SimVessel:

Physiology and pharmacology of smooth muscle contractions – a brief introduction

The virtual SimVessel Laboratory is for recordings of smooth muscle contractions of small muscle stripes of different organs during applications of different substances. The preparations are from the antrum of the rat stomach and the aorta of the same specimen, Among the substances to apply are the physiological modulators acetylcholine (Ach) and adrenalin (Adr) as well as their competitive receptor blockers atropine (Atr), phentolamine (Phe) and propranolol (Pro). Additionally, the Ca-channel blocker verapamil (Ver) is provided. There are also 2 weights, each of 0.5 g, to examine the effects of pre-stretching the muscle stripes (Bayliss-effect). The muscle contractions are plotted on a chart recorder. Switching to "Analysis" gives access also to page through the "paper" also to check previous recordings since entering the SimVessel lab. Specific parts of interest can be selecting and stored – also as jpg files on your hard disc.

A short description of the "experiments" lab (Fig. 1) is given below. For the "drugs" laboratory and the "analysis" tools, at this state, please, check the corresponding chapters of SimHeart. We soon will provide specific SimVessel chapters of these parts and also a more detailed description of the "experiments" lab.

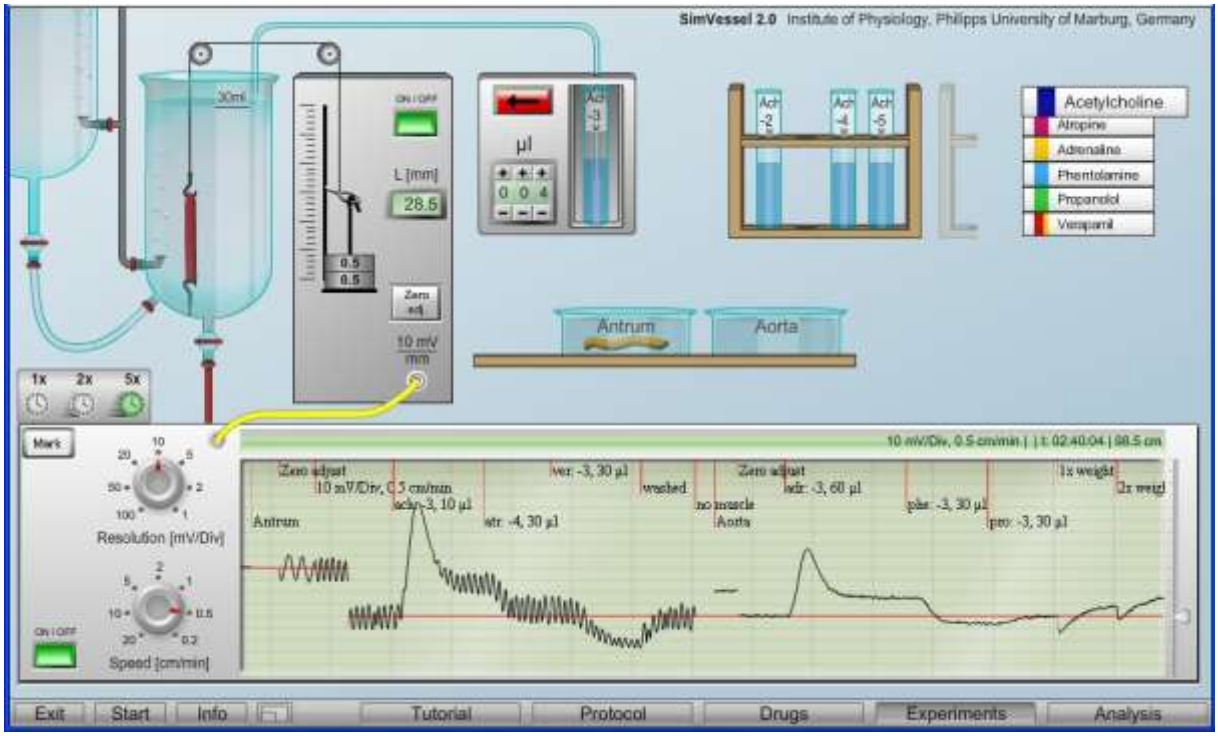


Fig. 1: SimVessel, "Experiments" Lab

1. The SimVessel laboratory

Entering the SimVessel lab you will find two **smooth muscle stripes** stored in separate Petri dishes on a shelf. With left mouse click you can grasp one of them and drag it to the **organ bath** where it, relieving the mouse button, will be fixed at the hooks. In Fig. 1, the aorta preparation is currently hanging in the organ bath. You can it bring back to the Petri dish with again with drag and drop.

