# SimVessel:

## A virtual laboratory for recordings of smooth muscle contractions

### in response to physiological signal substances and functionally relevant drugs

The virtual SimVessel Laboratory simulates real-life recordings of smooth muscle contractions, allowing experimentation with small muscle strips from different organs with the application of different substances. In programming, the focus has been to simulate the classical experiment as precisely as possible to provide students and junior instructors with a scenario with maximal opportunities for learning to independently design and carry out experiments. Accordingly, the virtual equipment allows adjusting the resolution, the speed and the offset. The experimental protocol - including the dosage of the drugs and the order in which they are applied - can be freely varied.

The preparations are from the antrum of the rat stomach and the aorta of the same species; the responses are modeled based on decades of experiences in a real-life teaching laboratory. The current version allows application of the physiological modulators acetylcholine (Ach) and adrenalin (Adr) as well as their competitive receptor blockers atropine (Atr), phentolamine (Phe) and propranolol (Pro). Additionally, verapamil (Ver) is provided, which blocks channel-mediated uptake of $Ca^{2+}$. To examine the effects of pre-stretching the muscle strips (Bayliss-effect), two weights of 0.5 g each are provided.

The muscle contractions are plotted on a chart recorder. Switching to “Analysis” allows the user to page through all data recorded since entering the SimVessel lab. Specific parts of interest can be selected and stored as jpg files on the hard disc for later reference.

A short description of the virtual “experiments” laboratory (see Fig. 1) with the analysis tool to document the results (Fig. 2) is given below. The “drugs” laboratory is largely self-explanatory, but you can also check out the corresponding chapters of SimHeart. The second chapter gives a brief overview on the physiological and pharnacologicl mechanisms controlling smooth muscle contractions. The tutorial concludes with some suggestions for experiments.

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# 1. The SimVessel laboratory

## 1.1 Experiments

After entering the SimVessel lab by clicking on the icon labeled “Experiments”, you can see the organ bath to the right of the display (Fig 1a ). On a shelf in the middle, you will find two **smooth muscle strips** stored in separate Petri dishes (Fig 1b). With left mouse click you can grasp one strip and drag it to the **organ bath**, where after relieving the mouse button, it will be fixed at the hooks. In Fig. 1, the antrum preparation is already hanging in the organ bath. You can return it to the Petri dish by drag and drop.

