

SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY
(PHD)

The role of functional transcranial Doppler method in the investigation of cerebral hemodynamics – The function of visual cortex in blind people and the effect of alcohol on cerebral circulation in healthy subjects

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The Examination took place at the Library of Department of Neurology, Faculty of Medicine, University of Debrecen at 1:00 p.m., 12 December, 2016

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The PhD Defense takes place in the 315. Lecture Room of the Department of Neurology (Augusztia building), Faculty of Medicine, University of Debrecen at 09:30 a.m., 23 February, 2017

1. INTRODUCTION AND THEORETICAL BACKGROUND

1.1. MECHANISMS INFLUENCING THE DIAMETER OF CEREBRAL ARTERIES AND CEREBRAL BLOOD FLOW

It has been well known, that while the cerebral blood flow (CBF) is relatively constant despite the large variations in blood pressure, the regional cerebral blood flow is rapidly adjusted to the actual metabolic needs during the neuronal activation. These mechanisms are very precisely regulated by vasoconstriction and vasodilatation of cerebral resistance vessels.

The regional cerebral blood flow is adjusted by the tone of cerebral vessels, which is regulated by three main mechanisms: the intrinsic characteristics of smooth muscle cells in the vascular wall (myogen regulation), the metabolic activity of neurons and astrocytes adjacent to the cerebral resistance vessels (metabolic regulation), and the effects of perivascular neurons on the microvessels (neurogenic regulation). Moreover, the significant effects of other humoral agents derived mainly from the endothelial cells, as well as extrinsic factors have to be considered in the CBF regulation.

Although the effect of different factors (intraluminal pressure, neuronal activation, perivascular nerves, humoral factors, blood gases and extrinsic agents) on CBF can be investigated separately in experimental studies, the diameter of the cerebral resistance vessels and thus the regional CBF are determined by an integrated regulation of the different mechanisms. In the present work my aim was to study the neurovascular coupling and autoregulation, as the essential factors responsible for CBF regulation. Effect of reading was investigated on the occipital cortex activation and the consequent changes of flow parameters in the posterior cerebral arteries (PCA) in sighted and blind subjects. Regarding the autoregulation, effect of alcohol was evaluated on the flow velocity changes in the middle cerebral arteries (MCA) at decreased perfusion pressure evoked by orthostatic stress (head-up tilt test, HUT). In the next paragraphs, the role of different factors affecting the tone of cerebral microvessels in CBF regulation will be discussed.

1.1.1. Myogen regulation

One of the main characteristic features of the precapillary vessels is that the smooth muscle cells or pericytes in the vascular wall respond with constriction to the increase and with dilatation to the decrease of intraluminal pressure. This is the so-called

Bayliss-effect, which phenomenon can also be demonstrated in vitro in separated vessels.

1.1.2. Metabolic regulation

The metabolites of the cells in the central nervous system as well as other humoral factors play one of the most important roles in regulation of the diameter of cerebral resistance vessels and thus the regional cerebral blood flow. According to the metabolic regulation theory, a regional neuronal activation leads to an increased metabolism of the activated tissue, and the release of by-products causes vasodilation of the microvessels in the activated area.

1.1.3. Neuronal regulation

It has been well known that perivascular nerves containing synaptic vesicles run to the cerebral resistance vessels. In synaptic vesicles of these nerves several neurotransmitters were shown, which receptors were also found in the wall of the cerebral microvessels. Besides the myogenic and metabolic regulation, the effect of perivascular nerves was also shown to play an important role in the regulation of cerebral blood flow.

1.2. THE ROLE OF EXTRINSIC FACTORS IN THE REGULATION OF CEREBRAL CIRCULATION

1.2.1. Caffeine

Effects of coffee on cerebral microvessels and cerebral blood flow have not been known in details. Coffee drinking induces several physiological alterations such as increase in sympathetic nerve activity, elevation of arterial blood pressure, vasoconstriction of cerebral resistance vessels, and consequently decrease in cerebral blood flow in the main intracranial arteries. Scientific experiments proved that the effect of caffeine is based mainly on neural and less on vascular mechanisms.

1.2.2. Alcohol

Chronic alcoholism was shown to cause decreased cerebral metabolism and blood flow, similarly to the effect of sedatives. Alcohol decreased the flexibility of erythrocytes, leading to impaired hemorheology. Chronic alcohol consumption was reported to increase the risk of ischemic stroke by 1.5-3.5-fold, and the risk of hemorrhagic stroke by 2-3-fold.