The upper hook is connected via two rollers to a slider of a **mechano-electrical transducer** (on the right side of the organ bath). The slides position gives the length of the muscle strip, shown in the display. The mechano-electrical transducer converts the slider position into a voltage providing *10mV/mm* to the cable output that is connected to the chart recorder. Above the cable connector there is a "Zero adj" button to compensate for the changes of the slider's position and the accompanying voltage offset when a muscle is brought in or when the preparation is changed.

Further to the right there is an **application device** to administer a defined quantity of a drug (in µl) from one of the test tubes that are provided by several **test tube stands**. The test tube stands can be selected in the same way as in SimHeart, e.g. with click on the list of substances. A test tube of the desire concentration can then be brought into the application device by drag and drop.

In the example of Fig. 1, a test tube stand for Acetylcholine is currently chosen and a test tube containing Acetylcholine in 10^{-4} M concentration (Ach -3 M) has been inserted. The pre-selection counter is set to 30 which means that 30 µl of 10^{-4} M Ach will be applied to the organ bath when else the button with the insert arrow is pressed.

Please note: Different from SimHeart, this is not a continuous perfusion but a singular **bolus injection**. If the insert button for Ach is pressed the first time, 30 µl of 10^{-4} M Acetylcholine will be diluted in 30ml nutrient solution which gives an Ach concentration in the organ bath of 10^{-7} M. If you wish to go up, for example, to 10^{-6} M you should consider that the organ bath already contains 30 µl of 10^{-4} M Ach and therefore only apply 270µl instead of 300µl.

Washing out all previously applied substances you can click on the faucet below the organ bath. The current solution will be replaced by nutrient solution, free of drugs, from the storage container that is hanging in the left upper corner of the lab.

In addition to the effects of diverse substance you can also check the effects of muscle **pre-stretching** (Bayliss effect, at the end of the recording in Fig.1) dragging the weights that are provided on the shelf to the suspension at the slider by drag and drop. In the same way, drag and drop, they can be removed.

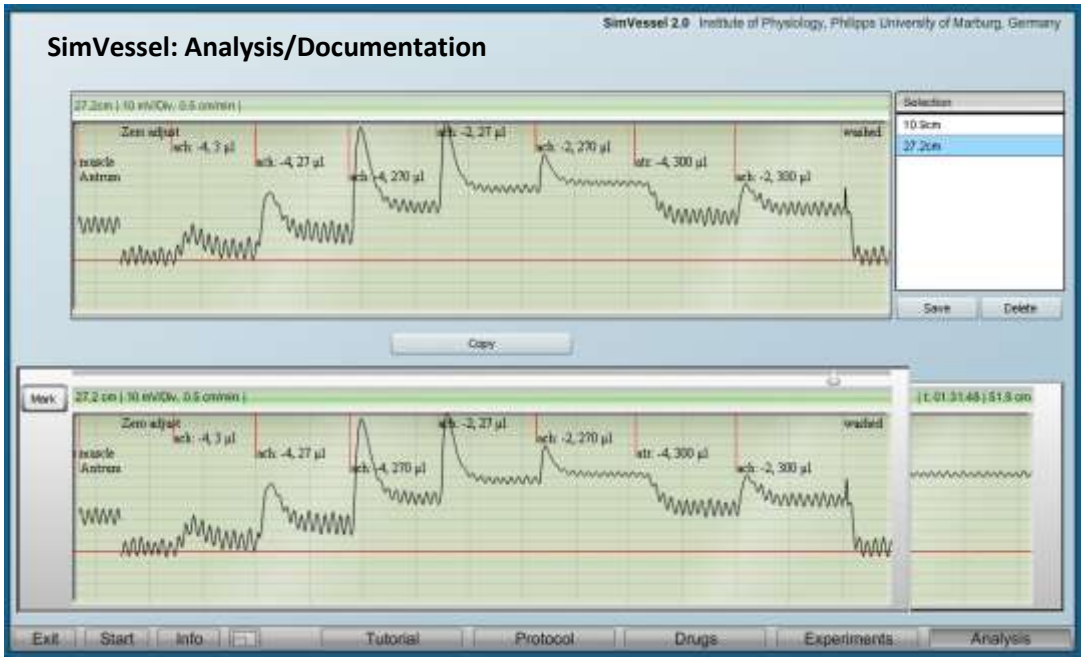
As a particular feature, not possible in real life, this virtual lab allows **time acceleration**. This can be done by selecting the clock buttons placed above the chart recorder on the left. 1x is real time while 2x or 5x let the time pass 2 or 5 times faster, respectively. This has been invented because smooth muscle reactions are very slow. It may become boring waiting for the muscle reactions in real time - especially on the computer screen. Moreover, you can conduct more experiments in the time given for your practical course.

