Diploma Thesis

Hormonal Changes during Orthostatic Challenge in Patients with Stroke

submitted by

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Graz, June 14, 2017

Declaration

I hereby declare that I have authored this thesis independently, that I have not used other than the declared sources / resources, and that I have explicitly marked all material that have been quoted either literally or by content from the used sources.

Graz, June 14, 2017 ***Thomas Niehaus eh***

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Abstract

Background: Orthostatic intolerance is a common complication in patients with stroke. Frequent falls are the result, which often leads to severe injuries and consequently, longer periods of immobilization and hospitalization. Orthostatic intolerance can arise when there are inadequate neuro-hormonal responses to an orthostatic challenge. Therefore, it is important to assess cardiovascular, autonomic and hormonal responses to orthostatic challenge in patients with stroke. It has previously been shown that hormonal responses such as those of the novel neuro-peptide adrenomedullin increase due to orthostatic challenge in a stimulus-dependent manner, but changes in the hormone galanin only occur at the end point of cardiovascular stability leading to orthostatic intolerance.

Aims: The objective of this study was to investigate the effects of an orthostatic challenge on adrenomedullin and galanin responses in patients with a history of ischemic stroke and to see how they compared with age-matched healthy controls. We hypothesized that i) *baseline adrenomedullin and galanin levels in stroke patients are higher than in healthy controls;* ii) *adrenomedullin and galanin levels increase due to orthostatic challenge;* and iii) *adrenomedullin and galanin responses during an orthostatic challenge differ in stroke versus healthy controls.*

Methods: Thirteen patients with a history of mild ischemic stroke (NIHSS ≤ 3) and eighteen age-matched healthy controls performed a sit-to-stand test (five minutes sitting and six minutes standing period). Venous blood was sampled prior to - and at the end of - standing.

Results: At baseline, no significant differences in plasma adrenomedullin and galanin concentrations between the two groups were seen. Furthermore, changes in posture from sitting to standing did not induce a significant change in hormonal concentrations across both groups.

Conclusions: In patients with a history of mild ischemic stroke up to one year, the baseline status of adrenomedullin and galanin was similar to healthy patients. Additionally, mild orthostatic challenge did not influence plasma adrenomedullin and galanin concentrations in patients with histories of ischemic stroke and healthy controls. Our results differ from previous observations regarding these hormones in stroke patients as we used patients with histories of stroke of up to one year.

Zusammenfassung

Hintergrund: Orthostatische Intoleranz ist als Komplikation bei Schlaganfallpatienten weit verbreitet. Als Folge davon kommt es häufig zu Stürzen, welche wiederum oft zu schweren Verletzungen und damit zu längeren Immobilisationen und Hospitalisationen führen. Orthostatische Intoleranz tritt auf, wenn die neuro-hormonelle Antwort des Körpers auf einen orthostatischen Reiz nicht angemessen ausfällt. Aus diesem Grund ist es wichtig, kardiovaskuläre, vegetative und hormonelle Reaktionen von Schlaganfallpatienten auf orthostatische Reize zu untersuchen. Frühere Studien haben gezeigt, dass die Hormonkonzentrationen des Neuropeptids Adrenomedullin im Plasma durch orthostatische Reizung in Abhängigkeit von der Reizintensität ansteigen. Demgegenüber traten Änderungen des Hormons Galanin erst bei Erreichung eines Endpunktes kardiovaskulärer Stabilität und damit verbundener orthostatischer Intoleranz auf.

Zielsetzung: Ziel dieser Studie war, den Einfluss einer orthostatischen Reizung auf die Hormone Adrenomedullin und Galanin im Plasma von Schlaganfallpatienten zu untersuchen und herauszufinden, wie sich die Hormonantwort von der gesunder, altersangepasster Individuen unterscheidet. Wir gehen davon aus, dass *i) die Basiskonzentration von Adrenomedullin und Galanin bei Schlaganfallpatienten höher ist, als bei den Kontrollpersonen; ii) die Adrenomedullin- und Galaninkonzentrationen durch orthostatische Reizung steigen; iii) sich die Reaktionen der beiden Hormone auf die orthostatische Reizung zwischen Schlaganfallpatienten und gesunden Kontrollpersonen unterscheiden.*

Methoden: Dreizehn Patienten, die innerhalb des vergangenen Jahres einen leichten, ischämischen Schlaganfall (NIHSS $\leq$ 3) erlitten, sowie achtzehn altersangepasste, gesunde Kontrollpersonen unterzogen sich einem Sit-to-stand Test (fünf Minuten sitzen, sechs Minuten stehen). Am Ende der Ruhephase und am Ende der Standphase wurde venöses Blut abgenommen.

Ergebnisse: Es konnte kein signifikanter Unterschied zwischen den Basiskonzentrationen von Adrenomedullin und Galanin im Plasma der Schlaganfallpatienten und der Kontrollgruppe erkannt werden. Außerdem bewirkte die Lageänderung in beiden Gruppen keine signifikanten Änderungen der Hormonkonzentrationen.

Schlussfolgerung: Patienten, die innerhalb des vergangenen Jahres einen leichten ischämischen Schlaganfall erlitten, zeigten eine ähnliche Adrenomedullin- und Galanin-Basiskonzentration wie gesunde Kontrollpersonen. Außerdem hatte leichte orthostatische Reizung in beiden Gruppen keinen Einfluss auf die Plasmakonzentrationen der beiden Hormone. Unsere Ergebnisse unterscheiden sich von den Ergebnissen früherer Untersuchungen dieser Hormone bei Schlaganfallpatienten, da der Schlaganfall unserer Patienten bis zu einem Jahr in der Vergangenheit lag.

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Abbreviations

ACE	Angiotensin Converting Enzyme
Ach	Acetylcholine
ADH	Antidiuretic Hormone
ADM	Adrenomedullin
ANP	Atrial Natriuretic Peptide
ANS	Autonomic Nervous System
AV	Atriovetricular
Bpm	Beats per Minute
CGRP	Calcitonin gene-related Peptide
CNS	Central Nervous System
CO	Cardiac Output
CV	Cardiovascular
CVP	Central Venous Pressure
F	Flow
GAL	Galanin
GFR	Glomerular Filtration Rate
HR	Heart Rate
HUT	Head-up Tilt
LBNP	Lower Body Negative Pressure
MAP	Mean Arterial Pressure
MRI	Magnetic Resonance Imaging
mRS	Modified Rankin Scale
NE	Norepinephrine
NO	Nitric Oxide
NIHSS	National Institutes of Health Stroke Scale
NTS	Nucleus Tractus Solitarius
OH	Orthostatic Hypotension
OI	Orthostatic Intolerance
P	Pressure
pCO_2	CO_2 Partial Pressure
PNS	Peripheral Nervous System
pO_2	O_2 Partial Pressure

R	Resistance
RAAS	Renin-Angiotensin-Aldosterone-System
SD	Standard Deviation
SNS	Sympathetic Nervous System
SV	Stroke Volume
TIA	Transient Ischemic Attack
TPR	Total Peripheral Resistance
VVS	Vasovagal Syncope

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1 Introduction

1.1 Overview of the Circulatory System

The cardiac cycle has several functions. The primary role is the transport of oxygen and nutrients such as glucose to the cells. In return it removes metabolic waste such as carbon dioxide from the cells.

Second, it regulates the volume and composition of extracellular fluid. Furthermore the circulatory system organizes hormonal control of the organism by transporting the hormones to their destinations.

Another function is to provide the immune defense by circulating white cells and immunoglobulins in the blood.

Last but not least the circulation plays an important role in the regulation of body temperature (1).

1.1.1 Components

The circulatory system consists of the heart and a series of interconnected blood vessels. From the right side of the heart blood is pumped through the lungs (pulmonary circulation) to the left side of the heart. Thence blood is pumped to the rest of the body (systemic circulation). Finally, blood returns via the superior and inferior venae cavae to the right heart (1).

An overview of the circulatory system is given in Figure 1.

1.1.1.1 Heart

The heart is a muscular organ that is coated by a protective fibrous sac, the pericardium. It is located in the mediastinum of the thoracic cavity (2).

It consists of four muscular chambers: two atria and two ventricles. Atria and ventricles are arranged in pairs so that each half of the heart forms a functionally separate pump. In an adult heart, the two halves are completely separated by the septum. The septum is divided into the atrial septum and the interventricular septum. The atrial septum is thin and of fibrous tissue, the interventricular septum is thicker and largely consists of muscle tissue.

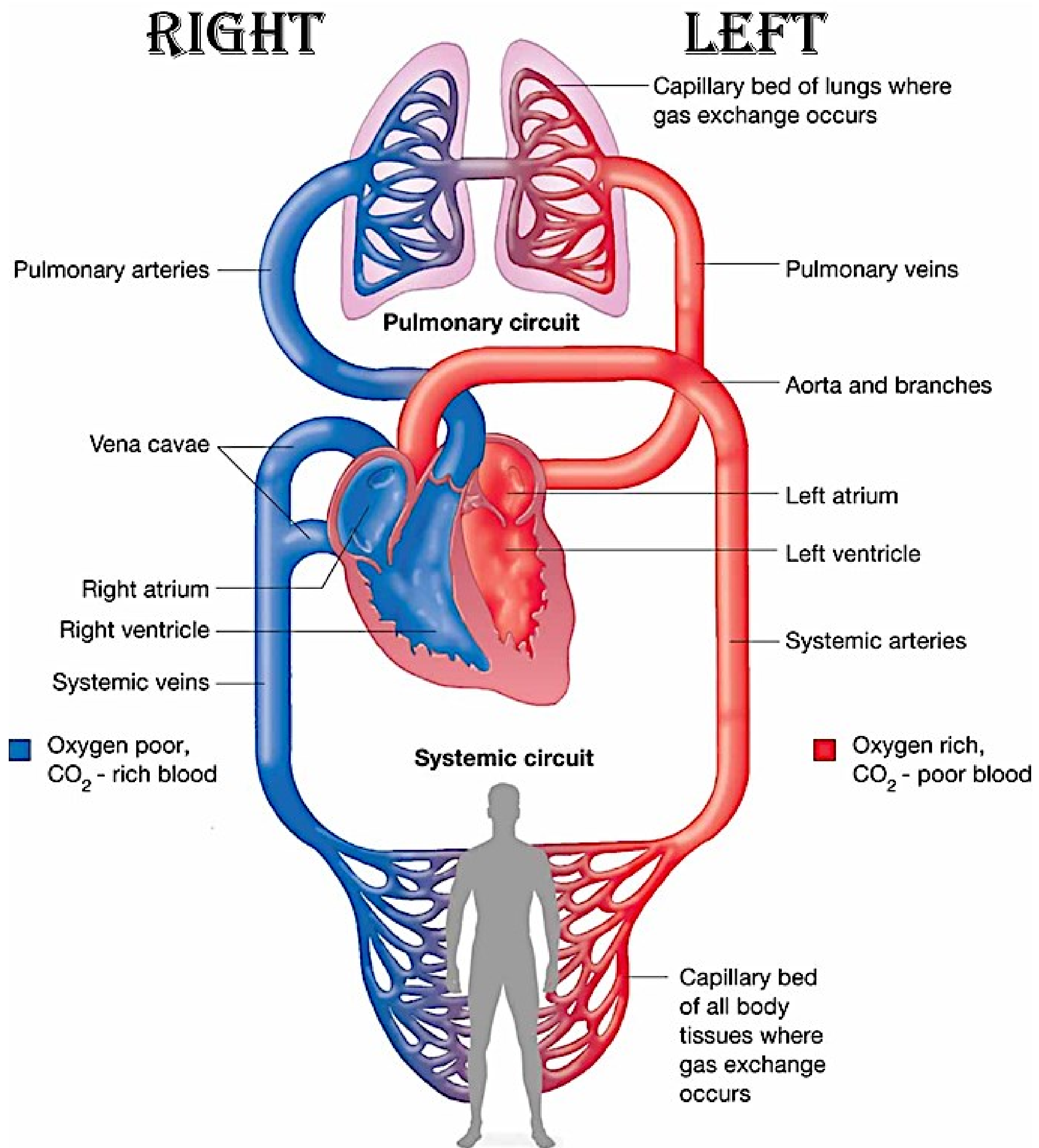

Figure 1: The systemic and pulmonary circulation

(Obtained from: https://classconnection.s3.amazonaws.com/437/flashcards/1106437/png/circulation_21328809825509.png)

The septum prevents mixing of oxygenated and desoxygenated blood.

The atria have a thin muscle wall. They receive blood from the large veins (right side: venae cavae; left side: pulmonary veins) and deliver it to the ventricles.

The ventricles have a thicker muscle wall. Particularly the left ventricle has the thickest wall.

Atria and ventricles are separated by atrioventricular (AV) valves, to ensure that the blood flows from the atria to the ventricles and to prevent reflux into the atria when the ventricles contract. The right sided AV valve is called tricuspid valve because it consists of three triangular flaps of fibrous tissue. The left sided AV valve is called bicuspid valve (also known as mitral valve) because it consists of two flaps.

To ensure that the blood flows from the ventricles to the arteries without any backflow, ventricles and arteries are separated by semilunar valves. On the right side the pulmonary valve isolates the right ventricle from the pulmonary trunk. On the left side the aortic valve isolates the left ventricle from the aorta (1). It is important to know that all valves open and close passively as a consequence of pressure gradient across the valves (2).

1.1.1.2 Vascular System

Generally speaking, the vascular system consists of arteries that carry blood away from the heart, and veins that carry blood to the heart. As the blood leaves the heart, it first passes through a series of *arteries* that become progressively smaller. To reach different tissues the blood passes through *arterioles* and finally through *capillaries*. From the capillaries blood flows through *venules* and enters the *veins* that become progressively bigger. Finally the veins drain into in the superior and inferior venae cavae that return the blood to the right atrium (1).

The different types of blood vessels have different structural and functional characteristics.

Arteries can be subdivided into two groups.

The *elastic arteries* are the largest vessels in human's body (aorta and pulmonary arteries, major branches of these) with a diameter of 1 - 2.5 cm.

Characteristic for elastic arteries is the high distensibility because they have a lot of elastic tissues in their walls. This feature prevents abrupt changes in blood pressure because energy is stored in the walls during the systole and delivered during the diastole (pressure reservoir function).

The *muscular arteries* are smaller. Their diameter ranges from 1 - 10 mm. Whereas elastic artery walls consist of 40% elastin, muscular artery walls only

contain about 10%. Therefore they contain a higher proportion of smooth muscle, which helps not to collapse at sharp edges and corners, for instance at articulations.