Acute effects of ethanol on systemic and cerebral hemodynamic parameters are rather heterogeneous depending on the actual alcohol concentration. Recent results has proved that acute alcohol intake may increase not only the risk of syncope, but also the risk of stroke.

1.2.3. Nicotine

Cigarette smoking is a well-known risk factor for the development of cardio- and cerebrovascular diseases. The risk of stroke was shown to be increased with the amount of cigarettes and the duration of smoking.

Investigation of the acute effect of cigarette smoking suggested a significant correlation between regional cerebral blood flow (rCBF) and blood nicotine concentration in certain brain areas. Smoking of cigarettes with low-nicotine level produced significantly smaller changes in rCBF than smoking of cigarette containing larger nicotine doses.

Chronic cigarette smoking causes endothelial dysfunction, decreased NO synthesis and bioactivity, increased platelet function, inflammatory processes, hypercoagulability, and eventually atherosclerosis, leading to either functional or structural damage of the vessels.

1.3. NEW CONCEPTS OF REGULATION OF NEUROVASCULAR COUPLING

In the last 2 decades the primary role of metabolic regulation in the neurovascular coupling has been queried. Its one main reason is that the flow increase evoked by neuronal activation is much higher than the increase of tissue metabolism and oxygen consumption. Moreover, the flow increase develops much faster than the release of the vasodilatative metabolic end-products. It means that the increases of flow and metabolism are not proportional either in time or intensity. It does not mean that the role of metabolic factors in maintenance of blood flow in neurovascular coupling could be neglected, but suggests that the very fast initial changes in metabolism and flow are regulated by the rapid neural mechanism through the perivascular nerves.

Maintenance of cerebral functions require a very precise regulation between the neuronal activation and regional cerebral blood flow, which mechanism is known as neurovascular coupling. During this process increase of tissue metabolism and synaptic activity lead to dilation of microvessels. Neurovascular coupling requires function of different cells, including the endothelial cells, neurons, vascular smooth muscle cells, as well as astrocytes. Astrocytes are the dominant glial cells of the central nervous system, which modulates the synaptic transmission and the local

cerebral blood flow. The close spatial relationship between the astrocytes, neurones and microvessels suggests that astrocytes play a functional role in the neurovascular coupling.

1.4. HUMAN EXAMINATION OF NEUROVASCULAR COUPLING

The neurovascular coupling (functional hyperaemia) refers to the increase of regional cerebral blood flow evoked by neuronal activation. An illustrative process of the neurovascular coupling is the increase in cerebral blood flow in the visual cortex evoked by a visual stimulus.

The neurovascular coupling is a complex and precisely regulated process that could be investigated by fMRI (functional Magnetic Resonance Imaging), PET (Positron Emission Tomography), SPECT (Single Photon Emission Computed Tomography), NIRS (Near-infrared Spectroscopy), and TCD (Transcranial Doppler) in humans.

In the present studies, the transcranial Doppler (TCD) method was used to investigate the cerebral circulation. The transcranial Doppler that was developed in 1982 by Aaslid and co-workers allowed to measure non-invasively the blood flow velocity in the main intracranial arteries. Moreover, this method is suitable to determine the pulsatility index and thus to evaluate the cerebrovascular resistance. Although the spatial resolution of the TCD is poor, its temporal resolution is excellent. In addition to the excellent temporal resolution, the TCD has several other advantages. It is non-invasive, free of dangerous side-effects, and therefore this method can be repeated at any time. It should be mentioned that the TCD examinations are cheap, and due to the easy transportation, it can easily be used anywhere and anytime.

The TCD equipments are usually applied with 2 MHz TCD transducers. This ultrasound frequency is able to penetrate through the certain points of the skull, and the flow velocity in the vessels can be calculated by the frequency shift (difference in the frequency of the transmitted ultrasound and the received echo) caused by the moving blood cells.

It is important to know that diameter changes evoked by different stimuli happen in the cerebral resistance vessels (arterioles) and not in the main intracranial arteries. It means that although the blood flow velocity is measured in the large intracranial arteries (MCA, or PCA), the neuronal activation, endogenous or exogenous chemical agents, as well as changes in blood pressure evoke diameter changes in the microvessels. Dilation of resistance vessels results in decrease in vascular resistance, and thus blood flow increases in the main cerebral artery with

constant diameter. Certainly, constriction of resistance vessels causes opposite flow changes.