Recordings and Analysis/Documentation

The smooth muscle contractions can be monitored on a **chart recorder** located in the lower part of the lab. You can select the **resolution** [mV/Div] as well as the chart recorder's **speed** [cm /min]. "Div" refers to the divisions as marked on the paper sheet of the chart recorder.

All actions (substance application etc.) are documented on the virtual paper. For example, "pro -3, 30µl" at the red bar indicates that at this time 3µl from a test tube containing 10^{-3} propranolol have been added to the bath solution. It is the task for the experimentalist to keep track of the current drug concentrations – a best by a clearly designed experimentation protocol. Additional marks can be set by pressing the "Mark" button in the left upper corner of the chart recorder.

With click on the "**analysis**" button, for further analysis of your recordings you can page through the paper (clicking on the paper or using the slider). You will find all your previous recording since you have been entering the lab. You can Copy selected sequences into the "notebook" above and also can save these sequences as .jpg files on your hard disk to overtake them into your study protocol.



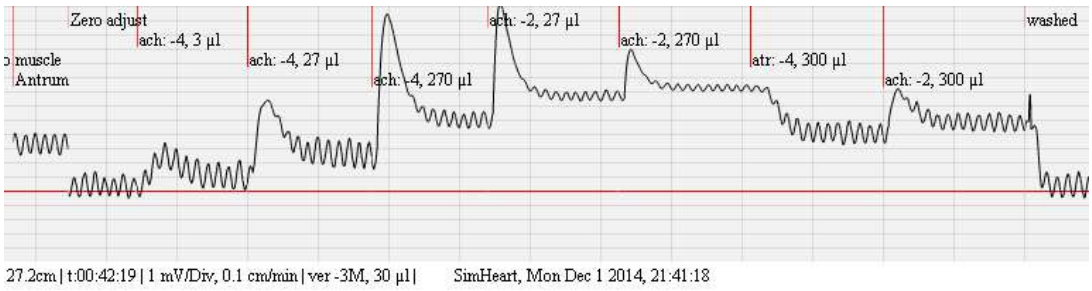


Fig.2 Analysis: A selected sequence of the recordings have been copied into the notebook. The lower part shows how it appears when saved as .jpg file

2. Physiological and Pharmacological Background – in brief

The following is an only brief summary of the main points to explain smooth muscle reactions as shown above (see also Figure 3 below).

The **antrum** shows spontaneous rhythmic contractions due membrane potential oscillations arising from the interplay of depolarizing Ca^{++}-currents with time delayed activation of Ca^{++}-dependent K^{+}-currents. Gap junctions between the smooth muscle cells of this so-called single-unit type lead to synchronized contractions. In the multi-unit type cells as of blood vessels (here: **aorta**) each cell acts separately and also the Ca^{++}-K^{+}-systems seems to be lacking.