Arterioles have a diameter from 30 - 300 μm and act as resistance vessels. They regulate blood flow through particular tissues and organs and thereby take part at the regulation of arterial blood pressure (see subsection 1.1.4) (3).

Capillaries are the major sites of nutrient, metabolic waste, and fluid exchange between blood and tissues (2). They have a maximum length of 1mm and a diameter of about 5 - 8 μm. Depending on the innermost layer of the wall (the endothelium), capillaries are subdivided into three types:

1. Continuous capillaries: most common, continuous endothelium with small clefts.
2. Fenestrated capillaries: perforated endothelium with circular pores, especially in areas of fluid and salt exchange such as kidneys.
3. Discontinuous capillaries: gaps between endothelial cells, that allow passage of blood cells and plasma proteins, especially in liver, spleen and bone marrow (1).

Postcapillary venules are about 20 μm in diameter and still take part in capillary exchange whereas **venules** (up to 200 μm in diameter) do not. The venules give rise to the **veins** with a diameter of 0.5 - 3 cm. The walls of the veins are very distensible. Therefore they are an excellent blood reservoir that built more than 50% of the total storage capacity of the circulatory system (3).

Except for the capillaries and the postcapillary venules, the walls of the vessels have three layers. Starting from the inside: the tunica intima, the tunica media, and the tunica adventitia (see Figure 2).

Tunica intima consists of a layer of flat endothelial cells, which are in direct contact with the blood. The endothelial cells are overlying a thin basal lamina. In the arteries, the arterioles, larger venules and veins the tunica intima is separated from the Tunica media by the internal elastic lamina. In limb veins the intima forms venous valves at intervals to prevent retrograde flow.

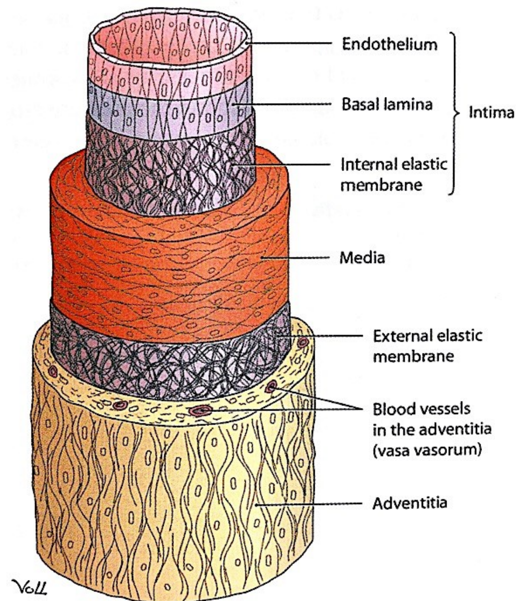

Figure 2: Different layers of an artery
(obtained from (3))

Tunica media consists of concentric layers of smooth muscle cells. Being interspersed with elastin and collagen fibres, the tunica media is responsible for the mechanical strength of the blood vessels. Sympathetic nerve fibres innervate the smooth muscles. It is important to remember, that there is no tunica media in capillaries and postcapillary venules.
In the arteries the tunica media is separated from the tunica adventitia by the external elastic lamina.
Tunica adventitia is formed by elastic and collagenous fibres in a loose connection. Its major task is to fix the vessels in place. In capillaries and postcapillary venules it is responsible for the mechanical strength (1).

1.1.2 Function of the Circulatory System

1.1.2.1 Systemic and Pulmonary Circulation

Based on its anatomy the circulation can be divided into two main circuits: The systemic (high pressure) and the pulmonary (low pressure) circulation (4).
In the *systemic circuit*, blood leaves the left ventricle via the ascending aorta. Around the aortic arch arteries for the upper part of the body - such as head, neck,

brain, and upper limbs - branch off the aorta. Afterwards the aorta descends to supply the lower part of the body, such as the organs of the abdomen, and the lower limbs. Blood from the upper part of the body returns to the right atria via the vena cava superior, whereas blood from the lower part of the body returns to the right atria via the vena cava inferior (1).

In the *pulmonary circuit,* blood leaves the right ventricle via the pulmonary trunk. Pulmonary trunk divides into the two pulmonary arteries, one supplying the right lung (three lobes) and one supplying the left lung (two lobes). Blood takes the well-known way via arterioles, capillaries and venules until it leaves the lungs via four pulmonary veins that lead into the left atrium.

As blood flows through the lung capillaries, it picks up oxygen from the alveoli and delivers carbon dioxide to the alveoli. Therefore, blood flowing through the pulmonary veins, the left side of the heart and the systemic arteries is called oxygenated blood. As this blood flows through the capillaries of the systemic circuit, oxygen leaves the blood and enters the cells. In return the cells deliver carbon dioxide to the blood (2).

1.1.2.2 Events of the Cardiac Cycle

The cardiac cycle is divided into two major phases: the *systole* and the *diastole* (Figure 3).

Systole is the period of ventricular contraction and blood ejection. The first part of systole is called *isovolumetric ventricular contraction.* In this period all valves are closed while the ventricles are contracting. As there is no blood movement in this phase, ventricular blood pressure increases. At the point when ventricular blood pressure reaches the level of pressure in the aorta and pulmonary trunk, the semilunar valves open. At this moment the period of *ventricular ejection* - the second part of systole - occurs. As long as the contracting ventricular muscle fibres shorten, blood is squeezed into the aorta and pulmonary trunk. The volume of blood ejected from the ventricles during systole is called *stroke volume* (SV) (2).

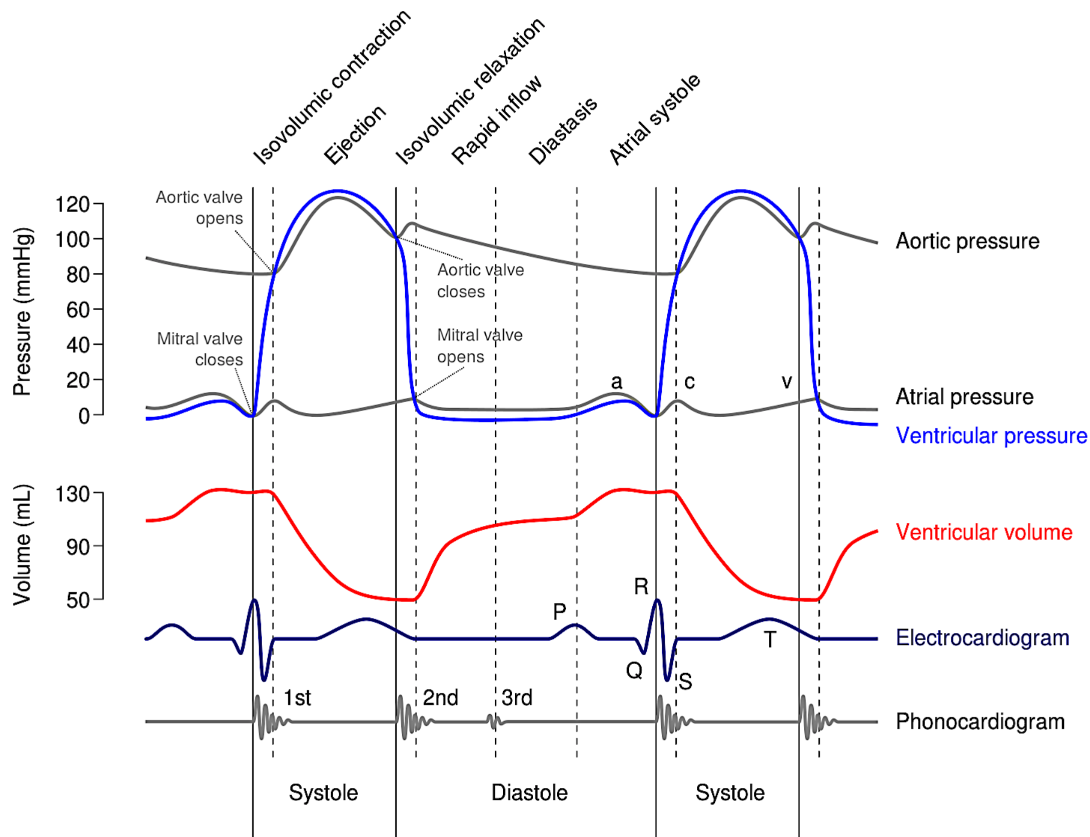

Figure 3: The cardiac cycle

(obtained from: https://commons.wikimedia.org/wiki/File:Wiggers_Diagram.svg)

In the **Diastole** the ventricles relax and are filled with blood. The first part of diastole is called *isovolumetric ventricular relaxation.* In this period the ventricles begin to relax and the semilunar valves close because of the higher pressure in aorta and pulmonary trunk. At the same time the AV valves are still closed because ventricular blood pressure is still higher than atrial blood pressure. Therefore ventricular volume does not change in this period.

As relaxation of the ventricles goes on, the ventricular blood pressure decreases under the level of atrial blood pressure. Thus, the AV valves open and blood flows from the atria to the ventricles. This period is called *ventricular filling* - the second part of diastole. About 80% of ventricular filling happens passively. Only the last 20% of blood flows to the ventricles via atrial contraction.

It is worth mentioning that only during the diastole the smooth muscle cells of the heart can be supplied with oxygenated blood via the coronary arteries.

For a normal heart rate of 72 beats per minute (bpm), each cardiac cycle takes about 0.8 seconds. In this case systole takes 0.3 seconds and diastole takes 0.5 seconds (2).
As described, the heart pumps blood intermittently into the arteries. Therefore, as a function of the cardiac cycle the pressure in the arteries varies. At the peak of ejection, pressure is named systolic pressure; at its lowest point during ventricular relaxation pressure is named diastolic pressure (1).
Typical pulmonary arterial pressures are 25 mmHg (systolic) to 10 mmHg (diastolic) compared to systemic arterial pressures of 120 mmHg to 80 mmHg (2).

1.1.3 Hemodynamics: Pressure, Flow, Resistance

The major function of the cardiovascular (CV) system is to guarantee sufficient blood flow in the capillaries dependent on tissue needs (2).
To understand the regulation of the circulation, it is necessary to understand the rough determinants of hemodynamics.
Blood flow (F) means the amount of blood passing a specific point in the circulation in a specific period of time. It is driven by two factors:

1. Pressure differences (ΔP) between the arteries and veins (perfusion pressure).
2. Vascular resistance (R)

Therefore the flow can be calculated in the following equation.

$$F = \frac{\Delta P}{R} \quad \text{(Ohm´s law)}$$

This states that the blood flow is direct proportional to the pressure differences, but inversely proportional to the vascular resistance (5).
Thus, blood flow increases when either perfusion pressure increases or vascular resistance decreases. This relationship is true for an individual vascular bed as well as to the whole circulation (1).
Pressure difference (ΔP) means the difference between the mean arterial pressure (MAP) and the central venous pressure (CVP).

$$\Delta P = MAP - CVP$$

However, the CVP is physiologically close to zero and does not change significantly during the cardiac cycle. Therefore, ΔP is nearly equal to the MAP (1).
Vascular resistance (R) means the impediment to blood flow through the vessels. It is determined by the viscosity (η) of the blood, the length (ι) of the vessel and the fourth power of the radius (r) of the vessel.

$$R = \frac{8\eta\iota}{\pi r^4} \text{ (Poiseuille´s law)}$$

One example to illustrate the significance of this formula: a reduction of the vessel radius by half enhances the resistance to blood flow 16-fold.
Cardiac output (CO) is the amount of blood each ventricle pumps in one minute, means it is the sum of the blood that flows to all tissues of the body (5).
It is the product of the stroke volume (SV) - the volume ejected by each ventricle with each beat - times the heart rate (HR) - the number of beats per minute (2).

$$CO = SV \text{ x } HR$$

Therefore again Ohm´s law shows the relationship between pressure, flow and resistance. As we talk about the sum of blood flow, we have to consider the total peripheral resistance (TPR) as it is the sum of all the vascular resistances within the circulation.

$$CO = \frac{MAP}{TPR}$$

To ensure that every tissue and cell receives what it needs, the cardiovascular system regulates the pressure present in the arteries and then apportions flow between various tissues by varrying the flow resistence (3).

1.1.4 Regulation of the Cardiovascular System

Control of the heart and the vessels is provided by both, humoral and neuronal mechanisms. Both are essential for the cardiovascular homeostasis (3). The neurohumoral mechanisms act through changes in systemic vascular resistance, in cardiac function and in blood volume (6).

1.1.4.1 Neuronal Regulation of the Cardiovascular System

The autonomic nervous system (ANS) is the part of the nervous system that controls most visceral functions of the body. ANS operates unconsciously, means that it is not under voluntary control. It works very fast, for instance it is able to double the heart rate within 3 to 5 seconds (5). Therefore, the ANS is part of the short term regulation of the circulation. An overview of the ANS gives Figure 4.

The ANS operates as a series of negative feedback loops, composed of the following components:

1. **A sensor** that measures the controlled variable and transduces it into an electrical signal
2. **Afferent neural pathways** that transport the sensor signals to the central nervous system (CNS)
3. **A coordinating centre** that compares the incoming signal to a setpoint and coordinates the appropriate response
4. **Efferent neural pathways** that transport the response from the coordinating centre to the periphery
5. **Effectors** that realize the response and adjust the controlled variable (4)

At first I would like to focus on the efferent pathways as they are made up of the two opponents: sympathetic and parasympathetic nervous system.

The sympathetic nervous system (SNS) originates from the spinal cord between the segments T1 and L2/3. In general, each sympathetic pathway on its way from the spinal cord to the target organ is composed of two neurons that synapse in a sympathetic ganglion. The neuron originating from the spinal cord is the *preganglionic neuron*; the neuron to the stimulated tissue is the *postganglionic neuron* (3). Most of the sympathetic ganglia are located on both sides close to the

vertebral column (paravertebral ganglia). They are longitudinally interconnected by bundles of nerve fibres to form the two sympathetic trunks (1).

When a preganglionic neuron reaches a ganglion, there are three possible ways to go on:

1. The preganglionic neuron can synapse with the postganglionic neuron
2. It can pass upwards or downwards in the sympathetic trunk and then synapse with a postganglionic neuron in another ganglion
3. It can pass upwards or downwards in the sympathetic trunk and then leave the trunk via radiating sympathetic nerves to synapse in a peripheral sympathetic ganglion.

Therefore, the postganglionic neuron either has its source in one of the sympathetic trunk ganglia or in one of the peripheral ganglia (5).

The parasympathetic nervous system (PNS) is also composed of preganglionic and postganglionic neurons. The preganglionic fibres arise from two different regions of the central nervous system.