Monitoring of flow velocity changes induced by different stimuli is called functional transcranial Doppler (fTCD). This method is suitable for investigation of vasoreactivity, autoregulation, or neurovascular coupling. Vasoreactivity means flow velocity changes evoked by acetazolamide, orthostatic stress, or changes of pCO₂, while autoregulation refers to the flow response to blood pressure changes. Neurovascular coupling is defined as flow changes induced by neuronal activation. Depending on the applied stimulus and the activated cerebral region, flow velocity changes can be monitored in the MCA (stimulation of speech centre, or motor cortex), or PCA (stimulation of visual cortex). In our studies, flow velocity changes in the PCA evoked by visual stimulus were examined. Due to visual stimulation (reading) the visual cortex is activated, which results in dilation of cerebral microvessels in the activated brain tissue leading to increased flow and consequently flow velocity in the supplying PCA. The main advantage of this examination technique is that administration of external agent is not required, the examination can be repeated at any time, and the flow velocity changes can be detected by the non-invasive TCD method.

In our other study, the orthostatic stress induced flow velocity changes were investigated. The investigation is performed with use of a tilt-table that allows changing the vertical position of the patients/volunteers. At first, the different instruments (TCD transducers, ECG electrodes, blood pressure monitor) are placed on to the volunteer in supine state, which procedure is followed by detection of resting hemodynamic parameters (heart rate, blood pressure, flow parameters in the MCAs). After measurement of resting parameters, the volunteer is tilted to a 60-80 degree vertical position, and the same measurements are repeated. In healthy people, short-lasting, transient blood pressure drop is followed by a fast normalization of the blood pressure. This compensation is regulated by the so-called baroreceptor reflex that involves compensatory tachycardia (cardiac effect) and increased peripheral vascular resistance (vasomotor reflex) caused by constriction of peripheral resistance vessels. Physiological orthostatic reaction can be disturbed by several diseases with autonomic dysfunction (e.g. polyneuropathy, diabetes mellitus), as well as by different exogenous agents (e.g. alcohol, medicines with vasodilatory effects, cardioinhibitory drugs).

2. AIMS

In the present work my aim was to study the neurovascular coupling and autoregulation, as the essential factors responsible for CBF regulation. Cerebral circulation is regulated by different mechanisms. The autoregulation is responsible for maintenance of relatively constant cerebral blood flow despite huge fluctuations of systemic blood pressure, while the neurovascular coupling means adjustment of regional cerebral blood flow to the increased neuronal activity evoked by certain stimuli.

Investigation of neurovascular coupling was performed in early blind volunteers and age-matched control subjects. The aim of this study was 1) to investigate whether Braille-reading in blind subjects causes visual cortex activation and significant flow response in the supplying PCA; 2) to determine and compare the measure and dynamics of flow changes evoked by reading Braille in blind people and reading print in sighted subjects; and 3) to measure separately the effect of light stimulus necessary for reading and the effect of letter- and word recognition on PCA flow increase evoked by print reading in sighted volunteers. To answer these questions we applied two experimental protocols in both the sighted and blind groups, which allowed investigating separately the effect of “light stimulus + letter- and word recognition” and “letter- and word recognition alone” in sighted, and the effect of “hand/finger movement + letter- and word recognition” and “letter- and word recognition alone” in blind subjects.

Cerebral autoregulation was investigated in healthy students during orthostatic stress. It is well known that realignment of circulating blood volume in vertical position results in a decrease in blood pressure and cerebral perfusion, however, the blood pressure is restored within few seconds. This compensation is mainly induced by the rapid activation of the baroreceptor reflex leading to increased sympathetic activity, which induces tachycardia and increases the sympathetic vasomotor tone and thus systemic vascular resistance. If these compensatory mechanisms are not effectively regulated, patients may display orthostatic hypotension, and consequently, decreased cerebral perfusion. Blood-pressure lowering medicines, decreased fluid intake, and drinking alcohol are known triggering factors for orthostatic dysfunction, leading to neurally-mediated syncope.

Our specific aim was to investigate the acute effects of alcohol on systemic and cerebral hemodynamic changes induced by the HUT test in healthy volunteers. In addition to the blood pressure and heart rate measurements, flow velocities in the

middle cerebral arteries were also recorded in the recumbent and vertical positions before and after alcohol intake.