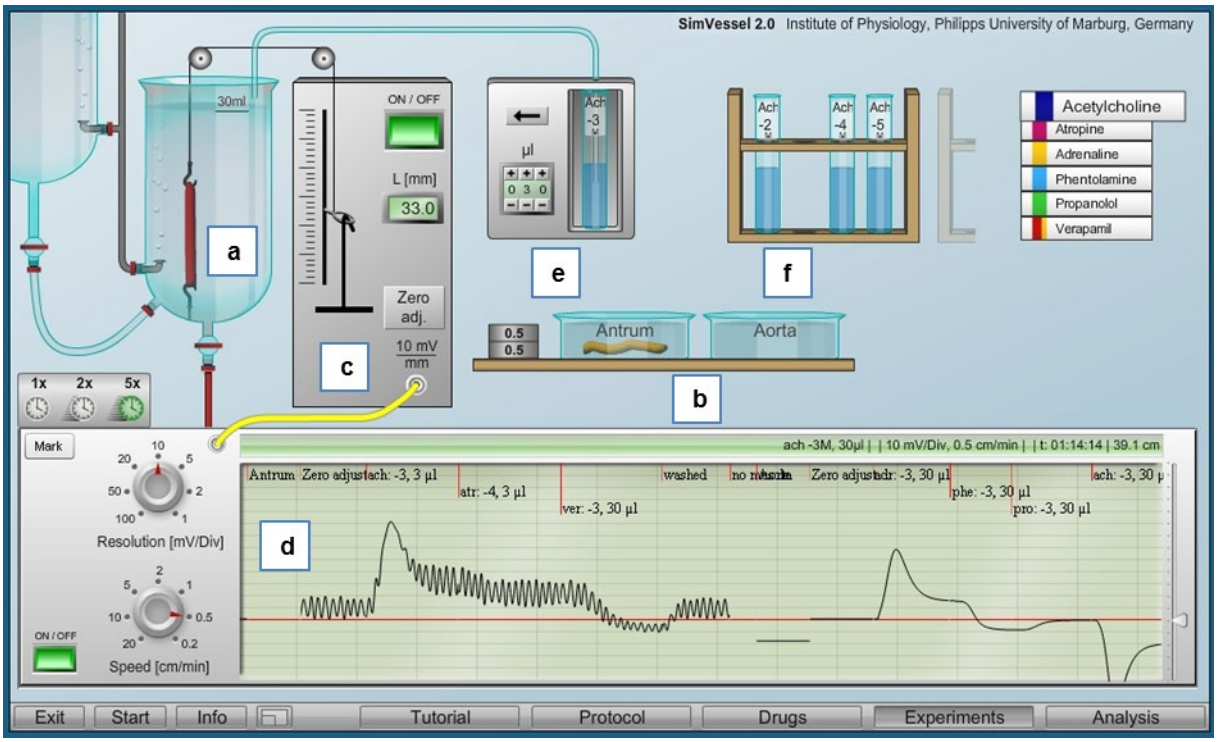



**Figure 1:** Setup of the virtual laboratory. a) organ bath with strip of aorta. The solution in the bath can be exchanged by clicking on the faucet below the organ bath. b) shelf with weights of 0.5 g each, and a fresh strip of antrum and a petri dish for the aorta strip. c) mechano-electrical transducer. Press “zero adj.” for offset correction. d) Chart recorder. As in a real-life chart recorder, the resolution and speed can be adjusted. e) Device for application of drugs. The counter has to be set to the desired amount (in µl), which is released upon pressing the button with the arrow insert. f) Rack with agonists /blockers that can be exchanged by clicking on the menu to the right.

The upper hook is connected to a string which passes to the slider of a **mechano-electrical transducer** (Fig 1c) via two rollers (on the right side of the organ bath). The position of the slider 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 at the bottom.

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. As in a real-life setup, this “Zero adj” button must be clicked in many situations to return

the trace on the chart recorder to the middle position. If you see no signal, also make sure that both the transducer and the recorder have been switched on (green "on-off" button)!

Further to the right there is an **application device** (Fig 1e) to administer a defined quantity of a drug from one of the test tubes that are provided by several **test tube stands** (Fig 1f). When first entering SimVessel, a test tube stand for Acetylcholine is provided as a default option. Other substances can be selected in the same way as in SimHeart, e.g with click on the list of substances to the left of the stand. A test tube of the desired concentration can then be brought into the application device by drag and drop. The quantity of the drug (in µl) can be altered by clicking on the "+" or "-" signs above and below the three-digit number of the counter on the application device. Application of the drug is started by clicking on the arrow above this counter. If the number in the counter is set to "000", no drug is applied and you cannot expect to see any drug effect.

In the example of Fig. 1, a test tube stand for Acetylcholine has been chosen and a test tube containing Acetylcholine in $10^{-3}$ M concentration (Ach $^{-3}$ M) inserted into the application device. 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 the button with the insert arrow is pressed.

Please note: in contrast to SimHeart, application does not occur in the form of a continuous perfusion but as 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 to $10^{-6}$ M, you must 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 occurs by clicking 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 substances, you can also check the effects of muscle **pre-stretching** (Bayliss effect) by 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. For the same reason, also washout of substances is faster than in real life and is always complete.

