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Hypocapnia induced vasoconstriction significantly inhibits the neurovascular coupling in humans ☆

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ABSTRACT

Background/aims: Previous studies proved that vasodilation, caused by hypercapnia or acetazolamide, does not inhibit the visually evoked flow velocity changes in the posterior cerebral arteries. Our aim was to determine whether vasoconstriction induced by hypocapnia affects the neurovascular coupling.
Methods: By using a visual cortex stimulation paradigm, visually evoked flow velocity changes were detected by transcranial Doppler sonography in both posterior cerebral arteries of fourteen young healthy adults. The control measurement was followed by the examination under hyperventilation. Visual-evoked-potentials were also recorded during the control and hyperventilation phases.
Results: The breathing frequency increased from 16 ± 2 to 37 ± 3/min during hyperventilation, resulting in a decrease of the end-tidal CO_2 from 37 ± 3 to 25 ± 3 mm Hg and decrease of resting peak systolic flow velocity from 58 ± 11 to 48 ± 11 cm/s ($p<0.01$). To allow comparisons between volunteers, relative flow velocity was calculated in relation to baseline. Repeated measures analysis of variance revealed significant difference between the relative flow velocity time courses during hyper- and normoventilation ($p<0.001$). The maximum changes of visually evoked relative flow velocities were $26 \pm 7\%$ and $12 \pm 5\%$ during normoventilation and hyperventilation, respectively ($p<0.01$). Visual-evoked-potentials did not differ in the control and hyperventilation phases.
Conclusion: The significantly lower visually evoked flow velocity changes but preserved visual-evoked-potential during hyperventilation indicates that the hypocapnia induced vasoconstriction significantly inhibits the neuronal activity evoked flow response.

1. Introduction

Maintenance of cerebral homeostasis requires dynamic regulation of oxygen and glucose supply so as to match nutrient delivery to metabolic demand of active neurons [1]. This is achieved by a tight spatial and temporal coupling between neuronal activity and blood flow, called neurovascular coupling [2–5]. Although neurovascular research has made significant strides toward understanding how the brain neurovascular unit accomplishes rapid and spatial increases in blood flow following neuronal activation, the exact mechanisms remained unclear. In order to match regional cerebral blood flow with neuronal activity, the cerebral microcirculation was shown to be equipped with control mechanisms, regulated by different mediator systems and cell types such as neurons, endothelial cells as well as astrocytes [6–11]. The regulation involves both vasodilating and vasoconstricting components [12], in which the pH may have a strong effect in both directions. An increase in pH (alkalosis) leads to vasoconstriction and thus decreased flow, whereas an acidosis induces vasodilation and increased cerebral blood flow (CBF).

According to the functional anatomy of the brain, neurovascular coupling can be easily and reliably examined in humans by measurement of the visual stimulation-evoked flow velocity changes in the posterior cerebral arteries [13]. Visual stimulation activates the neurons in the visual cortex, which activation induces vasodilation of arterioles and consequently leads to increase in regional CBF [14,15]. These regional changes result in increased flow and flow velocity in the supplying artery, in this case in the posterior cerebral artery. Transcranial Doppler (TCD) allows to measure the changes of cerebral blood flow velocity in the intracranial arteries, which was shown to be proportional to the changes of regional cerebral blood flow, within one individual [16].

Our previous studies proved that significant vasodilation, caused by either hypercapnia or acetazolamide administration, did not affect the neurovascular coupling since the visually evoked relative flow velocity in the posterior cerebral arteries increased by the same measure under the effect of vasodilating agents as under the control

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condition [13,17]. These data suggest that despite a significant cerebral vasodilation, caused by hypercapnia or acetazolamide, cortical neuronal activation evokes further local vasodilation, maintaining the adaptation of CBF according to neuronal activity.

In the present study, our aim was to examine whether a hypocapnia-(alkalosis-) related cerebral vasoconstriction inhibits the neuronal activation evoked microcirculatory response. In order to determine the effect of the hypocapnia induced cerebral vasoconstriction on the neurovascular coupling, visually evoked flow velocity changes were measured in the posterior cerebral arteries of young healthy subjects during normoventilation and controlled hyperventilation (HV). To obtain a measure of neuronal activity, visual-evoked-potentials (VEP) under conditions of normo- and hyperventilation were also examined.

2. Subjects and methods

Fourteen young healthy adults (7 males, 7 females) between 20 and 35 years of age (mean age: 25 ± 4 years) were included in the study. The study was approved by the local ethics committee, and each volunteer gave written, informed consent. Cerebrovascular risk factors such as smoking habit, arterial hypertension, obesity (body mass index), diabetes mellitus (fasting glucose levels), and hyperlipidemia (levels of total cholesterol, LDL, HDL), as well as history of migraine, coronary or peripheral artery diseases were screened, and subjects with risk factors were excluded. The included subjects did not take any medicine regularly. The study protocol included a complete neurological examination and routine clinical laboratory tests (serum ions, blood urea nitrogen, creatinine, fasting glucose, hepatic enzymes, creatinin-kinase, hemostasis screening test, serum lipids, and inflammatory markers). Blood was drawn after an overnight fast between 8 and 10 a.m.