Autoregulation can be evaluated by comparison of changes in blood pressure and MCA flow velocity evoked by the HUT test: in case of preserved autoregulation, cerebral blood flow remains constant despite decrease of blood pressure, however, similar decrease of cerebral blood flow and blood pressure indicates lack of compensatory cerebral vasodilation at lower blood pressure values, i.e. disturbance of cerebral autoregulation. Besides the heart rate, blood pressure and MCA flow velocities, the cerebrovascular resistance index, calculated from MCA flow velocity and blood pressure adjusted to the level of the MCA, was also determined in the supine and standing positions before and after alcohol consumption.

Our specific questions were the following:

- 1) Can visual cortex activation and significant flow increase be detected by TCD in the PCA in blind subjects during reading Braille? What is the measure of the flow response in the PCA evoked by reading Braille in blind and reading print in sighted volunteers?
- 2) What is the measure of the PCA flow response evoked separately by the light stimulus and the letter- and word recognition alone during reading print in sighted people?
- 3) How does alcohol influence the heart rate, blood pressure and the MCA flow velocity in healthy subjects?
- 4) How does alcohol influence the hemodynamic changes (heart rate, blood pressure, MCA flow velocity) evoked by the HUT test in healthy people?
- 5) Does alcohol influence the autoregulation assessed by the simultaneous changes of MCA flow velocity and blood pressure adjusted to the MCA level during the HUT test?

3. SUBJECTS AND METHODS

3.1. ACTIVATION OF THE VISUAL CORTEX IN SIGHTED AND BLIND SUBJECTS DURING READING

3.1.1. Subjects

Eleven healthy congenitally or early blind adults (8 males, 3 females, mean age: 23 ± 5 years) and 10 age- and sex-matched healthy sighted subjects (7 males, 3 females, mean age: 22 ± 4 years) were included in the study. Blind subjects were early blind having no sight at birth or by 5 years of age. They were blind as the result of peripheral lesions (eye disease, or optic nerve disease), but otherwise they were neurologically normal.

3.1.2. Functional TCD and VEP investigations

Two 2-MHz probes were mounted by an individually fitted headband. In all cases, the P2 segment of the PCA was insonated through the temporal cranial window on both sides at a depth of 58-60 mm.

As a stimulation paradigm, we used an emotionally neutral text that the volunteers could read freely. The stimulation protocol consisted of 10 cycles with a control phase of 20 s and a stimulation phase of 40 s for each cycle. Certainly, sighted subjects read print, while blind people read Braille text during the stimulation phase. The print and Braille texts were identical.

Half an hour after the fTCD examination, visual-evoked-potentials (VEP) were investigated over the occipital cortex (Neuropack, Nihon Kohden Corporation, Tokyo, Japan) and amplitudes and latencies of P100 waves were calculated.

3.1.3. Experimental design

Two experimental protocols were used in both the sighted and blind groups. In one of the two experimental protocols (Sighted/Rest-Reading protocol), sighted subjects were instructed to close their eyes for 20 s without doing or thinking about anything during the control period (Rest). After this 20-second period they opened their eyes and read an emotionally neutral text silently for 40 s during the stimulation phase (Reading print text). In the other experimental protocol (Sighted/NLC-Reading protocol), sighted volunteers were asked to “read” (look at) non-lexical characters (NLC) including the combination of dot, comma, semicolon, colon and hyphen (e.g.,-,:-:.....) for 20 s in the control period, after that they read an emotionally neutral text silently for 40 s during the stimulation phase (Reading print text).

Blind subjects were asked to keep their eyes closed during the whole experiment. Blind subjects in one of the two experimental protocols (Blind/Rest-Reading protocol) were asked to sit with eyes closed without doing or thinking about anything during the control period (Rest). After the control period they read an emotionally neutral Braille text silently (the same text as the sighted subjects read) for 40 s during the stimulation phase (Reading Braille text). In the other stimulation protocol (Blind/NLC-Reading protocol), blind subjects were asked to “read” (touch) signs of non-lexical characters (NLC) including the combination of dot, comma, semicolon, colon and hyphen (e.g.,-,:-:.....) for 20 s, after that they read an emotionally neutral Braille text silently for 40 s during the stimulation phase (Reading Braille text). As it has already been mentioned, 1 cycle consisting of 20 s control and 40 s stimulation phases was repeated 10-times.

Data were expressed as means \pm standard deviation (SD). Tests for normal distribution were performed, and the homogeneity of the variances was checked by an F test. Since no difference was found in the PCA flow parameters between the right and left sides, the results of bilateral measurements were averaged within one subject.