Acetylcholin has primarily constricting effects, as seen in the antrum, first of all via the muscarinic M3 receptor and activation of the IP3 system. In blood vessels like the aorta, the effect of Ach application is inversed, leading to dilatation, mainly due to dilating NO effects released from the blood vessel's epithelium on activation of the M2 receptor

Adrenalin and Noradrenalin exert their effects via α- and ß- receptors whereby the ß1 receptor, important for heart contractions (see SimHeart) does not play a role for smooth muscle control. Activation of **α-receptors** generally leads to constriction via activation of the IP3 system ($\alpha 1$) and inhibition of the cAMP system ($\alpha 2$). In contrast, activation of the ß2 receptors stimulates the cAMP system and leads thereby to dilatation. At the end, the result, constriction or dilatation, depends on which types of receptors predominate.

Adrenalin binds on all these receptors with higher affinity to ß2- than to α-receptors which explains muscle relaxation of the antrum and most other parts of the gut. By contrast, in smooth muscles of blood vessels, like the aorta, the α-receptors predominate which leads to contractions.

At very low adrenalin concentrations small relaxation effects induced via the high affinity ß-receptors might also be seen in the aorta which, however, will soon be overwhelmed by constricting effects from the α-receptors at slightly higher concentrations. Anyhow, with blockade of one type of receptor the effects can separately be studied. The effects of competitive inhibitors can be compensated with sufficiently high doses of the agonists. Such experiment can be done with application of competitive inhibitors for a- and ß-receptors like **phentolamine** and **propranolol**, respectively. **Atropin** is available as competitive inhibitor at muscarinergic Ach receptors.

For comparison, the effects of the Ca^{++}-channel blocker **Verapamil**, as a non-competitive inhibitor can be studied. Blocking the Ca influx leads to muscle relaxations in all situations. In contrast to competitive inhibitors, the effects of non-competitive inhibitors cannot be fully be compensated by accordingly higher doses of constrictive substances.

Furthermore, the effects of pre-stretching (**Bayliss effect**) can be examined using the weights. The Bayliss effect describes the smooth muscle phenomenon that pre-stretching of the muscle induces active contractions that even overcompensate the initial elongation. It is assumed that this is caused by the activation of excitatory, stretch sensitive ion channels but it is unclear why the effect sustain also after shortening of the muscle.

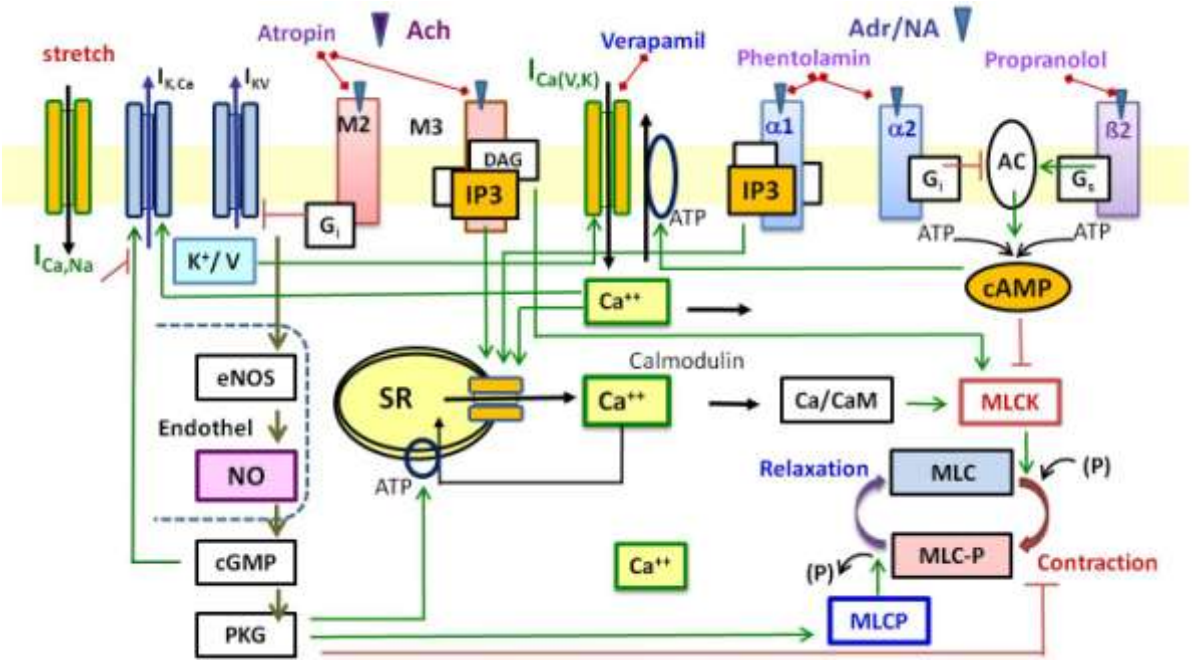


Fig. 3: Mechanisms of smooth muscle control.