The functional TCD tests were performed in the morning in a quiet room at about 23 °C while the subjects were sitting comfortably. All volunteers had abstained from caffeine overnight before the study. TCD examinations were always performed by the same examiners (L. O., K.SZ.).

3. Functional TCD study

Two 2-MHz probes were mounted by an individually fitted headband. In all cases, the P2 segment of the PCA was insonated on both sides at a depth of 58 mm. Peak systolic and end-diastolic blood flow velocities were recorded with a Multidop T2 Doppler device (DWL, Überlingen, Germany). The reason for the separated evaluation of systolic and end-diastolic blood flow velocities was that the indices show different time courses in dynamic blood flow regulation. Being less influenced by Doppler artifacts [18], the peak systolic velocity index was used for the following analysis. The other reason for the use of the peak systolic flow velocities in the present study was that this flow parameter reflects most appropriately the dynamic flow regulation [19].

As a stimulation paradigm, we used a news magazine with emotionally neutral text that the volunteers could read freely. This "reading" test has been previously validated against a checkerboard stimulation paradigm [20]. The stimulation protocol consisted of 10 cycles with a resting phase of 20 s and a stimulation phase of 40 s for each cycle. During the resting periods, volunteers were instructed to close their eyes; during the stimulation phases, they opened their eyes and read silently (Fig. 1). Changes between phases were signaled acoustically with a tone.

Beat-to-beat intervals of cerebral blood flow velocity data were interpolated linearly with a "virtual" time resolution of 50 ms for averaging procedures. Within one person, flow velocity data of 10 cycles were averaged. To ensure independence from the insonation angle and to allow comparisons between volunteers, absolute data were transformed into relative changes of cerebral blood flow velocity in relation to baseline. Baseline was calculated from the blood flow velocity averaged for a time span of 5 s at the end of the resting phase, before the beginning of the stimulation phase. With a short time delay at the beginning of the visual stimulation cerebral blood flow velocity increased rapidly, overshooted and then stabilized at a constant but lower level. To analyze the maximum increase of relative flow velcity changes (v_{max}), the highest of the relative values obtained during the overshooting phase was taken from each subject.

After the examination with normocapnia, the same volunteers were taught to hyperventilate at a rate of 35–40 breaths per minute and the visually evoked flow test was repeated under hyperventilation without changing the position of the TCD probes. As a side effect of hyperventilation, the subjects felt dizziness, therefore they were asked to maintain hyperventilation only for 5 min. Since the measurement was started 1 min after the beginning of hyperventilation, only 4 cycles were performed and averaged during the hyperventilation phase (Fig. 1). Relative flow velocities in the control and hyperventilation phases were calculated in each individual and expressed as percent of the control and hyperventilation baseline flow velocity values, respectively.

To control the effectiveness of hyperventilation, end-tidal CO_2 was recorded (Capnograd, Novametrix Medical Systems Corp., Wallingford, USA) during the whole examination period, and capillary blood gasses were checked before and at the end of the hyperventilation phase. Blood pressure was measured noninvasively in the sitting position before and at the end of both normo- and hyperventilation.

Besides visually evoked flow velocities, visual-evoked-potentials (VEP) were also investigated over the occipital cortex under normo- and hyperventilation conditions (Neuropack, Nihon Kohden Corporation, Tokyo, Japan) and amplitudes and latencies of P100 waves were calculated.

4. Statistical analysis

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. Results of bilateral measurements were averaged within one subject.

Repeated measures analysis of variance (ANOVA) with Greenhouse–Geisser adjustments for the degrees of freedom was applied to compare absolute and relative changes of visually evoked cerebral blood flow velocities between the normo- and hyperventilation phases. Paired t-test was used to compare the pulse rate, breathing frequency, end-tidal CO_2, resting flow velocity and resting pulsatility index (PI), maximum relative flow velocity change (v_{max}), amplitude and latency of the visual-evoked-potential (P100 wave), and blood gasses before and during hyperventilation. A difference of $p<0.05$ was considered statistically significant.

5. Results

5.1. Effects of hyperventilation on blood gasses, resting flow velocity, and visual-evoked-potential

The breathing frequency increased significantly ($p<0.001$) during hyperventilation and resulted in a significant decrease ($p<0.001$) of the end-tidal CO_2, capillary blood pCO_2, and a significant increase of blood pH and capillary blood pO_2 (Table 1). According to the hypocapnia induced vasoconstriction, the resting flow velocity decreased (Table 1, Fig. 2), while the pulsatility index increased significantly as a result of HV ($p<0.01$). Blood pressure did not change during hyperventilation, however, pulse rate increased significantly ($p<0.001$). Parameters of the visual-evoked-potentials (amplitude and latency of P100 wave) were similar under normo- and hyperventilation (Table 1).