Repeated measures analysis of variance (ANOVA) was applied to compare absolute and relative changes of cerebral blood flow velocities in the stimulation phases between the blind and sighted groups as well as between the different experimental settings.

Analysis of variance with Scheffe post-hoc test was used to compare the age, pulse rate, blood pressure, capillary blood gases and pH as well as baseline and maximum relative flow velocity differences between the blind and sighted groups. Differences between data from the same group obtained at different time points of the experiment (control phase vs stimulation phase) or during different experimental protocols (“Rest reading” vs “NLC-reading” protocols) were analyzed by paired t-test. A difference of $p < 0.05$ was considered statistically significant.

3.2. EXAMINATION OF ORTHOSTATIC HYPOTENSION IN HEALTHY SUBJECTS BEFORE AND AFTER ALCOHOL INTAKE

3.2.1. Subjects

Twenty healthy, young students (11 males, 9 females, mean age: 23 ± 2 years, body mass index: 23.3 ± 3.5 kg/m²) were included in the study.

During the experiment, non-invasive, continuous monitoring of hemodynamic parameters including heart rate (HR), systolic (sBP), diastolic (dBP)

and mean (mBP) arterial blood pressure values was performed using Task-Force Monitor (CN Systems Medizintechnik GmbH, Graz, Austria) which incorporates electrocardiography and devices for oscillometric and continuous blood pressure measurements. Bilateral continuous recordings of mean flow velocity (MFV) in both middle cerebral arteries (MCAs) were also obtained with transcranial Doppler ultrasound (Multidop T2, DWL, Überlingen, Germany). An index of cerebrovascular resistance (CVRi) was also calculated as the quotient of mBP adjusted for the MCA level (mBPMCA) and the MCA mean flow velocity (MFVMCA), i.e. $CVRi = mBPMCA/MFVMCA$. In order to assess the mBP adjusted for the MCA level, a correction of mBP was necessary during the HUT phase, because the mBP at the level of MCA insonation was decreased due to the hydrostatic reduction of pressure values in the upright position. During the calculation, the vertical distance between the levels of the heart and the MCA after raising the subjects to an inclination of 70° was considered.

3.2.2. Experimental protocol

First, each volunteer was positioned in the supine position on an electrically-driven tilt table equipped with a footboard. After proper positioning, the instruments (3-lead ECG, blood pressure recorder, TCD probes) were placed on the volunteers. The experimental protocol included a 30-minute supine rest and a 10-minute HUT phase both during the control period before, and during the test period after alcohol intake. After a 30-minute supine rest, the HUT test was performed by raising the subjects to an inclination of 70° for a period of 10 min. After the control measurements, alcohol (vodka, 37.5% alcohol content) was administered orally over a 10-minute period. Our aim was to investigate the effects of mild-to-moderate drunkenness on hemodynamic parameters, therefore the target blood alcohol level was chosen to be 100mg/dL (1 g/L = 0.1 g/dL, i.e. 1.0‰).

During the 10-minute drinking period and for an additional 30 min the subjects were sitting. Subsequently, they were positioned again on the tilt table in the supine position, and the same protocol was performed as before alcohol ingestion. Briefly, after 30 min of supine rest, the HUT test was performed again for a period of 10 min. It means that 1 h passed between the end of alcohol intake and the start of tilting to the upright position under the effect of alcohol. At the end of the 10-minute HUT test during the post-alcohol test period, blood was drawn for the measurement of blood alcohol concentration and blood gas values.

Heart rate, blood pressure values, and MFVs in both MCAs were continuously recorded and beat-to-beat data were averaged separately in the last 5 min

of the resting phase in the supine position (baseline) and over the last 9 min of the 10-minute HUT phase both in the control period before and in the test period after alcohol intake.

Values were expressed as median and ranges. Since variables were not normally distributed, the non-parametric Wilcoxon signed-rank test was used for the comparison of paired data in the supine and HUT positions before and after alcohol intake. The relative changes of hemodynamic parameters induced by orthostatic stress (HUT test) were expressed in the percentage of the baseline value, which was calculated using the following formula: Percentage change= [(value in HUT position–value in supine position)/value in supine position] × 100. The relative changes of hemodynamic parameters caused by the HUT test before and after alcohol intake were also compared by Wilcoxon signed rank test. P values <0.05 were considered to be statistically significant.

4. RESULTS

4.1. ACTIVATION OF THE VISUAL CORTEX IN SIGHTED AND BLIND SUBJECTS DURING READING

In both groups and experimental protocols the PCA flow velocity increased with a short time delay at the beginning of the stimulation period, overshoot and then stabilized at a lower level than the maximum, but at a higher level than the control value.