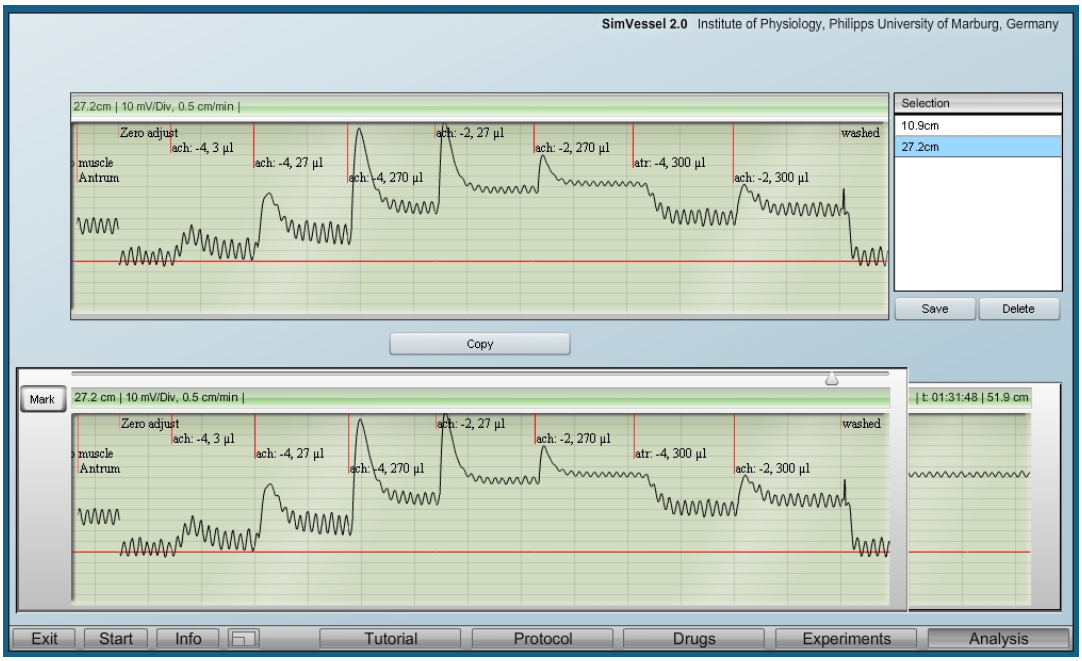
## 1.2 Recordings and Documentation

The smooth muscle contractions can be monitored on the **chart recorder** located in the lower part of the lab (Fig. 1d). 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 of the chart recorder.

All actions (substance application, chart recorder adjustments, etc.) are documented on the virtual. For example, "atr -4, 3ml" at the red bar indicates that at this time 3ml from a test tube containing $10^{-4}$ atropine have been added to the bath solution. It is the task of the student to keep track of the

current drug concentrations – at 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 “**analysis**” you can page through the chart recording not only after the experiment, but also while the experiment is running. This makes it possible to keep track of all manipulations since 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 and incorporate them into an experimental protocol. You can scroll through the recordings that have so far been obtained moving the mouse along the



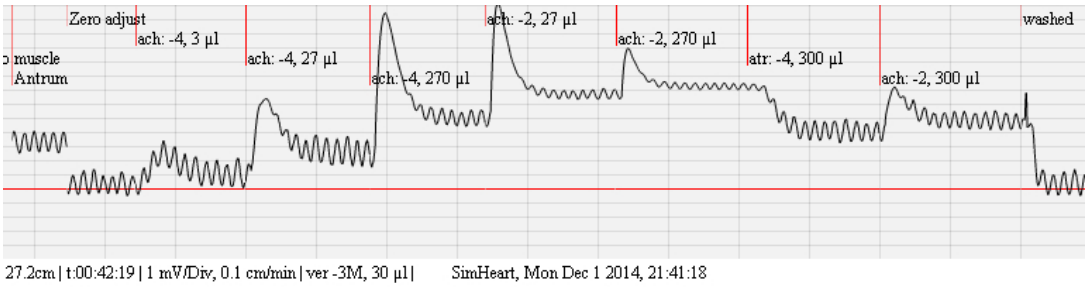




**Figure 2:** Analysis and documentation: By pressing the “Copy” button, the currently visible sequence of the chart recorder (lower part of the analysis window) is overtaken into the notebook (upper part) and can be saved as .jpg file, including documentation, in the form as shown in the figure below,.

## 2. Physiological and Pharmacological Background – in brief

The regulation of smooth muscle contractility is extremely complex, differing not only with the species and the tissue studied, but also frequently showing variability within different parts of an individual preparation. This diversity in regulation mirrors the complex movement patterns of many types of smooth muscle tissue ( e.g., the propulsion ingesta through the gut) and complicates attempts to understand all responses seen in an experimental situation. Nevertheless, it is possible to identify some main points which we will attempt to briefly summarize to explain smooth muscle reactions as shown above (see also the Figure 2 below).