In the *cranial part* of the PNS the preganglionic fibres leave the CNS through the cranial nerves III (N. oculomotorius), VII (N. facialis), IX (N. glossopharyngeus), and X (N. vagus). Thus, the origin of the cranial PNS is located in the corresponding nuclei of the cranial nerves in the brain stem.

The preganglionic fibres of the *sacral part* are part of the pelvic nerves that leave the spinal cord between S2 to S4.

In contrast to the SNS, the preganglionic neurons of the PNS usually travel all the way to the target tissues. The parasympathetic ganglia are located very close to or even embedded in the target organ where pre- and postganglionic neurons synapse.

The vagus nerves (tenth cranial nerve) transport about 75 % of all parasympathetic nerve fibres. Target tissues of the vagus nerves are located in the thoracic and abdominal region of the body (3,5).

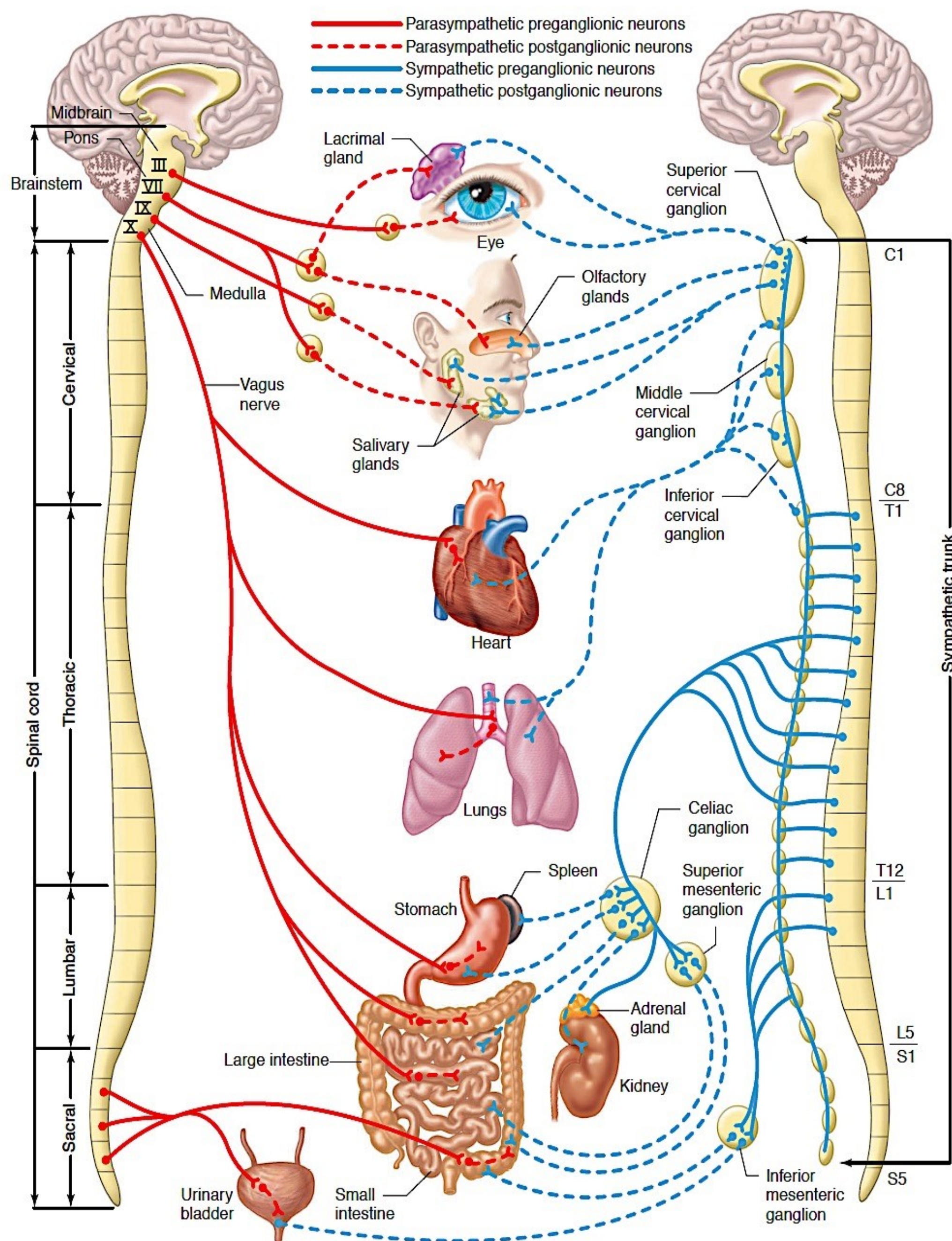

Figure 4: Overview of the autonomic nervous system
(obtained from (2))

Neurotransmitters of the autonomic nervous system are acetylcholine (ACh) and norepinephrine (NE). Fibres that secrete acetylcholine are called *cholinergic*; fibres that secrete norepinephrine are called *adrenergic* (adrenalin is another name for epinephrine).

Figure 5 shows, that all preganglionic fibres, both sympathetic and parasympathetic, are cholinergic. This means, that ACh - which was released by the preganglionic fibres into the synaptic cleft - excites all postganglionic fibres of the PNS and the SNS.

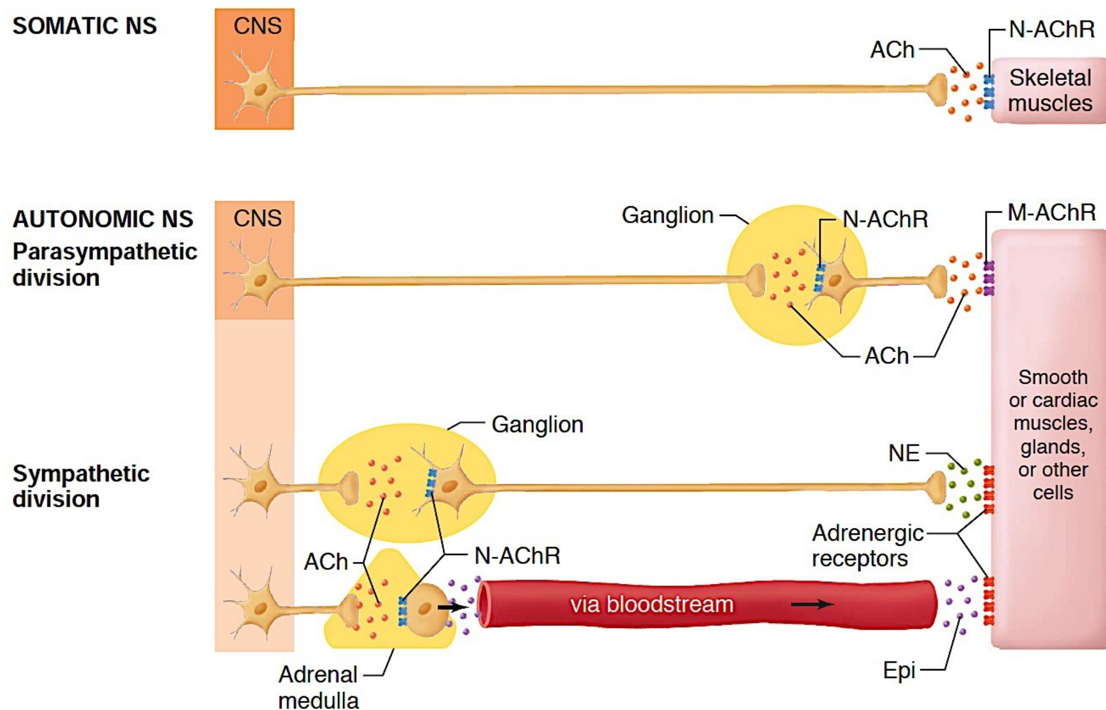

Figure 5: Neurotransmitters of the autonomic nervous system
(obtained from (2))

Furthermore, almost all of the postganglionic parasympathetic neurons are cholinergic. Therefore, it is as well ACh that excites the target tissues at the terminal nerve endings of parasympathetic fibres.
While all postganglionic parasympathetic neurons are cholinergic, most of the postganglionic sympathetic neurons are adrenergic. This means, that they secrete norepinephrine at their nerve endings in order to excite the target tissues. There are only few exceptions regarding the sympathetic neurotransmitters. Postganglionic sympathetic neurons that innervate the sweat glands or the piloerector muscles of the hairs release ACh into the synaptic cleft. Thus they are cholinergic (5).

In order to stimulate a target organ or to transmit a signal, the neurotransmitter needs to bind on a specific receptor.

ACh primarily activates two different types of receptors: *Muscarinic* and *nicotinic* receptors.

Muscarinic receptors are found on all target tissues that are innervated by the postganglionic cholinergic neurons. They are divided into three subclasses: M_1, M_2, M_3. However, only M_2 receptors are relevant for cardiovascular regulation because they are located on the heart.

Nicotinic receptors are acting in the synapses between pre- and postganglionic neurons of both the PNS and SNS.

Norepinephrin binds on two major types of **adrenergic receptors**:

alpha (subclasses: $alpha_1$, $alpha_2$) and *beta* (subclasses: $beta_1$, $beta_2$, $beta_3$) receptors. However NE has a higher affinity to the alpha receptors (5).

The below mentioned locations of adrenergic receptors are limited on the relevant organs for the cardiovascular regulation.

$alpha_1$ and $alpha_2$ receptors are located on smooth muscle cells of the blood vessels. Activation of these receptors leads to vasoconstriction.

$beta_1$ receptors can be found in the heart. Activation of this receptor results in an increased heart rate (chronotropic) and an increased force of contraction (inotropic).

$beta_2$ receptors are located in the smooth muscles of blood vessels, especially coronary arteries. Contrary to the alpha receptors, activation of $beta_2$ receptors results in vasodilation.

$beta_3$ receptors are irrelevant for the CV regulation (1).

In consequence of the receptor locations, the PNS only has an influence on the innervation of the heart. Exceptions are the vessels that supply pelvic organs. By way of example parasympathetic innervation of genitalia erectile tissue leads to vasodilation. As the adrenergic receptors are not only situated on the heart but also on the blood vessels, the SNS is able to innervate both.

As mentioned before, the ANS includes afferent pathways for sensory input and a modulation of this information in higher centres of the CNS.

Two types of receptors deliver afferent information about the pressures and fillings within the circulation: *baroreceptors* and *volume receptors* (1).

Baroreceptors send information about the conditions in the high-pressure circulation, such as the arterial blood pressure. They can be found in the walls of arteries, especially above the carotid bifurcation (carotid sinus) and in the walls of the aortic arch (Figure 6).

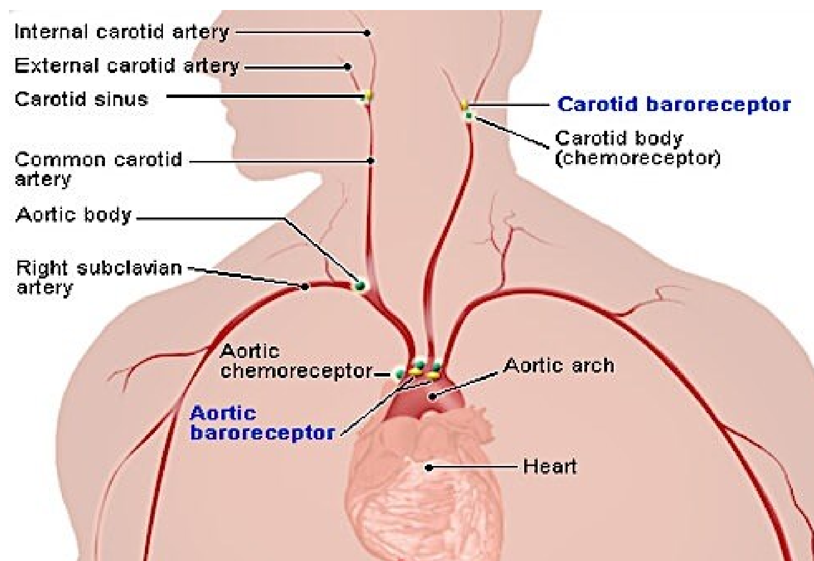

Figure 6: Locations of arterial baroreceptors
(obtained from:
http://www.medicinenet.com/image-collection/baroreceptors_picture/picture.htm)

Volume receptors supply information about the conditions in the low-pressure circulation, such as the cardiac filling pressure/the central venous pressure. They are located in the walls of the atria and ventricles.
Both receptor types are mechanoreceptors. Therefore, they are stimulated when stretched. This effects impulses from the receptors to the vasomotor centre (1).
In addition, the sensory input information are affected by information from **chemoreceptors**, that monitor arterial blood PO_2, PCO_2 and pH. Therefore, respiration and arterial blood gases are also involved in the nerval regulation of the circulation.
The vasomotor centre can be found in the lower third of the pons and in the medulla oblongata. The part of the centre that receives sensory nerve signals is called *sensory area.* The sensory area is situated bilaterally in the nucleus tractus solitarius (NTS) (5).

Afferent pathways from the aortic arch baroreceptors reach the NTS via the vagus nerves, those from the carotid sinus baroreceptors travel with the glossopharyngeal nerves to the NTS (1).

As the vasomotor centre receives information about the conditions in the circulation, it regulates the activity of the SNS and PNS and its influence on the heart and vasculature.

Besides the sensory area the vasomotor centre is organized in a *vasoconstrictor area* and a *vasodilator area.* The vasoconstrictor area excites preganglionic vasoconstrictor neurons of the SNS, whereas the vasodilator area inhibits the vasoconstrictor area.

When there is a need to increase heart rate and contractility, the vasomotor centre transmits stimulating impulses through the sympathetic nerve fibres to the heart. Conversely, when there is a need to decrease heart rate and contractility, the vasomotor centre transmits inhibiting impulses through the parasympathetic fibres of the vagus nerves to the heart.

The influence of the SNS on the heart and the vessels results in the capability to cause very fast changes in arterial pressure. To initiate a rapid increase the vasoconstrictor and the cardioaccelerator function of the SNS are stimulated together, whereas the inhibitory signals of the PNS on the heart are inhibited (5).

Cardioaccelarator function means, that sympathetic stimulation increases chronotropy, dromotropy and inotropy of the heart.

In the vessels, sympathetic stimulation constricts small arteries and arterioles (resistance vessels) as well as venules and veins (capacitance vessels). This increases the TPR and decreases the venous capacitance (increases the venous pressure).

The increased arterial blood pressure thus results from an increase in cardiac output and an increase in systemic vascular resistance (6).

1.1.4.2 Local Regulation of the Cardiovascular System

Besides the autonomic nervous system, paracrine factors play an important role in the short term regulation of the circulation. These factors only cause local circulatory effects (5).