4.1.1. Baseline absolute flow velocity values

The baseline peak systolic flow velocity in the PCA, measured in the last 5 s of the control phases, was significantly lower in the blind than in the sighted group. This difference between the two groups was observed either in the “Rest” (“Rest-Reading” protocol) or “NLC” (“NLC-Reading” protocol) phase. Within the sighted group, higher baseline flow velocity ($p < 0.001$) was measured during the “NLC” (volunteers' eyes were open and they “read” non-lexical characters) than in the “Rest” period (subjects' eyes were closed). However, in the blind group the baseline flow velocities in the PCA during the “NLC” (volunteers touched non-lexical characters) and “Rest” phases (volunteers sat and did not do anything) were similar ($p = 0.7851$).

4.1.2. Absolute flow velocity changes during reading

In the stimulation phase, reading resulted in a significant increase of absolute flow velocity in both groups and both experimental protocols. It means that, in both the blind and sighted subjects, either in the “Rest-Reading” or “NLC-Reading” protocols the flow velocity during reading was significantly higher ($p < 0.001$) than the baseline flow velocity measured in the “Rest” or “NLC” phase, respectively.

In both experimental designs (“Rest-Reading” and “NLC-Reading” protocols), repeated measures analysis of variance detected significant group main effect (sighted vs blind, $p < 0.001$), indicating significantly higher flow velocity during the stimulation phase in the sighted than in the blind volunteers.

4.1.3. Relative flow velocity changes during reading

In order to compare the effect of reading between the different groups and experimental designs, absolute flow velocity values were normalized to the corresponding baseline data (last 5 s of the corresponding control phase) and expressed in the percentage of the baseline flow velocity.

Repeated measures analysis of variance revealed significant group main effect (sighted vs blind, $p < 0.001$) between the two groups during the “Rest-Reading” protocol. In contrast, in the other experimental setting (“NLC-Reading” protocol), repeated measures analysis of variance did not detect significant difference in the group main effect (sighted vs blind, $p = 0.449$) between blind and sighted subjects. It indicated that the relative flow velocity values, normalized to the baseline flow velocity measured during the “NLC” phase, were similar in the blind and sighted groups during reading text. In the sighted group, comparison of the relative flow velocity changes between the two experimental designs (Sighted/Rest-Reading vs Sighted/NLC-Reading) revealed significant group main effect ($p < 0.001$), however, no significant difference ($p = 0.889$) could be detected between the two experimental designs in blind subjects.

4.1.4. Maximum relative flow velocity changes during reading

To analyze the maximum increase of relative flow velocity, the highest of the values obtained during the 40-second stimulation phase was taken from each subject during both protocols. The maximum increase of the relative flow velocity normalized to the “Rest” phase in the sighted group (Sighted/Rest-Reading) was significantly higher than that measured during the “Sighted/NLC-Reading” protocol in sighted or “Blind/Rest-Reading” protocol in blind people. However, the differences in the maximum increase of relative flow velocity values between the two experimental settings in blind subjects, as well as between the “Blind/NLC-Reading” protocol in blind and “Sighted/NLC-Reading” protocol in sighted subjects were not significant. The maximum increases of relative flow velocities in the middle cerebral arteries of both the blind and sighted subjects ($3.3\% \pm 1.4\%$ and $3.6\% \pm 1.5\%$, respectively) were significantly lower ($p < 0.01$) than in the PCA.

4.1.5. VEP

While VEP parameters in sighted subjects were within the normal range, no VEP signal could be detected in blind people.

4.2. EXAMINATION OF ORTHOSTATIC HYPOTENSION IN HEALTHY SUBJECTS BEFORE AND AFTER ALCOHOL INTAKE

Alcohol consumption resulted in an alcohol concentration of 0.91 ± 0.11 ‰ measured 70 min after drinking alcoholic beverage.

No significant difference was found between the resting hemodynamic parameters (heart rate - HR, systolic blood pressure - sBP, diastolic blood pressure - dBP, mean arterial blood pressure - mBP, mean flow velocity - MFV in the left and right MCAs) measured in the supine position before and after alcohol ingestion.

4.2.1. Effects of the HUT test on hemodynamic parameters measured before and after alcohol intake

Both before and after alcohol ingestion, orthostatic stress resulted in a significant increase in heart rate and systolic, diastolic and mean arterial blood pressures, while mean cerebral blood flow velocities significantly decreased in both MCAs.