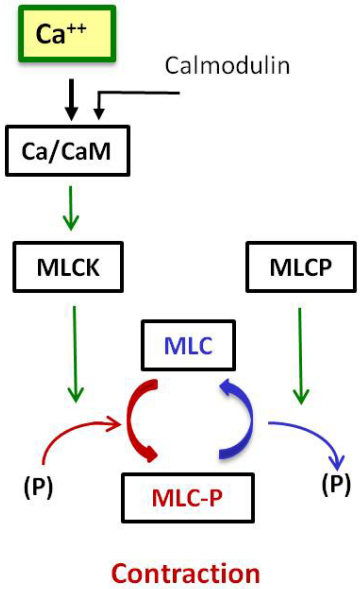
Smooth muscle cells of the single-unit type are interconnected by gap junctions, so that activation of one cell leads to a synchronized contraction of the entire syncytium. In multi-unit type cells, the activity of each cell is independent from that of neighboring cells and is determined by the receptors expressed and the transmitters released by surrounding autonomic neurons.

### 2.1 Mechanisms of smooth muscle contractions

In all types of smooth muscle tissue, a rise in cytosolic $Ca^{2+}$ initiates a signaling cascade that induces contraction. The rise in cytosolic $Ca^{2+}$ that precedes contraction of smooth muscle tissue can be initiated spontaneously, after a mechanical stimulus, or in response to a plethora of agonists. Many signaling cascades involve efflux of $Ca^{2+}$ from the sarcoplasmic reticulum, which may either precede or follow the activation of membrane channels.

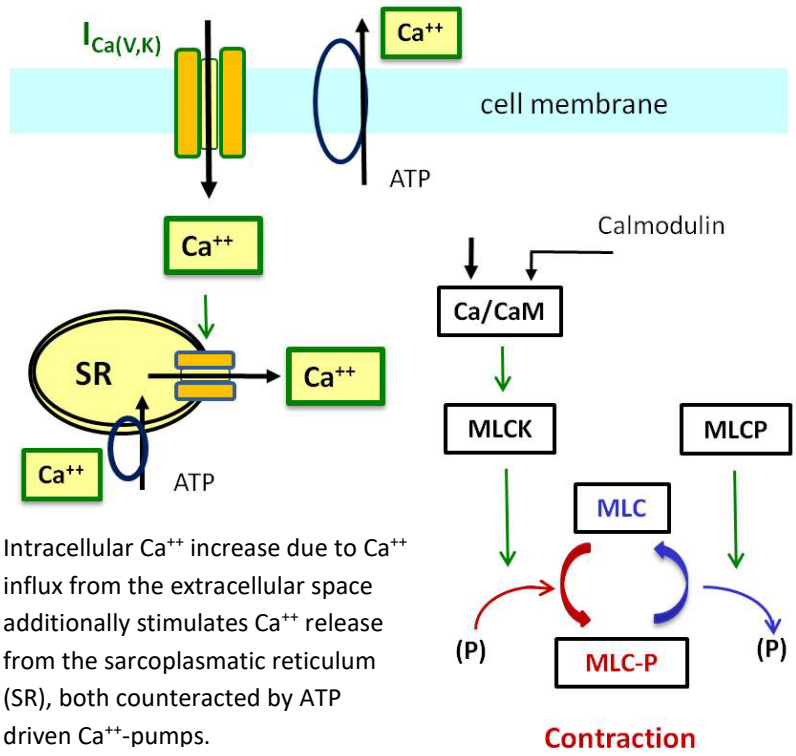
After binding of $Ca^{2+}$ to calmodulin, myosin light-chain kinase (MLCK) is activated and catalyzes the phosphorylation of myosin II. This triggers cross-bridge cycling of the myosin heads along the actin filaments, leading to a shortening or contraction of the smooth muscle cell. Each new cycle is initiated by a loosening of the bond between actin and myosin that follows the binding of a fresh ATP molecule, thus providing energy.

A peculiarity of smooth muscle tissue is the regulation of the contractile response by myosin light-chain phosphatase (MLCP). Following dephosphorylation of the light chain by MLCP, the affinity for binding of ATP decreases. Cross-bridge cycling ceases and myosin heads remain bound to actin in the “latched” state. This not only prevents any further contractile response but also enables smooth muscle tissue to sustain a basal tone for extended periods of time at minimal expense of energy. Falling levels of $Ca^{2+}$ finally lead to a dissociation of the latch-bridge state with subsequent relaxation.




Ca-induced smooth muscle contraction via MLCK dependent phosphorilation of MLC counteracted by MLCP dependen de-phosphorilaton (see text).

Both the activity of MLCK and MLCP are inversely modulated by a large number of intracellular signaling molecules that include $Ca^{2+}$, cAMP, cGMP, Rho-Kinase and many others, contributing to the complexity of responses that are observed.