Nitric oxide (NO) is released from the endothelial cells of the vessels as a reaction to physical and chemical stimulation. NO causes vasodilation in those tissues, where it is released.
Physical stimulation comprises shear stress on the endothelial cells through increased blood flow. By way of example, luminal narrowing of an artery or increased blood flow in coronary arteries due to exercise stimulates the release of NO and results in vasodilation.
Chemical stimulation of the endothelial cells comprises a variety of factors. Among other things, vasoconstrictor substances such as norepinephrine stimulate the release of NO. Thus, NO lowers the effect of vasoconstrictors (5,6).

Endothelin is also released by endothelial cells. Contrary to NO, Endothelin is a powerful vasoconstrictor that is particularly released when the endothelium is damaged in order to prevent severe haemorrhage (5).

1.1.4.3 Hormonal Regulation of the Cardiovascular System

At this point I would like to give a short overview of the most important endocrine factors. At a subsequent point of this subsection I will focus on the two hormones Adrenomedullin and Galanin, which seem to play an important role in the cardiovascular regulation and which are part of our investigations in the study.
As described above, an acute required increase of the mean arterial pressure is realized by the SNS, as it rises the heart rate, the stroke volume and the total peripheral resistance. However, in the event of hemorrhage or longer lasting physical strain the blood volume and simultaneously the venous return to the heart decreases. A solitary increased sympathetic innervation would not help in this situation, as there is not enough blood in the system to be pumped.
Therefore, the long term regulation is very important. Long term control of the circulation is closely connected with the homeostasis of body fluid volume. An increased body fluid volume increases the blood volume, which increases the cardiac output and therefore increases the arterial pressure. Keeping the excretion of salt and water according to the intake of salt and water in balance is one of the most important functions of the kidneys (5). Among other factors, the kidneys are controlled by various hormones.

Indeed, there are also hormones involved in the short term or in both, short and long term regulation.

Epinephrine and Norepinephrine

Besides the already described direct effects of the SNS neurotransmitter norepinephrine on heart and vessels, sympathetic stimulation owed to stress or exercise causes the adrenal medulla to secrete epinephrine and norepinephrine into the systemic circulation (5). Both hormones bind on the earlier described *alpha* and *beta* adrenergic receptors and effect similar results as direct sympathetic stimulation. Because norepinephrine has a higher attraction to alpha receptors it provokes vasoconstriction in most vessels. Epinephrine has a greater attraction to beta receptors. In addition to the cardioaccelarator function, binding on those receptors provokes a vasodilation in specific vessels, such as the coronary arteries or the vessels in skeletal muscle. Therefore, the blood is redistributed to those areas of the body that need more blood during exercise or stress (1).

Vasopressin is also called antidiuretic hormone (ADH). As these names indicate, the hormone has two major functions: vasoconstriction and water reabsorption. Vasopressin is secreted by the posterior pituitary gland into the blood as a reaction to decreased blood pressure, for instance due to haemorrhage or dehydration. As a short term reaction, vasopressin induces powerful constriction in several tissues, particular in the visceral circulation and therefore increases the TPR. At the same time vasopressin effects a dilation of cerebral and coronary arteries. This helps to raise the arterial blood pressure and to redistribute the blood to the vitally important organs (1).

However, it also has an effect on the kidneys and the regulation of the body fluid volume, as seen in Figure 7.

ADH stimulates the insertion of aquaporin water channels into the collecting ducts of the kidneys. This results in an increased diffusion of water through the collecting duct cells into the interstitial fluid and further into the blood. Finally, under the influence of ADH the kidneys produce a small volume of high concentrated urine (2).

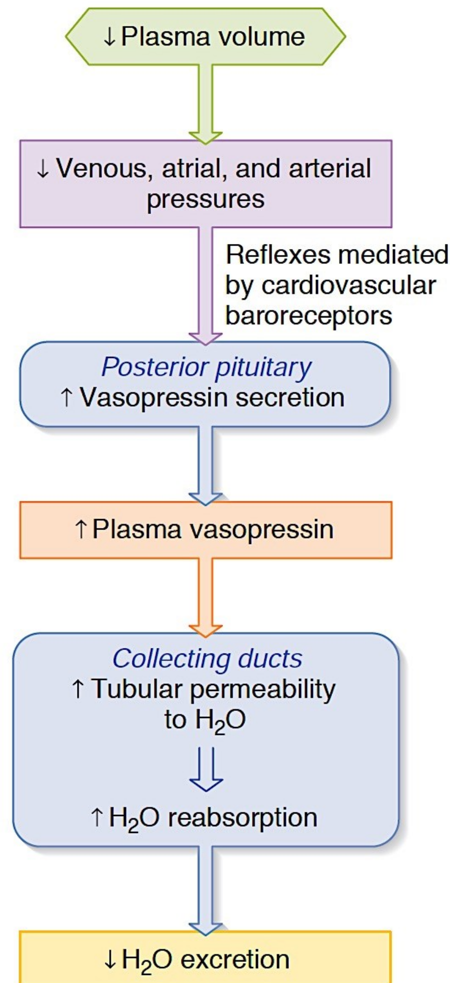

Figure 7: Effects of vasopressin on the kidneys
(obtained from (2))

Atrial natriuretic peptide (ANP) is produced by muscle cells in the cardiac atria. The secretion is a response to increased stretch of the atria in consequence of increased blood volume. ANP acts on the kidneys as it inhibits the sodium reabsorption and increases the glomerular filtration rate (GFR). In addition, ANP directly inhibits the secretion of aldosterone (2).

The Renin-Angiotensin-Aldosterone System (RAAS)

Renin is a proteolytic enzyme released by the kidneys. Stimuli for secretion of renin are low arterial pressure, low sodium concentration in the distal tubule or sympathetic innervation. Secreted to the blood, renin catalyses the reaction of the inactive peptide angiotensinogen to form angiotensin I. In the lungs, angiotensin

converting enzyme (ACE) converts angiotensin I to its active form angiotensin II (Figure 8).

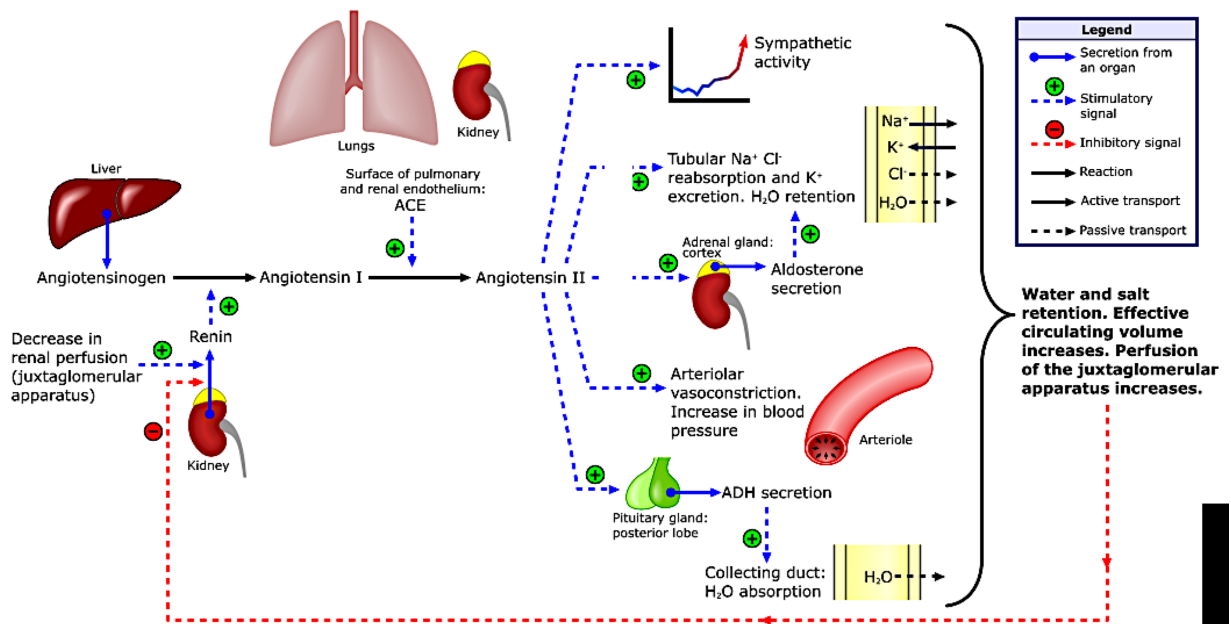

Figure 8: The renin-angiotensin-aldosterone system

(obtained from: https://en.wikipedia.org/wiki/Renin–angiotensin_system)

Angiotensin II has two major effects:

It causes vasoconstriction in the vessels (short term regulation) and it stimulates the adrenal cortex to release aldosterone (long term regulation). Besides, it increases thirst and stimulates the secretion of vasopressin.

Aldosterone causes an increased retention of salt and water in the distal tubule of the kidneys. Hence, all effects of the RAAS - directly or indirectly - increases the arterial blood pressure (1,3).

Adrenomedullin

Adrenomedullin (ADM) is an almost ubiquitous peptide, which is synthesized by a number of tissues and cell types (7).

For the first time, ADM was extracted in 1993 from human pheochromocytoma. It was named "adrenomedullin" because of its origin in the adrenal medulla.

Human ADM consists of 52 amino acids. It shows a resemblance to calcitonin gene-related peptide (CGRP) and amylin. Therefore, it was classified into the calcitonin/CGRP/amylin peptide family (8).

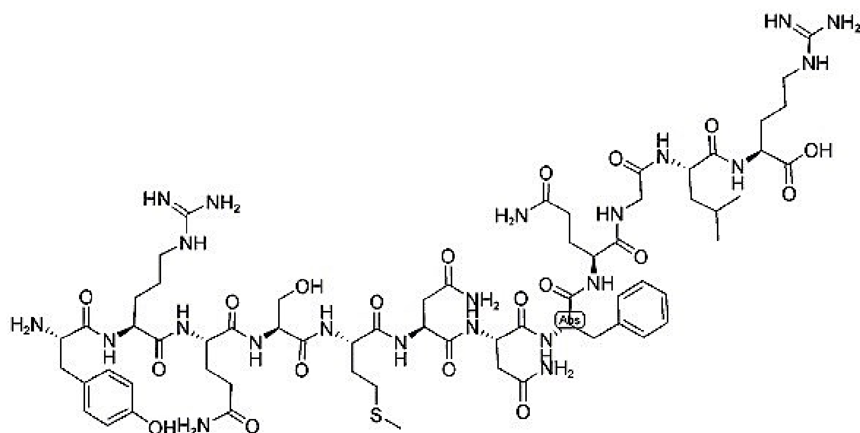

Figure 9: Structure of adrenomedullin
(obtained from: http://www.apexbt.com/adrenomedullin-1-12-human.html)

Adrenomedullin is synthesized as a part of the progenitor molecule preproadrenomedullin, consisting of 185 amino acids. Preproadrenomedullin is encoded by the adrenomedullin-gene, which can be found on chromosome 11. ADM is stored in the secretory granule of the pancreas (7).
At the very beginning, it was believed that adrenomedullin is mainly synthesized in adrenal medulla, ventricles, kidneys and lungs (9). Later it became apparent that it is highly expressed by the cells of the vascular endothelium (10). At last the expression of this peptide was seen in almost all tissues of the body and in many different cell types (7). In addition to the already mentioned tissues, ADM-gene expression was found in parts of the central nervous system such as the paraventricular, the supraoptic and the infundibular nucleus (11), in different blood cells like macrophages or granulocytes (12), in particular tissues and cells of the respiratory system (13), in the reproductive system (14,15), the skin (16) and in colorectal carcinoma of the gastrointestinal system (17,18).
Adrenomedullin circulates in many different fluids such as urine (19), liquor (20) or blood. Plasma half-life is 22.0 ± 1.6 minutes (21).
ADM provokes a strong vasorelaxant effect via local vasodilatory substances such as nitric oxide and endothelin (see subsection 1.1.4.2). Furthermore the vasorelaxant effects are caused by an inhibition of vasoconstrictors, as well as by a decrease of calcium sensitivity of contractile areas (22,23). Increased plasma ADM-levels therefore seem to lead to a significant decrease of total peripheral resistance, which can result in a fall of the mean arterial pressure.

Furthermore, adrenomedullin has a natriuretic and diuretic effect by increasing the renal blood flow and glomerular filtration rate while diminishing the renal vascular resistance (24,25).
Both effects, vasodilation and natriuresis, take part in the circulatory control. A sudden rise of ADM can lead to severe hypotension (8).
Elevated plasma ADM concentrations occur with strenuous exercise (26). However, higher plasma levels are also associated with a number of different diseases: Cardiovascular disorders such as cerebrovascular disease (27), hypertension (28), heart failure (29) or hemorrhagic shock (30) lead to elevated plasma levels as well as respiratory disorders (31,32), endocrine disorders (33–35), renal disorders (21,31,36) or other conditions like hepatic cirrhosis (37) or sepsis (38).
It has previously been shown that plasma ADM concentration rapidly increases due to orthostatic challenge and quickly returns to baseline values when the challenge is removed (39).

Galanin

Human Galanin (GAL) is a 30-amino acid peptide hormone. Originally Tatemoto and Mutt detected this hormone in 1983 in porcine intestine. GAL derives its name from the N-terminal glycine and the C-terminal alanine (40).

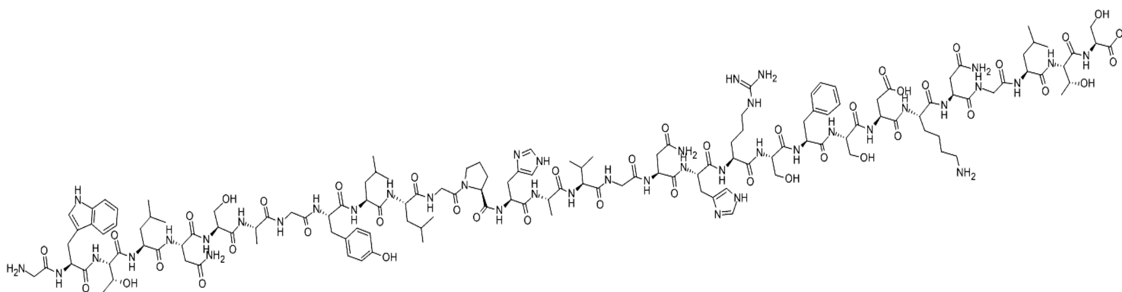

Figure 10: Structure of galanin
(obtained from:
http://www.chemicalbook.com/ProductChemicalPropertiesCB5234914_EN.htm)

Galanin is predominantly produced in the pituitary gland and hypothalamus (41). It is extensively spread in the central and peripheral nervous system, as well as in the intestines. Either galanin is delivered into the systemic circuit, or acts directly at its target organs via local release from nerve fibres (42). The gene that encodes

for GAL is located on chromosome 11. There are three Galanin receptors known: GALR 1-3. They are distributed in different areas of the body and have different functions in the neuroendocrine axis (43). In blood, galanin has a short half-life of 3 - 4 minutes (44,45). In the nervous system, it works as neurotransmitter or neuromodulator. For example it takes part in the control of neuroendocrine axis such as in the regulation of vasopressin. In the intestines GAL is - among other things - involved in the modulation of insulin release (46,47). It has been shown that plasma levels undergo no circadian rhythm (48), however increased levels occur with moderate exercise (49). Under certain conditions, GAL seems to play an important role in cardiovascular regulation. In the event of presyncope evoked by severe orthostatic challenge, GAL levels increase significantly and could be used as a marker of an imminent collapse (50). Increased GAL levels lead to an increased heart rate and have vasodilative effects (51) as well as they seem to inhibit parasympathetic acetylcholine release (42). Last but not least, GAL lowers plasma norepinephrine levels (52). Consequently, increased plasma GAL concentrations can lead to severe hypotension.