The analysis of relative changes induced by the HUT test showed that the increase in heart rate was significantly larger, while the increase in mean arterial blood pressure was significantly smaller during the HUT test after alcohol intake compared with the control period. Despite the increase in systemic mean arterial blood pressure (mBP), MCA flow velocity values (MFVMCA) significantly decreased in both MCAs in the upright position compared to the supine state. This decrease in MFVMCA was significantly more prominent in both MCAs after drinking alcohol than before alcohol ingestion.

4.2.2. Blood pressures at the level of the heart and brain

Although mean arterial blood pressure increased during orthostatic stress both in the control period and after alcohol ingestion, blood pressure values at the level of the brain (mBPMCA) had to be corrected in the upright position according to the decreased hydrostatic pressure due to the vertical distance between the heart and brain. Despite the increase in mean arterial blood pressure in the HUT position, blood pressure values adjusted to the level of the MCA (mBPMCA) were significantly lower in the vertical position compared to the horizontal state. The relative increase in the mBP was smaller (11% vs. 15%; $p < 0.05$), while the relative decrease in the mBPMCA was larger (-17% vs. -14%; $p < 0.05$) in the post-alcohol period, than before alcohol intake.

4.2.3. Comparison of relative changes in mBPMCA and MFVMCA values induced by the HUT test in the control and the post-alcohol periods

The comparison of relative changes of mBPMCA and MFVMCA values during the HUT test showed that the relative decrease in MFVMCA (-8 and -9 mmHg in the left and right MCAs, respectively) was significantly smaller than the reduction in mBPMCA (-14%) in the control period. In contrast, the relative decrease of

MFVMCA (-15 cm/s in both MCAs) was similar to the drop of mBPMCA (-17%) under the effect of alcohol.

4.2.4. Effects of alcohol on the changes in cerebrovascular resistance provoked by the HUT test

In order to determine the cerebrovascular resistance, the adjusted mean arterial blood pressure at the level of MCA insonation (mBPMCA) was divided by the mean flow velocity values measured in the MCAs (MFVMCA). While the calculated cerebrovascular resistance decreased after head-up tilt in the control period before alcohol intake, similar changes could not be detected after alcohol ingestion.

5. DISCUSSION

In the present work, transcranial Doppler (TCD) was used to investigate the neurovascular coupling and the autoregulation. Investigation of PCA flow response evoked by occipital cortex activation in blind and sighted subjects, as well as the evaluation of systemic and cerebral hemodynamic changes during orthostatic stress before and after alcohol intake proved that the TCD is a sensitive method for investigation of both the neurovascular coupling and the autoregulation.

Neurovascular coupling in the occipital cortex induced by visual stimuli in sighted and tactile stimuli in blind volunteers was investigated in the present study. Our TCD data confirmed those earlier fMRI and PET findings, which proved that occipital cortex can be activated not only by visual stimulus and not only in sighted subjects, but also by sensory stimulus (Braille reading) in blind people. The use of two different experimental protocols in the present study allowed to investigate the flow velocity changes in the PCA induced by letter- and word recognition alone (“NLC-Reading” protocols) as well as evoked by the combined effect of eye opening (light stimulus) + letter- and word recognition in sighted and hand/finger movement + letter- and word recognition in blind subjects (“Rest-Reading” protocols). Our results proved that in case the “Rest” phase served as the control condition, eye opening (light stimulus) + letter- and word recognition in sighted people induced a significantly larger flow velocity increase ($25.9 \pm 6.9\%$) in the PCA than hand/finger movement + letter- and word recognition did in early blind subjects ($10.0 \pm 5.0\%$). However, when sighted and early blind subjects “read” NLC as a control condition, i.e. only the effect of letter- and word recognition was investigated, print reading in sighted ($8.1 \pm 3.5\%$) and Braille reading in blind subjects ($10.5 \pm 4.5\%$) resulted in a similar increase in maximum relative flow velocity in the PCA. On the one hand, these findings indicated that reading alone (letter- and word recognition) was responsible for the increase in flow velocity by approximately 8-10% in the artery supplying the occipital cortex in both early blind and sighted subjects. On the other hand, opening the eyes (light stimulus) elicited the remaining 18% increase in the PCA flow response in the sighted group. Our results suggested that approximately two-thirds of flow increase in the PCA evoked by print reading in sighted people was caused by light stimulus, while only a third of the flow response was due to letter- and word recognition. Since light stimulus did not influence the PCA flow increase in blind subjects, it must have been the effect of reading by itself that elicited the PCA flow response in this group. On evaluating the effect of reading alone (letter- and word recognition) on the PCA flow

response (“NLC-Reading” protocol), it was found quite similar in the sighted and blind subjects, indicating a similar degree of activation in the occipital cortex.