Intracellular $Ca^{++}$ increase due to $Ca^{++}$ influx from the extracellular space additionally stimulates $Ca^{++}$ release from the sarcoplasmatic reticulum (SR), both counteracted by ATP driven $Ca^{++}$-pumps.

In many parts of the gut, special pacemaker cells (interstitial cells of Cajal) express cation channels that are active at the resting potential. Influx of $Na^+$ and $Ca^{2+}$ leads to a spontaneous depolarisation with subsequent opening of various types of voltage- and calcium activated $Ca^{2+}$ channels in the cell membrane. Alternately, membrane stretch (Bayliss effect) or the application of a suitable agonist induces the opening of cation channels and membrane depolarisation with subsequent $Ca^{2+}$ influx.

Repolarisation of the tissue is driven by the opening of $Ca^{2+}$ dependent $K^+$ and $Cl^-$ channels ($K_{Ca}$ and $Cl_{Ca}$) with subsequent closing of voltage dependent $Ca^{2+}$ channels. Removal of $Ca^{2+}$ from the cytosolic space via pumps and transporters (e.g. $Ca^{2+}$-ATPases, $Na^{2+}/Ca^{2+}$ exchangers) completes the cycle. The channels and transporters involved in this cascade are again fine-tuned by $Ca^{2+}$, cAMP and cGMP and other signalling molecules, so that in summary, these membrane proteins act in synergy with MLCK/MLCP to regulate the contractile response.

The **antrum** shows spontaneous rhythmic contractions due membrane potential oscillations essentially arising from the interplay of depolarizing pacemaker currents, including voltage dependent $Ca^{2+}$ channels, and time delayed activation of $Ca^{2+}$-dependent $K^+$-currents. Cells are interconnected via gap junctions (single-unit type), allowing the signals originating in individual pacemaking cells to spread out over the entire syncytium. Conversely, cells of the A**orta** show a continuous tone without spontaneous contractions. The tone is regulated by the neurons in the vicinity of the cells of this multi-unit tissue.

Usually, a good way to start an experiment is to stretch the tissue with one or both of the weights provided. An initial downward inflection of the trace on the chart reflects the mechanical stretching of the muscle. This “stretch” activates mechano-sensitive cation channels, leading to a higher influx of $Ca^{2+}$ with subsequent increase in smooth muscle tone.

## 2.2 Drug Effects

**Acetylcholine** typically has constricting effects, via the muscarinic M3 receptor as seen in the antrum. Activation of the IP3 system with release of $Ca^{2+}$ from stores and activation of phospholipase C (PLC) with opening of $Ca^{2+}$ channels leads to a rapid contraction of the tissue. The partial relaxation that follows application of acetylcholine is usually attributed to the activity of $Ca^{2+}$ pumps and transporters that normalize the cytosolic calcium level. A further relaxation can be observed after

application of **atropine**, which is provided as a competitive inhibitor at muscarinergic Ach receptors, or **verapamil**, which blocks channels mediating influx of $Ca^{2+}$. Typically application of verapamil will reduce spontaneous contractions, reflecting the central role of $Ca^{2+}$ channels in this response.

In certain blood vessels like the aorta, the effect of Ach application is inversed, leading to dilatation via release of nitric oxide (NO) from vessel's epithelium after activation of muscarinic receptors with initialization of the IP3-PLC cascade. After diffusion into smooth muscle, NO leads to production of cGMP with stimulation of MLCP and opening of potassium channels ($K_{Ca}$). Cross-bridge cycling is arrested, the membrane hyperpolarizes, and cytosolic $Ca^{2+}$ levels fall, leading to a dissociation of the actin-myosin complex and relaxation.

**Epinephrin (adrenaline) and norepinephrin (noradrenaline)** exert their effects via $\alpha 1$- and ß2-receptors. Activation of **$\alpha 1$-receptors** generally leads to constriction via activation of the inositol-3-phosphate (IP3) system with release of $Ca^{2+}$ from the sarcoplasmic reticulum, an activation $Ca^{2+}$ channels via phospholipase C (PLC), upregulation of MLCK and downregulation of MLCP.