1.2 Orthostasis

Taken literally, orthostasis simply means upright standing (53). Due to a changing posture from sitting or recumbency to standing, up to one liter of blood moves to the lower extremities. The loss of central blood volume results in a decreased venous return and therefore in a decrease of cardiac output and blood pressure. In healthy people, compensatory mechanisms ensure that these events do not affect any severe problems. Their orthostatic tolerance secures an upholding consciousness during postural changes. One reaction to the decreased venous return to the heart is the stimulation of the baroreceptors. As is known from subsection 1.1.4.1, baroreceptors activate the sympathetic nervous system. Via the neurotransmitters norepinephrine and epinephrine, the heart rate and peripheral vasoconstriction increase, which restores the cardiac output and blood pressure (54).

However, there is a large amount of people in which these compensatory mechanisms fail. Impairment of autonomic nervous system or antihypertensive medications are only two possible reasons for orthostatic intolerance (OI) (55). As a consequence of the absent compensatory mechanisms, blood pressure decreases dramatically. Orthostatic hypotension (OH) is defined as a reduction of systolic blood pressure bigger than 20 mmHg or of diastolic blood pressure bigger than 10 mmHg within three minutes of standing (56).

As the cerebral blood flow is markedly reduced, typical symptoms of orthostatic hypotension include dizziness, light-headedness and syncope but also nausea and transient ischemic attacks (TIA) (54).

Especially patients with a history of stroke show abnormalities of the baroreceptor reflexes, thus are predisposed for orthostatic hypotension (57).

In order to simulate orthostatic stress and to investigate the dimension of orthostatic hypotension, researchers use different methods:

1. **Head up tilt (HUT):** The patient lies on a special table (tilt table), then the table performs a change from lying to a more upright posture
2. **Lower body negative pressure (LBNP):** The patient is placed in a LBNP-chamber. The chamber covers the patient´s lower abdomen and lower extremities and is enclosed with an airtight seal at the patient´s waist. A

pump creates a negative pressure inside the tube, which results in a movement of blood to the lower extremities

3. **Sit-to-stand test:** At first the patient has to sit still for a defined resting period, afterwards he is requested to stand up and to stay upright for a defined time. After this period the patient is requested to sit down again
4. **Artificial gravity:** is induced by short arm centrifugation

For all these methods the test persons are connected to several devices that record different parameters, such as arterial blood pressure, heart rate and cardiac output. Furthermore the patient is continuously observed for symptoms like dizziness, lightheadedness or faint.

1.3 Stroke

The term "stroke" describes an acute ischemia in particular areas of the brain that persists longer than 24 hours (58). Neurons in that particular area do not receive any blood supply, which means a lack of nutrients and oxygen for the brain. Even a few minutes of under-supply lead to neuronal death, which results in a loss of function of this specific area (2).

Stroke can be subdivided into two groups: the *ischemic stroke* and the *hemorrhagic stroke*.

Ischemic stroke is the result of an occlusion of brain feeding arteries or hypoperfusion to the brain. There are several mechanisms leading to ischemia:

1. Atherothrombotic occlusion: based on an atherosclerotic narrowing in a brain-feeding artery, like the internal carotid artery.
2. Thromboembolism: sudden occlusion of a brain artery by embolic substances that have developed in another part of the body (heart, venous system etc.)
3. Systemic hypotension: when cerebral blood flow seriously decreases and cerebral autoregulation mechanisms are not able to compensate (e.g. severe hypovolemia or heart failure).

When systemic hypotension causes stroke, infarctions occur in the border zones between the anterior cerebral artery and the middle cerebral artery or between the

middle cerebral artery and the posterior cerebral artery. This kind of stroke is also called "watershed stroke" (58).

On the other hand, when stroke is caused by thrombotic occlusion or thromboembolism, the location of the affected vessel and the dimension of injured brain tissue determine the neurological signs and symptoms. The clinical symptoms of an ischemic stroke can be seen in Table 1.

Table 1: Clinical symptoms of cerebral artery occlusions
(modified from (59))

Carotid system	
Ophthalmic artery	Total or partial monocular blindness
Anterior cerebral artery	Contralateral weakness leg > proximal arm, urinary incontinence, behavioral changes
Middle cerebral artery	
Anterior division	Contralateral motor and sensory loss, if dominant hemisphere → nonfluent (Broca) aphasia
Posterior division	Contralateral hemisensory loss and homonymous hemianopsia; if dominant hemisphere → fluent (Wernicke) aphasia
Vertebrobasilar system	
Posterior cerebral artery	Hemianopsia, cortical blindness, alexia without agraphia, agitated delirium, memory deficit
Basilar branches	Crossed cranial nerve/contralateral limb deficits, bilateral bulbar weakness, dysphagia and loss of airway protection, diplopia, quadriparesis, ataxia, vertigo, nausea, vomiting
Vertebral artery	Ipsilateral sensory/contralateral weakness, dysarthria, diplopia, dysphagia, hiccups, nausea, vomiting

Hemorrhagic stroke is the result of a rupture of a brain vessel. As a consequence, adjacent brain areas are injured by increased local pressure or by lack of blood supply which results in local ischemia. Furthermore, the intracranial pressure increases, which leads to shifts of the brain tissue or to a compression of the brain stem (58).

2 Aims and Objectives

Orthostatic hypotension is often stated as a widespread complication in patients with stroke. Patients who suffered a stroke in their history seem to have a decreased orthostatic tolerance and therefore experience syncopes more frequently than healthy subjects (60,61).

Orthostatic challenge provokes cardiovascular and neuroendocrine alterations dependent on intensity and duration of the challenge. We suggest that there is a correlation between the declined orthostatic tolerance in stroke patients and their hormonal responses to orthostatic challenge.

This study investigates how the disease stroke affects the hormonal responses during changes in posture from sitting to standing.

The focus of my diploma thesis is the analysis of the two neuropeptides adrenomedullin and galanin, which seem to play an important role in the cardiovascular regulation. Although the study situation is inconclusive, it has been shown before, that there is a direct proportional, stimulus dependent increase of plasma adrenomedullin due to orthostatic challenge (39).

The situation is different for galanin. In a previous study, performed at the Institute of Physiology in Graz, plasma galanin did not change up to a point of presyncope, upon which it increased (50).

We hypothesize that:

- Baseline adrenomedullin and galanin concentrations are higher in stroke patients than in control group
- Plasma adrenomedullin and galanin levels increase due to an orthostatic challenge
- Responses of plasma adrenomedullin and galanin due to an orthostatic challenge differ in stroke patients versus healthy subjects

We hope to get a better idea of these specific hormones and their influence on the human body. The results of our investigations could improve medical care of stroke patients, including a better prophylaxis of syncope. Syncopes often lead to falls, resulting in serious injuries, hospitalization and long-term immobilization. Preventing syncope means to conserve an enhanced quality of life.

3 Methodology

The topic of my diploma thesis was part of a bigger pilot study called "Vascular stimulus response in health and disease: A longitudinal study", which was a cooperation of the Institute of Physiology and the Department of Neurology at the Medical University of Graz. The study has been approved by the local Ethics Committee of the Medical University of Graz and conformed to the standards set by the Declaration of Helsinki.

3.1 Study Participants

In this longitudinal study, two cohorts were investigated: One group of 13 older stroke patients and one control group of 18 age-matched healthy subjects. Within the stroke patients, 10 subjects were male and 3 were female. In the control group, there were 6 male and 12 female subjects (Table 2).

Stroke patients were recruited by the Department of Neurology, as they were in-patients in the past year. Diagnosis of a stroke was made by a neurologist and approved by magnetic resonance imaging (MRI). Healthy subjects were recruited by the Department of Neurology as well. Mostly they were partners of the stroke patients. All subjects received a 20 Euro allowance for the participation in the study.

Inclusion criteria for the group of stroke patients were:

- Ischaemic stroke with transient or mild neurological deficits within 12 months before testing
- Stroke severity defined as a National Institutes of Health and Stroke Scale (NIHSS) ≤ 3
- Cerebral ischemic infarction approved by MRI
- Minimum age of 55 years

Exclusion criteria for the group of stroke patients were:

- Further neurological disorder (epilepsy, dementia, Parkinson’s disease)
- Severe disability defined as modified Rankin Scale (mRS) > 2
- Intracranial vessel stenosis/occlusion

Control group consisted of age-matched (≥ 55 years) male or female subjects with no history of stroke or further neurological disorders.

3.2 Study Protocol

Setting

For the testing the participants were invited to the outpatient clinic of the Department of Neurology. Investigations were carried out in the months November 2014 till March 2015. All test subjects were first acquainted with the study protocol. They were clarified to have the right to quit the investigation at any given time. Before the testing started, all subjects gave verbal and written informed consent.

All measurements were performed between 7 to 10 am. To minimize the effects of postprandial hypotension, subjects were instructed not to have breakfast before the testing. Furthermore subjects were told to abstain from alcohol and other stimulants like coffee during the previous 24 hours of the testing as both affect the orthostatic tolerance (62,63).

All testing took place in an attraction-poor environment that was as free as possible from external influences.

Procedure

For this study we decided to use a sit-to-stand test in order to induce orthostatic challenge in each subject. The study protocol consisted of a 5-minute resting period at the beginning during which the subjects had to sit quietly, with arms relaxed, on an examination couch. After this period subjects were assisted into an upright position in which they had to stay for an additional 6 minutes. During the standing period study participants had to keep their eyes open with the focus on a marked point of the wall. To ensure that muscle pump of the lower extremities does not affect the orthostatic tolerance they were instructed not to alter foot placement. Following to the standing period subjects were requested to sit down on the examination couch again and to rest in a relaxed position for an additional 5 minutes (recovery period). An overview of the study protocol is given in Figure 11.

At any given time subjects' physical comfort was monitored, be it by monitoring of blood pressure and heart rate, or by keeping a watch on presyncopal signs like dizziness, paleness or sudden onset of sweating. In the event of presyncope or subjects feeling unwell, testing would have been terminated.

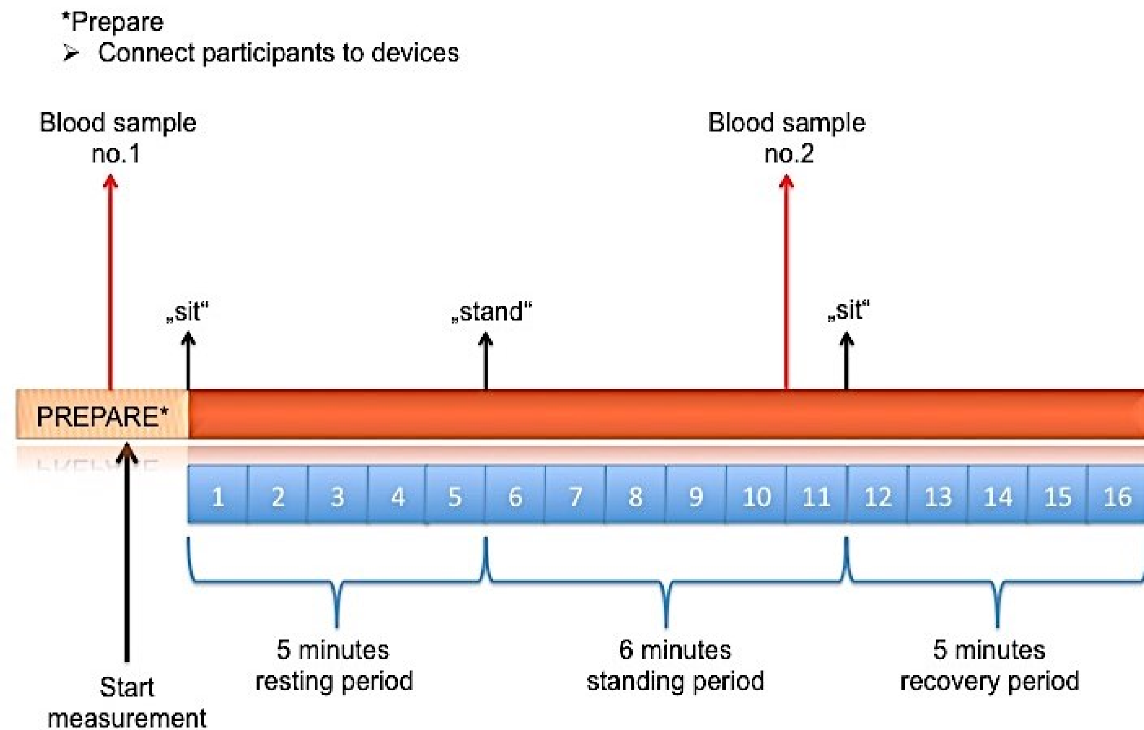

Figure 11: Timeline of the study protocol

Cardiovascular monitoring

As the effect of orthostatic challenge on cardiovascular parameters is not topic of my diploma thesis, I will only give a short overview of the cardiovascular measurements.

During the experiment hemodynamic parameters were monitored precisely. Therefore, before testing started all subjects were connected to various devices. The measurements included heart rate (3-lead electrocardiogram), continuous (finger plethysmography (Finometer)) and intermittent (upper arm oscillometry) blood pressure as well as arterial oxygenation (nasal alar pulse oximetry). Furthermore, thoracic impedance was measured which allows a continuously calculation of stroke volume, cardiac output and total peripheral resistance. All data were collected and visualized by a device called Task Force® Monitor, produced by CNSystems Austria.