Our results proved that alcohol intake, causing mild-to-moderate drunkenness, did not influence the baseline hemodynamic parameters in the resting phase, however, it modified the hemodynamic responses induced by orthostatic stress. The HUT test under the effect of alcohol resulted in a more significant increase in heart rate, but a less prominent increase in systemic mean arterial blood pressure, a more significant reduction in the adjusted mBPMCA, and a larger decline of MFVMCA, than in the control period. These data suggested that the cardiogenic part of the baroreceptor reflex was intact, moreover the HUT test induced increase in heart rate was more significant under the effect of alcohol than before alcohol intake. However, in contrast with the heart rate, blood pressure elevation under the orthostatic stress was significantly less pronounced after than before drinking alcohol, indicating disturbance of vasomotor reflex. One of the main findings of the study was that the decrease in MFVMCA during orthostatic stress was more prominent under the effect of alcohol than in the control period. Comparison of the HUT test provoked relative changes of mBPMCA and MFVMCA values indicated that the relative decrease in MFVMCA was significantly smaller than the relative reduction of mBPMCA before alcohol consumption, however, the extent of these changes was similar after alcohol ingestion. The other main result of our work was that while the calculated cerebrovascular resistance decreased during the HUT phase in the control period, it showed an increase under the effect of alcohol.

These findings indicated a functioning autoregulation during the HUT test in the control period, attenuating the reduction of MCA flow velocity at lower mBPMCA values. However, a similar decrease in mBPMCA and MFVMCA values during orthostatic stress and the lack of decrease in the calculated cerebrovascular resistance during the HUT test after alcohol ingestion suggested that alcohol inhibited the compensatory vasodilation of cerebral resistance vessels at a reduced mBPMCA value, i.e. impaired cerebral autoregulation. Our results suggest that acute alcohol ingestion may contribute to impaired orthostatic tolerance and stroke not only by its hypotensive effect, but also by the alteration of cerebral blood flow regulation.

6. NEW RESULTS, FINDINGS

1. Using two different experimental protocols, our data proved that **the measure and dynamics of flow velocity changes in the posterior cerebral arteries were similar when the effect of letter and word recognition (reading alone) was studied in blind and sighted subjects.** These results suggested that **letter and word recognition (reading alone) based on visual stimulus in sighted and tactile stimulus in blind people (Braille reading) caused similar occipital cortex activation.**
2. Using two different experimental protocols, our results indicated that **two-thirds of the flow increase in the posterior cerebral arteries evoked by reading in sighted subjects was due to the simple light stimulus and only one-third of flow response was caused by the much more complicated letter and word recognition.**
3. Investigating the acute effects of alcohol, our study showed that the **flow velocity decrease in the MCA, evoked by orthostatic stress, was more prominent after alcohol intake than in the control period.**
4. In contrast with the control period, **the cerebrovascular resistance index increased during the HUT phase in the post-alcohol period.** This finding suggested that **lower blood pressure at the level of MCA during the head-up-tilt test resulted in compensatory cerebral vasodilation in the control period, but lack of dilation of cerebral resistance vessels was observed after alcohol intake.**
5. While the relative decrease in MCA flow velocity was significantly smaller than the relative reduction of adjusted blood pressure at the level of MCA during the HUT test before alcohol ingestion, the measure of these changes was very similar in the post-alcohol period. Due to the **lack of compensatory cerebral vasodilation during the orthostatic stress after alcohol consumption, the flow decrease in the MCA followed passively the blood pressure reduction calculated at the level of MCA after alcohol intake.** It means that **cerebral blood flow failed to be compensated at lower blood pressure values under the effect of alcohol, indicating disturbance of cerebral autoregulation.** The impaired cerebral autoregulation caused by acute alcohol consumption may increase the risk of alcohol-related syncope and stroke.

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1. **Viski, S.**, Orgovan, D., Szabó, K. J., Rosengarten, B., Csiba, L., Oláh, L.: Effect of reading on blood flow changes in the posterior cerebral artery in early blind and sighted people: a transcranial Doppler study.
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8. PUBLICATIONS RELATED TO THE THESIS

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