3. Experiments:

3.1 Differences of smooth muscle contractions and drug effects

Major differences between the two different smooth preparations can be demonstrated in comparison of recordings as shown in Fig. 4. The recordings in the upper traces are taken from the Aorta strip , and the lower traces are recorded from the Antrum.

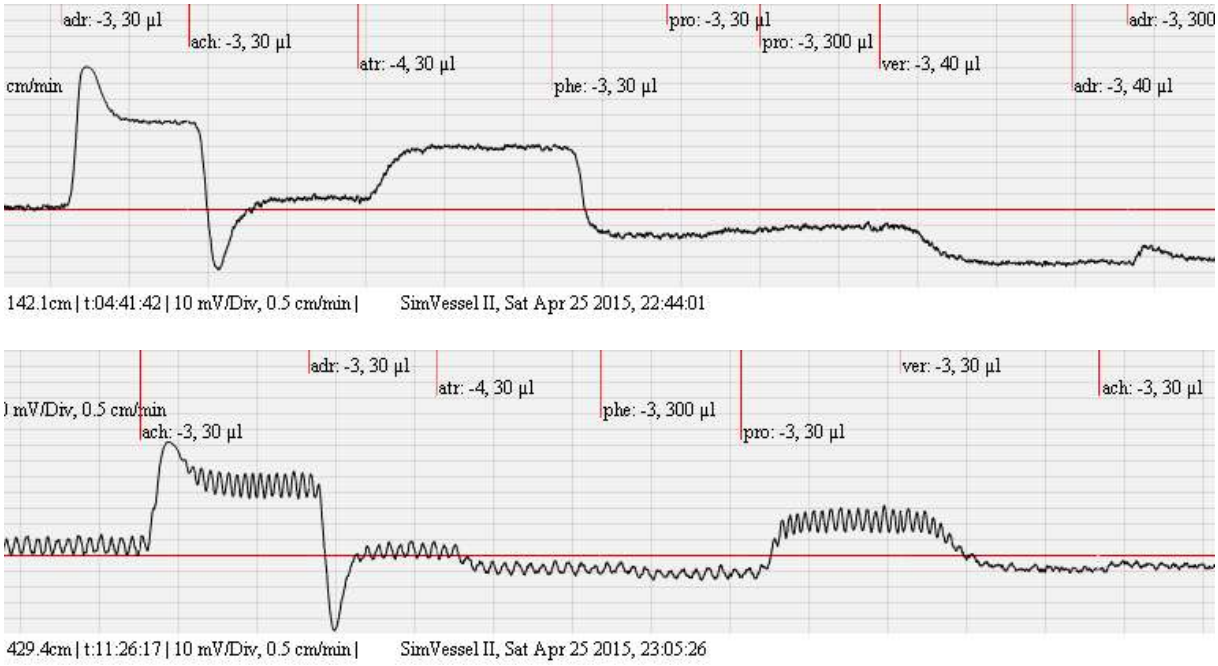


Fig. 4: Example recordings from the aorta (upper trace) and the antrum (lower trace) in the virtual SimVessel lab.

An immediately obvious difference is that the Aorta changes its length only gradually (**tonic** activity) while the Antrum additionally exhibits periodic, oscillatory changes (**tonic-phasic** activity).