Blood sampling and analysis

Once the participants were connected to the above-mentioned devices and before the experiment started, a 21G, 0.8x19 mm winged infusion set (Butterfly) was fixed

in a vein of their antecubital fossa or on the back of their hand. As the cannula was left in place for the whole experiment, it was fastened to the skin with adhesive tape. Altogether two blood samples à 10 ml were taken: One directly after the vein was canalized (“Baseline”) and one at minute 10 during the standing period (1 minute before the end of upright posture, "End of standing").

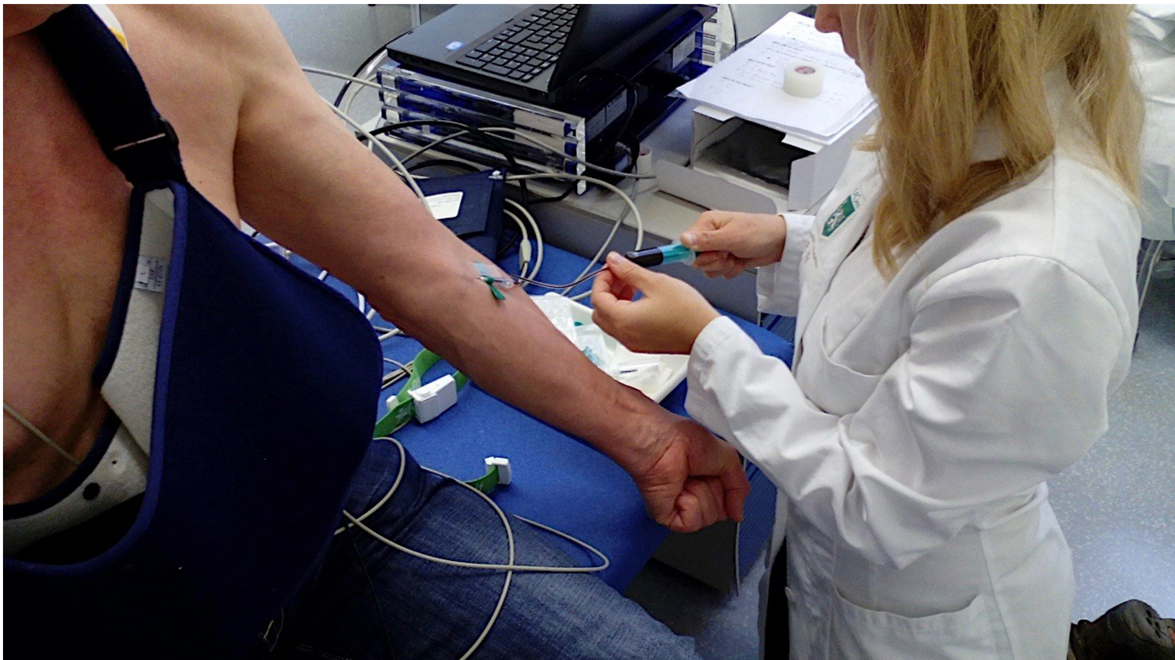

Figure 12: Male test subject during experiment

Blood was sampled into 10ml K3-EDTA (1,6mg/ml) tubes, which were pretreated with 500U/ml of aprotinin in order to avoid degeneration of proteins.
Short after the experiments the blood samples were centrifuged for 13 minutes at 2500g to separate blood plasma from blood cells. Afterwards plasma was removed with a pipette and transferred to Eppendorf-tubes. These plasma samples were stored at -70°C.
Due to logistical and financial reasons, hormone analyzes were performed after a larger amount of plasma samples was collected. Both Adrenomedullin and Galanin concentrations were analyzed with special enzyme-linked immunosorbent assay (ELISA) kits (ADM: CEA220HU; GAL: CEB084HU) designed by Cloud-Clone Corp., Houston, USA.

3.3 Statistical Analysis

All collected data were first listed in an Excel chart and sorted by time of measurement (baseline vs. end of standing) and groups (stroke patients vs. control group). One sample from stroke and one from control group, both taken at the end of standing period, were excluded from the analyzes as they were hemolytic and hormonal concentrations were out of range.

Goal was to analyze i) if there are any differences in hormone concentration during baseline between stroke patients and control subjects, ii) if hormone concentrations change due to orthostatic challenge, iii) if there are any differences in hormone concentrations between stroke and control group at the end of standing.

To test whether the concentrations of adrenomedullin and galanin are following normal distribution, Shapiro-Wilk´s test was applied, as it is powerful for small samples. The test was performed for each of the two measurements and for both groups. Thus, in total four tests were conducted for the data of each hormone. To keep the cumulative alpha error on a low level, an alpha of 0.0125 was chosen for each test. Hence, the cumulative alpha stayed at a reasonable level of 0.05.

In adrenomedullin and in galanin Shapiro-Wilk´s test pointed out, that all data are normally distributed.

However, in order to compare baseline levels with end of standing levels within stroke group, one baseline sample had to be removed from the analysis as it belonged to the same patient as the hemolytic sample from the end-of-standing blood draw.

Same procedure had to be performed with the data of control group. In order to compare baseline data with end of standing data, one baseline sample, which belonged to the same patient as the hemolytic sample from the end of standing blood draw, had to be removed.

For the comparison between baseline of stroke and control group the mentioned baseline samples remained in the analysis. For the distribution of the data, these interventions did not have any consequences.

As all data were following normal distribution, I decided to apply Student´s t-Test for paired samples to compare baseline with end of standing hormone concentrations. Furthermore, I applied Student´s t-Test for unpaired samples to

compare baseline hormone concentrations in stroke group with baseline hormone concentrations in control group, as well as to compare end of standing hormone concentrations between stroke and control group.

All statistical calculations were performed by SPSS Statistics 23, developed by IBM Corporation.

4 Results

As already mentioned above, two cohorts were investigated in the study:
One group of 13 elderly stroke patients and one control group of 18 age-matched healthy subjects. Within the stroke patients, 10 subjects were male and 3 were female. In the control group, there were 6 male and 12 female subjects.
Table 2 illustrates the different characteristics of the study participants.

Table 2: Characteristics of study participants

Stroke	Mean age ± SD	Mean height ± SD	Mean weight ± SD
Total (n=13)	68.24 ± 6.97 years	173.00 ± 7.99 cm	83.00 ± 15.56 kg
Males (n=10)	68.06 ± 6.71 years	175.40 ± 7.38 cm	82.70 ± 14.97 kg
Females (n=3)	68.84 ± 9.40 years	165.00 ± 3.61 cm	84.00 ± 21.00 kg
Control			
Total (n=18)	63.05 ± 6.83 years	167.83 ± 8.54 cm	76.67 ± 14.83 kg
Males (n=6)	62.36 ± 6.49 years	175.50 ± 6.25 cm	86.67 ± 13.74 kg
Females (n=12)	63.40 ± 7.25 years	163.92 ± 6.79 cm	71.67 ± 13.12 kg

In the stroke group, one blood sample that was drawn at the end of standing period was hemolytic and the value was out of range. Therefore, this one was excluded from the analysis. Thus, there were 12 subjects to be analyzed at both times of measurement and one additional subject with only a single measurement at baseline.
A similar situation arose in the control group. Likewise, one blood sample from the end of standing period was hemolytic and statistically seen an outlier, thus had to be removed from the analysis. Therefore, 17 samples were analyzed for both measurements and one more sample only for the measurement at baseline.

4.1 Adrenomedullin Results

Statistical analysis of the adrenomedullin data yielded the following results:

In both groups, mean plasma concentration slightly decreased from sitting to standing (Table 3).

Table 3: Mean concentrations of adrenomedullin

	Baseline Mean Concentration ± SD	**End of standing** Mean Concentration ± SD
Stroke	6.53 pg/mL ± 3.30	5.41 pg/mL ± 3.34
Control	5.83 pg/mL ± 2.59	5.58 pg/mL ± 3.42

Figure 13 displays some additional parameters of the data distribution by boxplots. These are interesting characteristics, as the diagram is not based on mean concentrations but on the median.

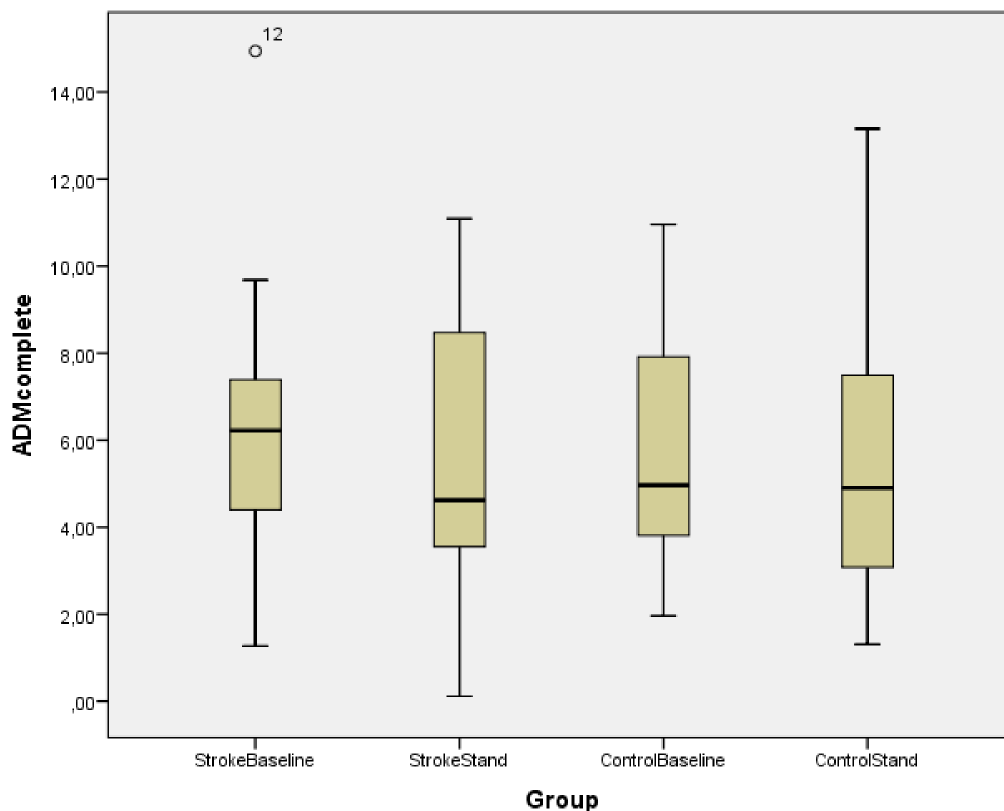

Figure 13: Distribution of adrenomedullin concentrations by boxplots

In the center of the plot the median is depicted. The median is surrounded by the box. Bottom and top of the box are the boundaries for the interquartile range. This means that the middle 50% of our data are located in the box. Two whiskers describe the minimum and maximum values (extreme scores) of the distribution. Values that are out of a range of 1.5 times the interquartile range are classified as outliers, which are tagged by a circle (64).

In order to analyze whether there are any significant changes between ADM concentrations, at first it had to be determined, if the data are normally distributed. Therefore, Shapiro-Wilk´s test was applied. With a cumulative alpha set at 0.05 (alpha of 0.0125 for each test), it turned out that all data were following normal distribution. As already described in chapter 3.3, for one part of the statistical analysis, one Baseline sample from stroke group and one from control group had to be removed, as they belonged to the same subjects as the hemolytic samples from the end-of-standing measurement. However, these changes in data did not change the fact of normal distribution.
As all data were normally distributed parametric tests were conducted.
Using Student´s t-Test for unpaired samples with an alpha of 0.05 it was found, that there is no significant difference between baseline ADM concentrations in stroke group and baseline concentrations in control group.
Furthermore, Student´s t-Test for unpaired samples turned out, that there is no significant difference between end of standing ADM concentrations in stroke and control group.
To analyze, if there is a significant difference within the stroke group between baseline concentration and concentration at the end of standing period, Student´s t-test for paired samples was conducted. With an alpha of 0.05, it turned out that there is no significant difference.
As well, Student´s t-test was applied to assess, whether significant differences occur within the control group from sitting to standing. This was not the case. The results of the different t-Tests are summarized in Table 4.

Table 4: Adrenomedullin results of t-Test

	t	df	Significance
Baseline Stroke vs. Baseline Control	0.658	29	0.516
Standing Stroke vs. Standing Control	-0.138	27	0.892
Baseline Stroke vs. Standing Stroke	1.402	11	0.188
Baseline Control vs. Standing Control	-0.109	16	0.915

4.2 Galanin Results

Statistical analysis of the galanin data revealed the following results: In stroke group hormonal levels at baseline and end of standing were nearly identical, whereas in control group hormonal levels decreased from sitting to standing (Table 5).

Table 5: Mean concentrations of galanin

	Baseline Mean Concentration ± SD	**End of standing** Mean Concentration ± SD
Stroke	61.65 pg/mL ± 19.60	62.18 pg/mL ± 17.81
Control	73.73 pg/mL ± 35.09	59.75 pg/mL ± 22.19

Moreover, the boxplots in Figure 14 depict the data through their quartiles, medians and extreme values.

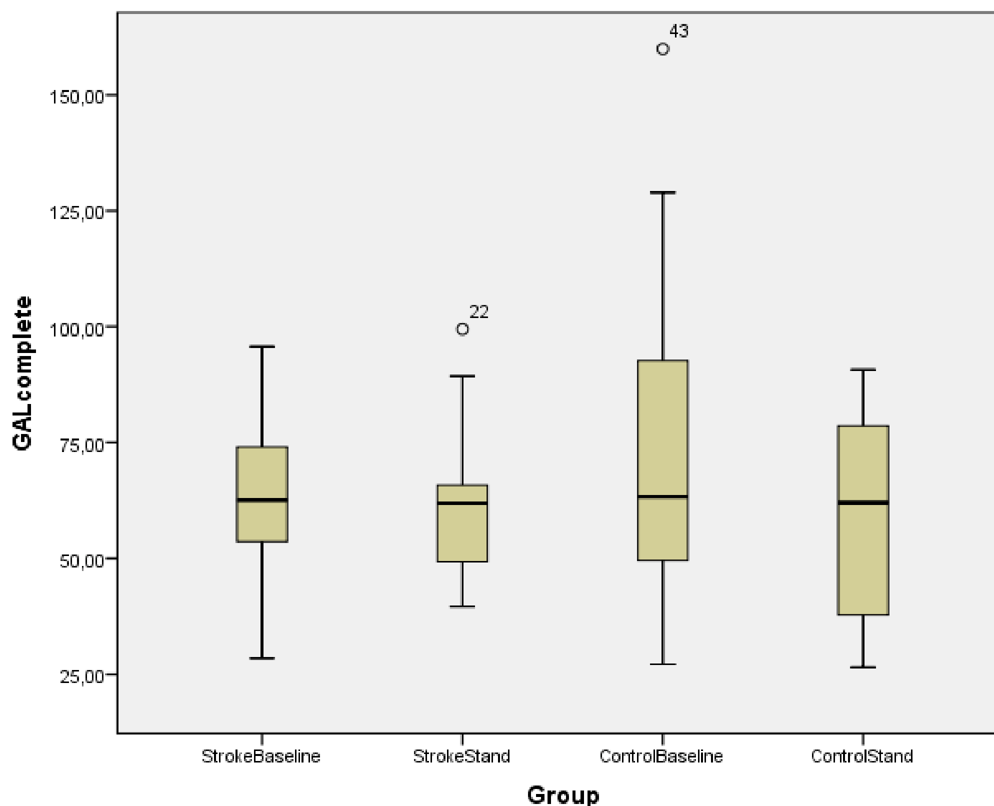

Figure 14: Distribution of galanin concentrations by boxplots

The legend of this figure is the same as in Figure 13*.*

All galanin data were normally distributed as was the case with adrenomedullin. Therefore, parametric tests were conducted.