In contrast, activation of ß2 receptors stimulates the cAMP system. Downregulation of MLCK, upregulation of MLCP, and activation of hyperpolarizing potassium channels ($K_{Ca}$) with hyperpolarisation and reduced influx of $Ca^{2+}$ all contribute to relaxation. In preparations with spontaneous contractility, the reduction in cross-bridge cycling can be seen by a reduction in the amplitude and frequency of these spontaneous contractions. Typically and as shown in SimVessel, spontaneous contractility soon resumes, albeit at lower muscle tone.

Smooth muscle cells typically only weakly express $\alpha 2$ receptors. However, a response may be seen due to an α2-induced decrease in transmitter release from neuronal synapses remaining in the tissue. Net effects depend on the local constellation of transmitter and receptor. Thus, stimulating α2 receptors expressed by a cholinergic terminal neuron adjacent to a smooth muscle cell expressing M3 receptors will lead to relaxation, blocking them will result in a contractile response. $\beta 1$ receptors are not typically expressed by smooth muscle tissue.

In many situations, smooth muscle cells will express multiple receptors and the net effect, constriction or dilatation, depends on which types of receptors predominate. **Epinephrine (adrenaline)** has higher affinity to ß2- than to $\alpha 1$-receptors which explains muscle relaxation seen in the antrum. Conversely, in the aorta $\alpha 1$-receptors predominate which leads to contraction.

At very low epinephrine concentrations, a slight relaxation induced via the high affinity ß2-receptors can sometimes also be seen in the aorta which, however, will soon be overwhelmed by constricting effects from the $\alpha 1$-receptors at slightly higher concentrations. The effects can be separately studied by the use of blockers. P**hentolamine** blocks α1 and α2 receptors, while $\beta$ receptors are not affected. P**ropranolol** has higher affinity to $\beta 1$ than to $\beta 2$ receptors, but small effects on $\beta 2$ receptors can be visible in experiments on the aorta when propanolol is given after epinephrine. This finding invites discussions concerning the possible side effects of giving propanolol as a "cardioselective" $\beta$-blocker. For comparison, the effects of the blocker **Verapamil**, a non-competitive inhibitor of $Ca^{++}$ uptake via cation channels, can be studied.