Both preparations react on application of their physiologically relevant control substances **Acetylcholin** (**ach**) and **Adrenalin** (**adr**) with a transient overshoot or undershoot before relaxing to a new steady state. However, both the transient as well as the steady state responses of the two preparations are of opposite direction. In the Aorta Adrenalin leads to contraction which can be compensated by Acetylcholin while the Antrum contracts on Acetylcholin application which can be counteracted by Adrenalin. This functional antagonisms between Adrenalin and Acetylcholin, more general, between the sympathic and parasympathic system, can be seen in most autonomic systems

Accordingly, with application of the competitive inhibitor **Atropin** (**atr**) of muscarinic cholinergic receptors, the relaxing Ach effects in the Aorta are reduced and the constricting Adrenalin effects predominate. By contrast, in the Antrum, where the Acetylcholin effects are constricting, their inhibition by Atropin leads to further relaxation.

Adrenalin exerts it effects via different types of receptors, alpha and beta receptors, noteworthy with opposite effects. This can be seen with application of their specific receptor agonists **Phentolamine** (**phe**) and **Propranolol** (**pro**), respectively.

In the Aorta, the alpha receptors pre-dominate and the constricting adrenalin effects will accordingly be attenuated by Phentolamine. Application of the beta-receptor agonist Propanolol leads to slight relaxation.
In the Antrum, the beta receptors predominate, there, however, inducing relaxation. Accordingly, their inhibition by propranolol attenuates the relaxing Acetylcholin effects. In this case, application of the alpha receptor agonist leads to further relaxation.

The non-competitive inhibitor **Verapamil** (**ver**), reducing the Ca-influx, induces relaxation in all situations. Without the necessary increase of Ca, of course, also the effects of the application of otherwise strongly constrictory substances, Ach or Adr, respectively, will be impaired.

The above shown recordings can only give a first impression how different smooth muscle preparations can be modulated by different signal substances and drugs. For a better understanding we recommend to do systematic experiments, e.g. for analysis of the effects of different substances and drugs, also in quantitative form, e.g. with recordings of dose-response curves of the physiological control substances Acetylcholine and Adrenalin, also in the presence of the diverse competitive and non-competitive inhibitors.