Student´s t-Test for unpaired samples was conducted to analyze the differences in GAL concentrations between stroke and control group at baseline as well as between stroke and control group at end of standing measurement.

With an alpha of 0.05 it was found that i) there is no significant difference between baseline concentration in stroke and control group and ii) there is no significant difference between stroke and control group at end of standing.

Now using Student´s t-Test for paired samples, the different galanin levels within stroke and control group at baseline and end of standing measurement were analyzed. With an alpha of 0.05 it became apparent, that there are no significant differences between the two measurements in each group.

Table 6 summarizes the results of the different t-Tests.

Table 6: Galanin results of t-Test

	t	df	Significance
Baseline Stroke vs. Baseline Control	-1.119	29	0.273
Standing Stroke vs. Standing Control	0.315	27	0.755
Baseline Stroke vs. Standing Stroke	-0.682	11	0.509
Baseline Control vs. Standing Control	1.724	16	0.104

5 Discussion

In this study the effects of an orthostatic challenge (sit-to-stand test) on hormonal responses in stroke and age-matched healthy older controls were investigated. The following findings were observed:

1. No significant differences in hormone concentrations at baseline measurement between the two groups were seen
2. Changes in posture from sitting to standing did not induce a significant change in hormonal concentrations across both groups
3. No significant differences in hormone concentrations at the end of standing measurement between the two groups

5.1 Hormonal Levels at Baseline

Adrenomedullin

The present study did not find any significant differences in plasma adrenomedullin concentration at baseline between stroke and control group.

These findings are not in agreement with the previous literature about adrenomedullin concentrations in stroke patients. There are few studies existing that investigated the influence of *acute* or *chronic* ischemic stroke on plasma adrenomedullin.

Serrano-Ponz et al. (65) investigated 76 patients with acute ischemic stroke. Compared to 14 age-matched controls, stroke patients had significant higher plasma adrenomedullin levels on day of admission and day two. However, on day seven levels returned to normal. In addition, they observed that adrenomedullin concentration measured on day two can predict neurological severity at day seven and three months post event. Thus, adrenomedullin could act as a predictor for clinical outcome. Zhang et al. (66) investigated acute stroke patients (n=138) compared to age-matched controls (n=138). Like Serrano-Ponz et al., they observed a significant higher level in stroke patients and a direct proportional relation between hormonal concentration and NIHSS score. Additionally they identified plasma adrenomedullin concentration as a predictor of 3-month mortality and clinical outcome. Somay et al. (67) made similar observations in patients with acute ischemic stroke (n=70). Compared to 32 healthy, age-matched controls,

plasma adrenomedullin levels were significantly higher in stroke subjects. However, they did not find a relation between the severity of neurological deficit and plasma adrenomedullin levels.

Hosomi et al. (68) investigated mature adrenomedullin concentrations in the plasma of 61 patients with atherothrombotic stroke in chronic phase (stroke event ≥ 6 months ago) compared to 50 age-matched controls. In their study, hormone concentrations showed to be significant higher in stroke patients than in controls. Furthermore, they observed significant positive associations between adrenomedullin levels and carotid atherosclerosis. Similar results were observed by Shinomiya et al. (69). They investigated a smaller number of chronic ischemic stroke patients (n=38; stroke event ≥ 6 months ago) compared to 10 much younger controls. Same as Hosomi et al., they observed a significant higher plasma mature adrenomedullin concentration in stroke patients and found that the extent of carotid atherosclerosis and plasma hormone levels directly correlate. Moreover, they observed a direct correlation between systolic blood pressure and adrenomedullin levels.

The previous findings about correlation of adrenomedullin levels and ischemic stroke could be explained by the determination, that hypoxia induces the transcription of the adrenomedullin gene in the brain (70). Further reason for higher hormone levels in stroke patients might be the association between adrenomedullin and carotid atherosclerosis. Vascular endothelium seems to secrete adrenomedullin to induce antiatherosclerotic actions (compensatory mechanism) (69). Moreover, in two different studies neuroprotective effects of adrenomedullin has been proved. Xia et al. (71) observed in rats, that after adrenomedullin infusion on acute ischemic stroke, reduced cerebral infarct size and reduced neurological deficit scores occurred. Hurtado et al. (72) investigated mice brains with lack of adrenomedullin compared to normal mice brains. After a longer period of cerebral artery occlusion, they observed a significant larger infarct size in brains with lack of adrenomedullin. These observations indicate that increased adrenomedullin levels seem to be an important part of recovery mechanisms after stroke.

In the present study, these significant differences were not observed. This could be due to different reasons. The most obvious reason is that all mentioned studies investigated patients either after acute event or after a period of approximately half

a year post stroke. As adrenomedullin seems to support recovery mechanisms it is possible that patients in this study already completed recovery and adrenomedullin levels returned to normal.

Galanin

This study did not observe any significant differences in plasma galanin concentrations at baseline between stroke and control group. These observations are completely novel results, as there is no previous study existing that investigated plasma galanin concentrations in post-stroke patients.

However, few researchers investigated galanin and galanin receptor distribution in rodent brains after middle cerebral artery occlusion, which is a common model for ischemic strokes. In certain areas of the brain, a significant higher expression of galanin and galanin receptor mRNA in the ipsilateral hemisphere of the stroke was observed (73–75). This indicates, that there is an influence of stroke on galanin responses.

As there seems to be an evident lack of knowledge about plasma galanin in post-stroke subjects, further investigations are warranted.

5.2 Hormonal Levels during Orthostatic Challenge

Adrenomedullin

Over the last years, just a few researchers followed up with the reaction of adrenomedullin to orthostatic challenge – with contradictory results. To our knowledge, except one study (76), all the other researchers investigated young and healthy study participants. According to that, the present work is the first that investigated the orthostatic challenge-induced reaction of plasma adrenomedullin concentrations in stroke patients and age matched older, healthy subjects.

My results demonstrate that adrenomedullin does not seem to play an instant role in the reaction to orthostatic challenge as in both groups no significant difference due to orthostatic challenge occurred.

Although the miscellaneous characteristics of adrenomedullin have been described in the introduction, it is important to remember, that it is a strong vasorelaxant (22,23), natriuretic and diuretic peptide hormone. Numerous studies have shown, that it is synthesized in an abundance of different tissues. Lots of

cells that are involved in the cardiovascular system (cardiac myocytes, vascular endothelial/smooth muscle cells) react to various stimuli, such as increased plasma volume, with an altered release (24,25,77). However, still not all mechanisms that influence plasma adrenomedullin levels are completely understood.

Orthostatic challenges effect up to one liter of blood to move to the lower extremities. The loss of central blood volume results in a reduced venous return and therefore in a decrease of cardiac output and blood pressure.

Healthy human bodies react to this central hypotension with different mechanisms. Neuronal control reacts very fast to central hypotension. Activation of sympathetic nervous system leads to a prompt vasoconstriction and an increase of cardiac output. Deferred hormonal reaction to central hypotension includes a proportional increase of renin-angiotensin-aldosteron system, as well as an increase of plasma catecholamines and vasopressin (more details in subsection 1.1.4). Earlier studies have shown that increased levels of norepinephrine (78,79) and angiotensin II (80) stimulate the release of adrenomedullin. The vasodilative effect of adrenomedullin counteracts the reactions of catecholamines and of the renin-angiotensin-aldosterone system on central hypotension. This indicates, that adrenomedullin plays a role as fine-tuner to buffer the effects of humoral responses.

The observations of the present study agree with those presented by Mallamaci et al. (76), who investigated uremic dialysis patients and healthy subjects. They found out that 70° head up tilt (HUT) did not significantly change plasma adrenomedullin in both groups. However, at baseline measurement, uremic patients had a significant higher adrenomedullin level than healthy subjects. Mallamaci et al interpreted this effect with the major role of the kidneys in the clearance of the hormone. Furthermore they suggested, that central hypovolemia was an inadequate stimulus for changing adrenomedullin release. Additionally, Hinghofer-Szalkay et al. (81) investigated changes in adrenomedullin concentration in healthy, young men by inducing orthostatic stress to a maximum level. 70° HUT plus increasing intensity of lower body negative pressure (LBNP) led to presyncope (orthostatic end point) in all test subjects. Even under those extreme conditions, they did not find a significant difference between baseline and presyncopal concentrations. Similar results were obtained by Nishikimi et al. (82). They investigated the behavior of two different molecular forms of adrenomedullin

in healthy, middle-aged men in function of 70° HUT and did not observe any significant change.

Opposite results have been observed by Rössler et al. (39) and Gasiorowska et al. (83). Rössler et al. observed in the plasma of healthy, young men that adrenomedullin immediately increases with orthostatic challenge in a stimulus-dependent manner. On five different days, study participants underwent four tests with different degrees of HUT and one in supine rest. It turned out that concentrations significantly increased from HUT ≥ 30° to a maximum at 70° and with course of time. Gasiorowska et al. investigated adrenomedullin changes in young, healthy men in function of increasing LBNP. Almost half of the cohort experienced presyncopal signs, whereas the other part of the cohort completed the test (maximum of -50 mmHg) without presyncope. Retrospective analyzes showed a significant higher baseline level in the “presyncope group” than in the “non-presyncope group”. Furthermore, in both groups plasma concentration significantly increased by rising LBNP. At the end of LBNP, no significant difference between the two groups was seen.

The role of adrenomedullin in response to orthostatic challenge remains unclear. In former studies we could not find a clear correlation between the quality of orthostatic stress and the appearance of significant changes in plasma concentration. As elucidated, conditions of presyncope apparently did not change adrenomedullin levels significantly (Hinghofer-Szalkai et al., 2011), whereas more moderate orthostatic stress without presyncope seemed to increase concentrations in a significant way (Rössler et al., 1999). On the other hand the same technique (70°HUT) did not induce significant changes in other studies (76,82). Additionally, stronger orthostatic stress, as applied by Gasiorowska et al. induced significant changes. Therefore, the initial assumption that our unchanged values might relate to an inadequate orthostatic challenge cannot be clarified.

Another consideration was, that in principal adrenomedullin might not react in a fast manner to orthostatic challenge. Due to the susceptible patients, in contrast to all previous studies, this study applied a sit-to-stand test with a 6-minutes standing period to induce orthostasis. It is possible, that changes only occur after a longer time of more severe orthostatic challenge.

Galanin

Same as for adrenomedullin investigation, we are not aware of any studies that have previously investigated the effects of orthostatic challenge on plasma galanin in stroke patients and age-matched older, healthy subjects.

Galanin seems to play a role in cardiovascular regulation. Generally speaking, it lowers plasma norepinephrine (52), increases heart rate and induces vasodilation (51). Furthermore it modulates the release of vasopressin (46).

My results demonstrate that mild orthostatic challenge does not influence the plasma galanin concentrations across both groups. These findings are in agreement with the few existing previous insights about orthostatic influence on this peptide hormone.

Hinghofer-Szalkay et al. (50) discovered that a significant difference only appear, when orthostatic challenge is driven to an end point of presyncope. They investigated 26 healthy subjects (n=12 females, n=14 males) whilst inducing progressive cardiovascular stress by 70°HUT plus increasing levels of LBNP. In every single subject, galanin levels increased significantly. Though, it is remarkable that increase in women was way stronger (4.9 fold) then in men (3.5 fold).

In contrast to these findings, the results of Bondanelli et al. (42) were not that distinct. They investigated two different groups whilst applying 60°HUT for 45 minutes to provoke orthostatis. One group consisted of 22 subjects (n=11 females, n=11 males) with a history of recurrent vasovagal syncope (VVS). The control group included 10 healthy subjects (n=5 females, n=5 males). In control group, every subject finished the test protocol without presyncopal symptoms, whereas in the VVS group 13 subjects developed presyncope/syncope. It turned out, that neither in the control group, nor in the 13 orthostatic intolerant subjects, any significant changes in plasma galanin occurred. However, in those 9 subjects of VVS group who finished the testing without presyncope, a significant increase appeared.

Therefore the question arises, whether orthostatic challenge in Bondanelli´s study was strong enough. As we know from Hinghofer-Szalkay et al. (50), significant galanin changes only occur, when a maximum of orthostatic stress is applied. Regarding the results of Bondanelli et al., one can assume that 45 minutes of 60°HUT was a too weak stimulus for healthy subjects. Against this, the 9 VVS

subjects who finished the test were presumably more challenged. It is conceivable that they finished the test close to their presyncopal point, which would explain the significant increase. The reason why galanin did not increase in the 13 presyncopal subjects is presumably related to the short episode of orthostatic stress until presyncope occurred (after 15.5 ± 2.7 minutes of HUT).

The findings about galanin in my study are consistent with the results of the above-mentioned studies. As my study is the first that investigated susceptible stroke patients, it was medically irresponsible to drive them to an orthostatical endpoint. However, this endpoint seems to be required to effect a significant hormonal response.

5.3 Limitations

As the present work was part of a pilot study, one intention was to test feasibility and limitations of the study design. My results support the need to implement careful study design for future investigations as well as they can be used for calculating the sample size for bigger epidemiological studies.

Additionally, we need to consider the small and unequal sample size in patients with stroke and healthy controls. Stroke group consisted of 13 subjects, whereas control group included 18 subjects. In addition, within the groups the gender distribution was quite unequal (Stroke: 3 females, 10 males; Control: 12 females, 6 males). Goswami et al. (84) found out, that women show higher trend for orthostatic intolerance. This finding indicates a possible confounder in comparing the reaction to orthostatic challenge across the two groups. However, the gender distribution is not surprising, as it is generally known that in the investigated age band incidence of stroke is much higher in men than in women (85). In addition, due to organizational reasons, control group mainly consisted of partners of the stroke patients.