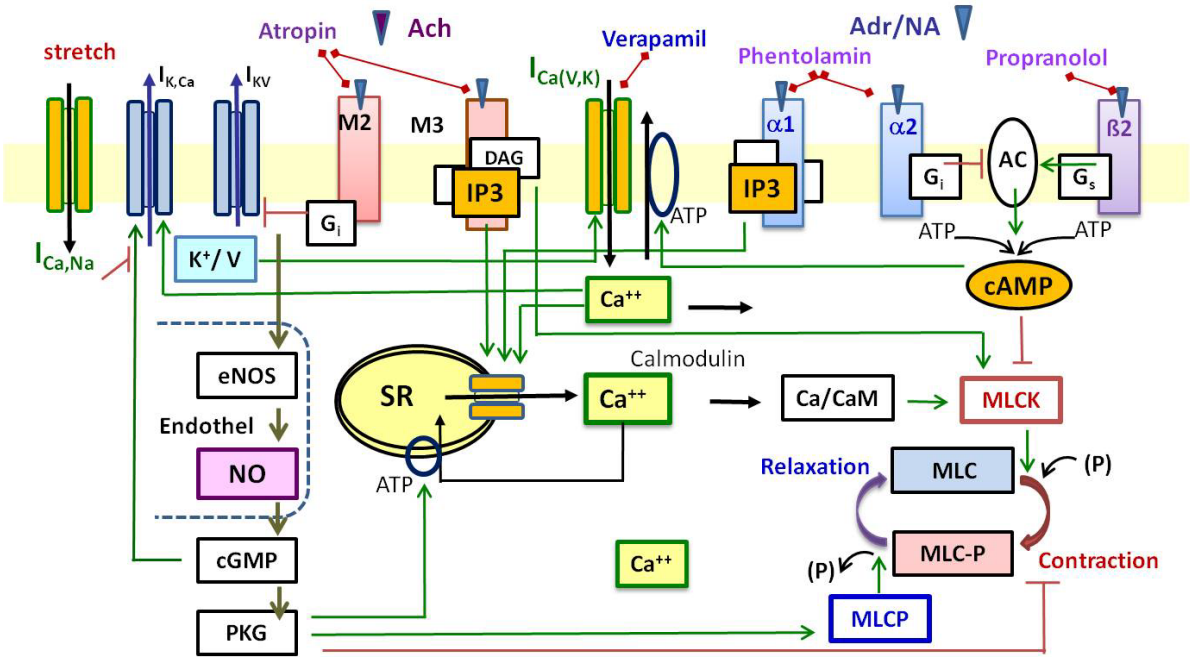



**Figure 3:** Schematic drawing showing signaling pathways in smooth muscle. Details see text.

## 3. Suggestions for Experiments

### 3.1 Differences of smooth muscle preparations: 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.

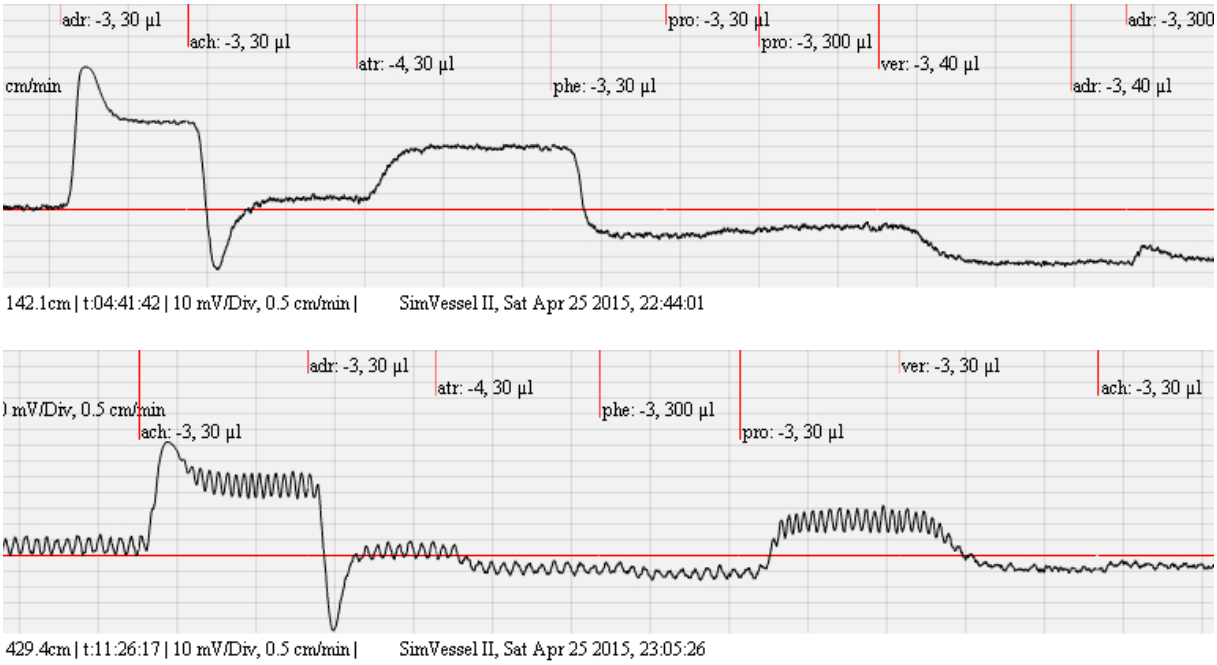



**Figure 4:** Example recordings from the aorta (upper trace) and the antrum (lower trace) in the virtual SimVessel lab.

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 Adrenalin 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