3.2 Dose-Response-Curves:

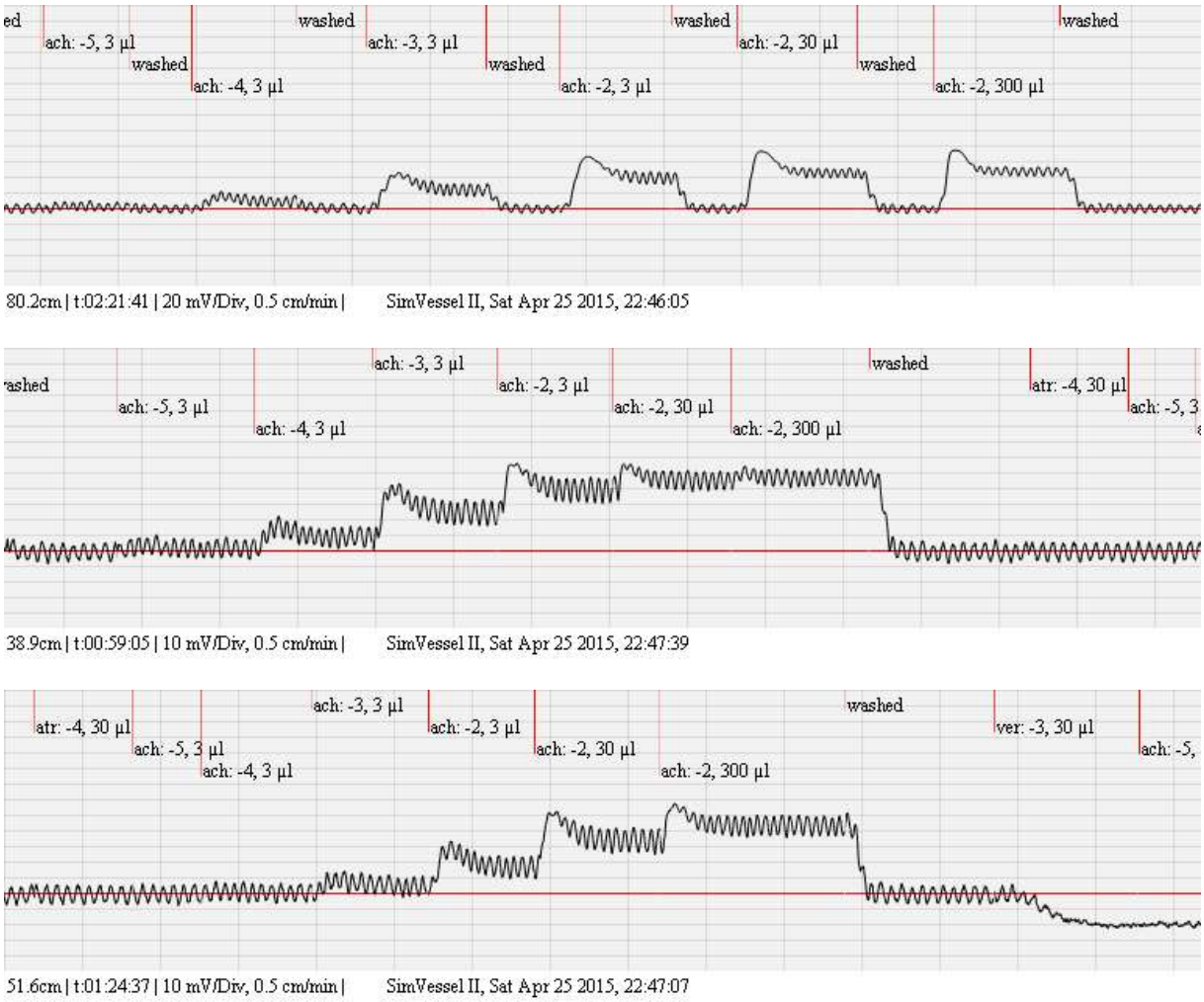


Fig. 5: Examples of dose-response curve of Acetylcholine effects on the Antrum.

Examples of **dose-response curves** of the antrum on application of acetylcholine in increasing concentration are shown in Fig. 5. The upper trace is with washing out the previous substance application. The mid-trace shows the effects of continuously increasing acetylcholine concentration, shown again in the lower trace when before the receptor agonist Atropine has been applied.

Similar experiments can be some with the aorta with application of Adrenalin, in this case in the presence of competitive inhibitors of both receptor types. In the same way, the effects of the non-competitive inhibitor Verapamil on the dose-response curves of both preparations can be examined and should be compared with those of competitive inhibitors. Likewise, the functional antagonism between Acetylcholine and Adrenalin, i.e. how the doe-response curves of the one is modified in the presence of the other one, can systematically be examined.

3.3 Bayliss-Effect:

Another functionally important effect, the **Bayliss effect** (Fig.6), can be examined by using the weights for pre-stretching of the smooth muscle preparations to demonstrate that the muscle elongation is even overcompensated by intrinsic mechanisms, i.e. leading to muscle contractions. In the case of the tonic-phasic contractions of the antrum (recording on the right in Fig. 6)) this is accompanied with increasing amplitude and frequency of the periodic contractions

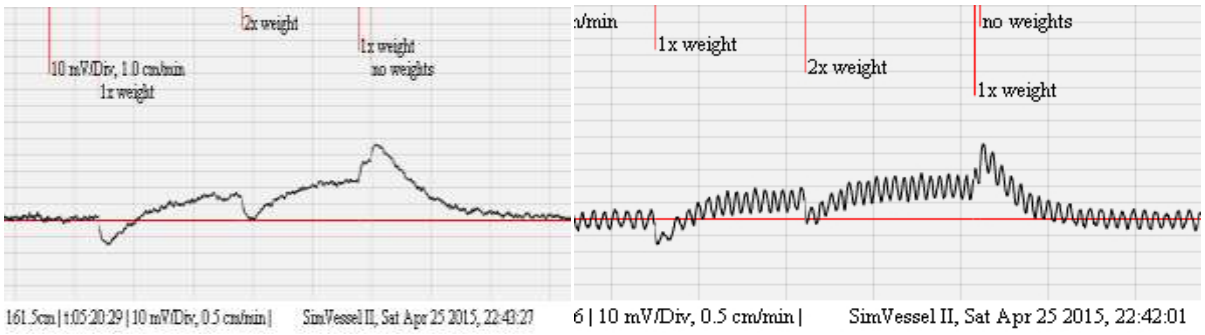


Fig. 6: Bayliss Effect