Furthermore, the applied model for provoking orthostatic challenge (sit-to-stand test) was probably not sufficient enough to elicit the hormonal responses as seen in other studies. There are several further possibilities to induce orthostatic challenge: head up tilt and lower body negative pressure, applied singly or in combination. However, as the participants in this project included those with

histories of stroke and older healthy controls, we did not want to induce severe orthostatic challenge, which could lead to falls in both groups.

Finally, stroke patients that were included in this study had sustained only mild stroke (NIHSS ≤ 3) that happened up to one year in the past. Thus, they were largely stable in their physiological state. Therefore, they have to be regarded as having similar physiological state as control subjects.

5.4 Conclusions and Outlook

In this pilot study neither at baseline, nor at the end of standing any significant differences between stroke and control group could be demonstrated. We also observed that mild orthostatic stress, as provided by a sit-to-stand test, does not induce any significant changes in plasma adrenomedullin and galanin concentrations.

For future studies, this pilot study is very valuable as it supports calculation of an adequate sample size in order to receive more reliable results. As we could carry out this rather intensive study in patients with mild stroke (NIHSS ≤ 3), future studies could investigate patients with more acute events (for instance six weeks post stroke) or more severe symptoms (NIHSS ≥ 3).

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Appendix

Probandeninformation und Einwilligungserklärung zur Teilnahme an der wissenschaftlichen Studie

„VASCULAR STIMULUS RESPONSE IN HEALTH AND DISEASE: A LONGITUDINAL STUDY“ (VESSELS)

„Gefäßstimulation bei Gesunden und Kranken: Eine Längsschnittstudie“

- Pilotstudie -

Sehr geehrte Teilnehmerin, sehr geehrter Teilnehmer!

Wir laden Sie ein an der oben genannten wissenschaftlichen Studie im Anschluss teilzunehmen. Die Aufklärung darüber erfolgt in einem ausführlichen Gespräch.

Die Teilnahme an dieser Studie ist freiwillig und kann jederzeit ohne Angabe von Gründen durch Sie beendet werden.

Wissenschaftliche Studien sind notwendig, um verlässliche neue Forschungsergebnisse zu gewinnen. Unverzichtbare Voraussetzung für die Durchführung einer wissenschaftlichen Studie ist jedoch, dass Sie Ihr Einverständnis zur Teilnahme an dieser Studie schriftlich erklären. Bitte lesen Sie den folgenden Text sorgfältig durch und zögern Sie nicht Fragen zu stellen.

Bitte unterschreiben Sie die Einwilligungserklärung nur

- wenn Sie Art und Ablauf der wissenschaftlichen Studie vollständig verstanden haben,

- wenn Sie bereit sind, der Teilnahme zuzustimmen und

- wenn Sie sich über Ihre Rechte als Teilnehmer an dieser wissenschaftlichen Studie im Klaren sind.

Zu dieser wissenschaftlichen Studie, sowie zur Probandeninformation und Einwilligungserklärung wurde von der zuständigen Ethikkommission eine befürwortende Stellungnahme abgegeben.

1. Was ist der Zweck der wissenschaftlichen Studie?

Das Projekt ist als Longitudinalstudie angelegt und untersucht Gefäßantworten auf orthostatische[1] Reizung bei gesunden jungen Probanden, Patienten mit Parkinson-Erkrankung (PD), Alzheimer-Krankheit (AD), Schlaganfall (S), sowie älteren gesunden Probanden.

Es wird ermittelt, wie orthostatische Belastung, Zeitfaktoren, Alter und Geschlecht das Gefäßverhalten beeinflussen und sich damit auf Blutdruckregulation und orthostatische Stabilität auswirken. Die Endothelfunktion[2] wird über reaktive Vasodilatation[3] der a. brachialis (Ultraschall-Dopppler), Fundus- und Blutuntersuchung abgeschätzt - d.h. es wird die Blutzirkulation in verschiedenen Körperregionen, z.B. Augen oder Unterarm, gemessen.

2. Wie läuft die wissenschaftliche Studie ab?

Diese wissenschaftliche Studie wird am Institut für Physiologie, MedUniGraz (Studie A) bzw. an der Univ.-Klinik für Neurologie, LKH Graz (Studie B und C) durchgeführt, und es werden **insgesamt** 180 Personen daran teilnehmen. Die Studie ist unterteilt in drei Probandengruppen (A, B, C). Ihre Teilnahme an dieser klinischen Studie wird voraussichtlich 1 Jahr dauern (4 Versuche in dreimonatigem Abstand) - siehe Table 1.

Studie A: 60 gesunde junge Personen werden (konsekutiv) passiver Kopf-hoch-Lagerung (head-up tilt, HUT) plus zunehmender Unterdruck am Unterkörper (lower body negative pressure, LBNP), intensitätsmäßig ansteigend bis zum Auftreten präsynlopaler Symptome, ausgesetzt.

Studie B: 60 ältere Patienten (20 PD, 20 AD, 20 S) durchlaufen einen orthostatischen Test (Sitzen > Stehen für 5 min) und ihre Gefäßreaktionen werden studiert. Im Anschluss an den ersten Studientag wird eine augenärztliche Untersuchung, bestehend aus einer Untersuchung an der Spaltlampe, einer Augeninnendruckmessung und eines Sehtests durchgeführt. Weiter werden die Gefäßdurchmesser mittels Retinal Vessel Analyzers (=eine spezielle Kamera zur Darstellung der Netzhautgefäße) für drei Minuten gemessen. In der zweiten Minute wird die Netzhaut mit flackerndem Licht beleuchtet und die Reaktion der Gefäße auf diesen Reiz bestimmt. Dabei müssen Sie ruhig auf eine Nadelspitze blicken. Die Messung der Gefäßweiten erfolgt berührungslos und absolut schmerzfrei. Sollten Sie oder ein Familienmitglied an einer Epilepsie leiden, können Sie an dieser Messung nicht teilnehmen. Zur Durchführung der augenärztlichen Untersuchung, sowie der Gefäßweitenmessung wird das Studienauge mit einem Mydriatikum (=eine pupillenerweiternde Substanz) eingetropft. Sie werden dadurch einige Stunden unscharf sehen, die Wirkung klingt nach 3-5 Stunden wieder ab. Sie dürfen daher nach Studientagende kein Fahrzeug lenken und keine Maschinen bedienen!
Sämtliche augenärztlichen Untersuchungen und Messungen werden an der Universtitäts-Augenklinik ausschließlich am ersten Studientag durchgeführt.

Erläuterungen: [1]orthostatisch = "in aufrechter Körperhaltung"
[2]Endothelfunktion = Steuerung des Kontraktionszustands von Blutgefäßen durch die innere Gefäßhaut
[3]Vasodilatation = Unter **Vasodilatation** versteht man die "Ausdehnung" bzw. „Erweiterung" (Dilatation) von Blutgefäßen

Studie C, Kontrolle: Wie B, aber mit gesunden Probanden der gleichen Altersgruppe (N=60). Wir erwarten uns wichtige neue Hinweise auf den Einfluss von Alter und Krankheit auf Endothelfunktion und orthostatische Stabilität, die für epidemiologische Studien und Risikoabschätzungen nutzbar sind.

Tabelle 1:
Zusammenfassung der drei Gruppen und detaillierte Auflistung der orthostatischen Herausforderung

Erklärung: HUT: Head up tilt = aufrechte Körperlage
LBNP: Lower body negative pressure = Unterdruck an Becken und Beinen
Präsynkope: Zeit vor Auftreten eines Kreislaufkollaps

Gruppe	Anzahl/ Geschlecht	Gesund	Alter	Orthostatische Herausforderung	Orthostat. Test / Jahr
JÜNGERE PERSONEN					
A	**Anzahl = 60:** 30 Männer 30 Frauen	JA	18 -35	HUT + LBNP →Präsynkope	4-mal alle 3 Monate
ÄLTERE PATIENTENGRUPPE					
B	**Anzahl = 60:** 10 Männer (mit Parkinson-Erkrankung) 10 Frauen (mit Parkinson-Erkrankung) 10 Männer (mit Alzheimer-Erkrankung) 10 Frauen (mit Alzheimer-Erkrankung) 10 Männer (Schlaganfall) 10 Frauen (Schlaganfall)	NEIN	60 -90	**Sitzen/Steh-Test** - sitzen (5 min) - stehen (5min) - wieder hinsetzen (5 min)	4-mal alle 3 Monate
C	**Anzahl = 60:** **(Kontrollgruppe)** 30 Männer 30 Frauen	JA	60 -90	**Sitzen/Steh-Test** - sitzen (5 min) - stehen (5min) - wieder hinsetzen (5 min)	4-mal alle 3 Monate

3. Worin liegt der Nutzen einer Teilnahme an der wissenschaftlichen Studie?

Es ist möglich, dass Sie durch Ihre Teilnahme an dieser wissenschaftlichen Studie keinen direkten Nutzen für Ihre Gesundheit ziehen. Bei dieser Studie (sind für den Probanden wertvolle kardioregulatorische (Werte die den Kreislauf betreffen: z.B.: Blutdruck, Herzfrequenz, Schlagvolumen der linken Herzkammer, Nervensystem ...) Parameter (Werte)

mit Hilfe des TFM (Task Force Monitor – Gerät zur kontinuierlichen Prüfung des Blutdruckes, Herzfrequenz, des Nervensystems, Schlagvolumen ... - Hersteller ist eine Grazer Firma mit dem Namen CNSystems) bestimmbar und werden dem Probanden auch in Form eines Diagnosebogens (dieser wird vollautomatisch mit Hilfe der Auswertesoftware des Task Force Monitor erstellt) zugänglich gemacht.

4. Gibt es Risiken, Beschwerden und Begleiterscheinungen?

Nein.

5. Zusätzliche Einnahme von Arzneimitteln?

Nein.

6. Hat die Teilnahme an der wissenschaftlichen Studie sonstige Auswirkungen auf die Lebensführung und welche Verpflichtungen ergeben sich daraus?

Die Studie hat keine Auswirkungen auf Ihre Lebensführung. Sollten Sie verhindert sein und einen Termin nicht wahrnehmen können, bitten wir Sie umgehend den Koordinator dieser Studie zu informieren.

7. Was ist zu tun beim Auftreten von Symptomen, Begleiterscheinungen und/oder Verletzungen?

Sollten im Verlauf der wissenschaftlichen Studie irgendwelche Symptome, Begleiterscheinungen oder Verletzungen auftreten, müssen Sie diese dem Studienleiter mitteilen, bei schwerwiegenden Begleiterscheinungen umgehend.

8. Wann wird die wissenschaftliche Studie vorzeitig beendet?

Sie können **jederzeit** auch ohne Angabe von Gründen, Ihre Teilnahmebereitschaft widerrufen und aus der wissenschaftlichen Studie ausscheiden.

9. In welcher Weise werden die im Rahmen dieser wissenschaftlichen Studie gesammelten Daten verwendet?

Sofern gesetzlich nicht etwas anderes vorgesehen ist, haben nur die Prüfer und deren Mitarbeiter Zugang zu den vertraulichen Daten, in denen Sie namentlich genannt werden. Diese Personen unterliegen der Schweigepflicht.

Die Weitergabe der Daten erfolgt ausschließlich zu statistischen Zwecken und Sie werden ausnahmslos darin nicht namentlich genannt. Auch in etwaigen Veröffentlichungen der Daten dieser wissenschaftlichen Studie werden Sie nicht namentlich genannt.

10. Entstehen für die Teilnehmer Kosten? Gibt es einen Kostenersatz oder eine Vergütung?

Die Teilnehmer an der Studie erhalten eine finanzielle Vergütung in der Höhe von 20 Euro pro Versuch. Überdies bekommen Sie einen ausführlichen Bericht über Ihr Kreislaufverhalten in Form eines TFM–Auswertebogens, der Ihnen genau erklärt wird.

11. Möglichkeit zur Diskussion weiterer Fragen

Für weitere Fragen im Zusammenhang mit dieser wissenschaftlichen Studie stehen Ihnen Ihr Studienleiter und seine Mitarbeiter gern zur Verfügung. Auch Fragen, die Ihre Rechte als Proband und Teilnehmer an dieser wissenschaftlichen Studie betreffen, werden Ihnen gerne beantwortet.

Name der Kontaktpersonen:

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12. Sollten andere behandelnde Ärzte von der Teilnahme an der wissenschaftlichen Studie informiert werden?

Nein

13. Einwilligungserklärung

Name der Probandin/des Probanden in Druckbuchstaben:

....

Geb.Datum: .... Code: ....

Ich erkläre mich bereit, an der wissenschaftlichen Pilot-Studie

„Vascular Stimulus Response in Health and Disease: A Longitudinal Study" (VESSELS) -

„Gefäßstimulation bei Gesunden und Kranken: eine Längsschnittstudie"

teilzunehmen.

Ich bin von Herrn Priv.Doz. Dr. Nandu Goswami ausführlich und verständlich über mögliche Belastungen und Risiken, sowie über Wesen, Bedeutung und Tragweite der Studie und sich für mich daraus ergebenden Anforderungen aufgeklärt worden. Ich habe darüber hinaus den Text dieser Probandenaufklärung und Einwilligungserklärung, die insgesamt sieben Seiten umfasst, gelesen. Aufgetretene Fragen wurden mir vom Studienleiter oder von seinen Assistenten verständlich und genügend beantwortet. Ich hatte ausreichend Zeit, mich zu entscheiden. Ich habe zurzeit keine weiteren Fragen mehr.

Ich werde den wissenschaftlichen Anordnungen, die für die Durchführung der wissenschaftlichen Studie erforderlich sind, Folge leisten, behalte mir jedoch das Recht vor, meine freiwillige Mitwirkung jederzeit zu beenden, ohne dass mir daraus Nachteile für meine weitere Betreuung entstehen.

Ich bin zugleich damit einverstanden, dass meine im Rahmen dieser wissenschaftlichen Studie ermittelten Daten aufgezeichnet werden. Beim Umgang mit den Daten werden die Bestimmungen des Datenschutzgesetzes beachtet.

Eine Kopie dieser Probandeninformation und Einwilligungserklärung habe ich erhalten.
Das Original verbleibt beim Studienleiter.

....

(Datum und Unterschrift der Probandin/des Probanden)

....

(Datum, Name und Unterschrift des Studienleiters)

(Die Probandin/der Proband erhält eine unterschriebene Kopie der Probandeninformation und Einwilligungserklärung, das Original verbleibt im Studienordner des Studienleiters.)