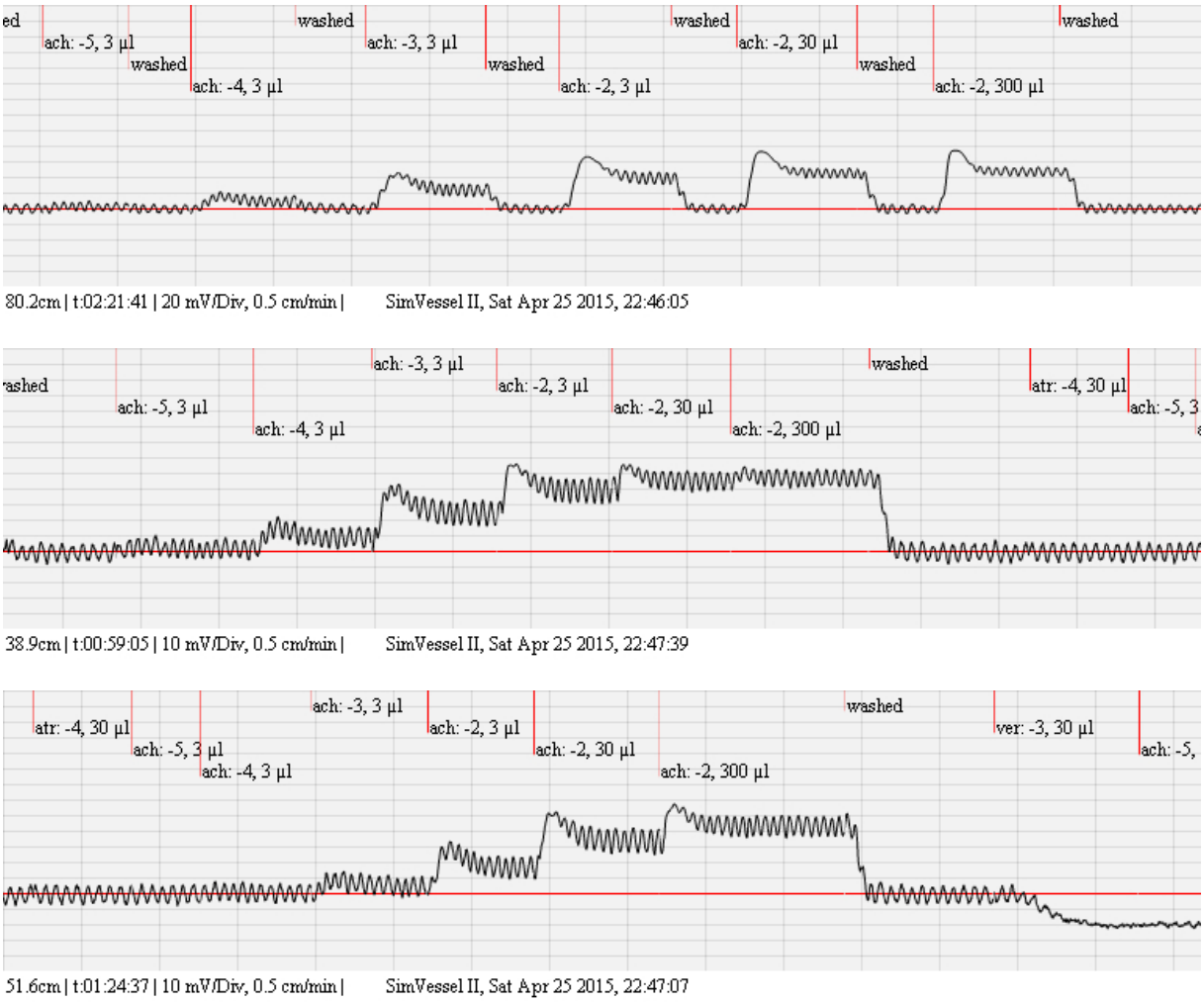



**Figure 5:** Examples of dose-response curve of Acetylcholine effects on the Antrum.

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

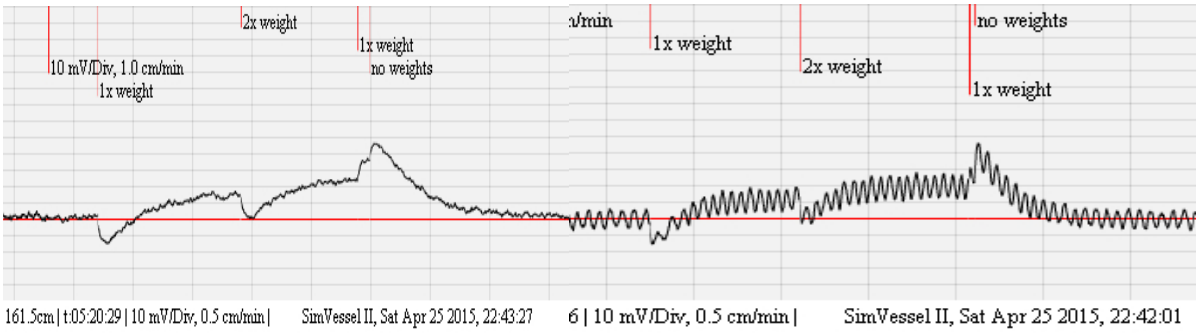



**Figure 6:** Bayliss Effect