective binding of the drugs, nicotine and muscarine, respectively.

Antagonist drugs are particularly useful pharmacological tools because they can be used to specifically inhibit the action of the physiological agonist. For nicotinic receptors, such an antagonist is **curare**, which is also known as Indian arrow poison. Curare-derived drugs are

in daily clinical use as muscle relaxants because they competitively inhibit the effect of Ach at the neuromuscular junction.

Another antagonist of Ach is **atropine**, which selectively binds muscarinic receptors, such as those on cardiac cells. It is has a long history as the active substance of the classic poison, **belladonna**, a name that is derived from its effect of dilating the pupils of the eye. The control of pupil size is a classic example of the physiological antagonism of the sympathetic and parasympathetic nervous systems; the papillary constricting effect of the parasympathetic nervous system is blocked by atropine.

As with cholinergic receptors, **adrenergic receptors** can be antagonized by specific drug substances. **Phentolamine** acts as an antagonist at α-adrenergic receptors, which play virtually no role in the heart. It acts on α-adrenergic receptors of smooth muscle cells of blood vessels and of the intestinal wall (see "SimVessel"). In contrast, **propranolol** is an adrenergic "**β-blocker**" that has potent effects on the heart and is an important heart medication. It directly affects the force of cardiac contractions by acting on β-adrenergic receptors and blocking the actions of NE and adrenaline, and can lower blood pressure and reduce tachycardia. It was in 1965 that the first β-blocker was introduced and, today, they are among the most widely prescribed heart medications.

3.5 Calcium channel-blockers: non-competitive inhibition

Calcium acts as a second messenger in the mediation of either adrenergic or cholinergic receptor activation but is also a key mediator of electromechanical coupling in muscle cells. In electromechanical coupling, it is the cytosolic Ca^{++} concentration that determines the strength of cardiac contractions, and the cytosolic Ca^{++} concentration is governed by the amount of Ca^{++} released from intracellular stores and the amount of extracellular Ca^{++} entering the cell from outside. **Calcium channel blockers** or **calcium antagonists**, such as **verapamil**, have a broad therapeutic utility in heart disease. They reduce the power of cardiac contractions (and so reduce blood pressure) and can also normalize an increased heart rate.

The β-blockers and Ca^{++} channel blockers have different effects on the heart and these can, in turn, vary depending on the underlying state of the heart. While β-blockers reduce the effect of stimulated release of sympathetic NA and of adrenal gland-derived adrenaline, while the effect of Ca^{++} channel blockade is independent of the sympathetic innervation and, therefore, acts non-competitively to the physiological transmitters, NA and adrenaline.

It should be noted, in particular, that Ca^{++}-channels are involved in electromechanical coupling in all muscle cells, although they are not always regulated by the same mechanisms. This explains why Ca^{++} channel blocker drugs is not selective for heart muscle but also act on the smooth muscles of blood vessels, causing vasodilation of peripheral blood vessels, including the coronary arteries. Thus, Ca^{++} channel blocker drugs are often used to reduce high blood pressure.

3.6 Cardiac glycosides

Another important group of cardioactive drugs are the digitalis glycosides, which include g-strophantin (or ouabain). These are an important group of cardioactive drugs because they are among the few drugs which can increase the power of the heart in, for example, heart failure.

The cardiac glycosides act in principle at every cell membrane, because they primarily inhibit the ubiquitous Na^+-K^+ pump. However, there is a therapeutic dose, at which the strength of cardiac contractions is increased without the biology of other cells, especially nerve cells, being affected.

This dose-dependent specificity is explained by the existence of digitalis glycoside receptors on cardiac cells. However, it is necessary to use cardiac glycosides with caution because of their very low **therapeutic index**. They have a therapeutic benefit that is essentially limited to heart failure and have, otherwise, a real risk of inducing cardiac arrhythmias.

Their mechanism of action is quite clear. Their enhancement of cardiac contractile force is explained by an ouabain-induced inhibition of Na^+-K^+ pump, which decreases the Na^+ membrane gradient and so indirectly reducing the Na^+-Ca^{++} membrane exchange and increasing cellular retention of Ca^{++}. This resultant increase in cytosolic Ca^{++} increases the degree of muscle contraction.

The narrow therapeutic index of this group of drugs is not due to their effects on intracellular Ca^{++} but to their primary effect, which is inhibition of Na^+-K^+ pump. This membrane protein exchanges Na^+ and K^+ at a ratio of 3 to 2 and is, therefore, electrogenic and serves to maintain the ion concentration gradients that underlie the membrane potential. Inhibition of the Na^+-K^+ pump can thus lead to membrane depolarization, which in the heart could lead arrhythmias.