Effects of Head-Down Tilt on Cerebral Blood Flow and Somatosensory-Evoked Potentials in Rabbits

Yasumasa ASAI*,†, Sadanori INOUE*, Kyouko TATEBAYASHI*, Yoshimitsu SHIRAISHI*, and Yasuaki KAWAI*

* Department of Physiology, and † Division of Neurology, Institute of Neurological Sciences, Faculty of Medicine, Tottori University, Yonago, 683–8503 Japan

Abstract: Changes in cerebral blood flow (CBF) and somatosensory-evoked potentials (SEPs) were studied in rabbits exposed to head-down tilt (HDT) at 45° and 75°. The animals were anesthetized with alpha chloralose and the lungs were artificially ventilated. CBF was continuously measured by laser Doppler flowmetry (LDF), and SEPs were recorded as responses of the cortex to median nerve stimulation. In the 45° HDT rabbits, CBF did not change significantly in the parietal cortex during 1 h of HDT. In contrast, in the 75° HDT rabbits, CBF did not change significantly within 5 min after the onset of HDT, but decreased gradually to 79% of the pre-HDT baseline value at the end of 1 h of HDT. The latency and amplitude of SEPs did not change significantly throughout the experiment in any group. These results suggest that CBF and SEPs do not change significantly during 1 h of 45° HDT and that 75° HDT disturbs the regulation of the cerebral circulation but does not affect cortical somatosensory response, at least for 1 h.

Key words: cerebral blood flow, head-down tilt, microgravity, somatosensory-evoked potentials, laser Doppler flowmetry.
We also investigated changes in somatosensory-evoked potentials (SEPs) during HDT. The effects of parabolic flight [9] or postural change [10, 11] on SEPs have been examined in human subjects, and relationships between SEPs and CBF have been studied in animals during ischemia [12, 13]. To our knowledge, however, there are no reports concerning the effects of actual or simulated microgravity on SEPs in experimental animals. Thus the second purpose of the present study was to examine the changes in SEPs and CBF simultaneously during HDT in rabbits. It was assumed that the SEP amplitude and latency would be altered according to the change in CBF.

MATERIALS AND METHODS

Thirty-two adult Japanese rabbits of both sexes, weighing 2.5 to 3.5 kg, were used in this study. All procedures were reviewed and approved by the Committee for Animal Experimentation of the Faculty of Medicine, Tottori University, Japan, and conformed to the Guiding Principles for the Care and Use of Animals approved by the Council of the Physiological Society of Japan.

Animal preparation. The rabbits were fasted overnight and anesthetized with 4% isoflurane in oxygen. When they were anesthetized adequately, a catheter was inserted in the ear vein for drug infusion. The anesthesia was replaced by an intravenous injection of 50 mg/kg chloralose. An additional dose (25 to 30 mg/kg/h) of chloralose was administered through the ear vein with a syringe pump (KDS100, KD Scientific, New Hope, USA). The depth of the anesthesia was monitored by the stability of blood pressure and heart rate and the state of pupillary constriction. The animals were then intubated, placed in the supine position, and artificially ventilated (tidal volume, 10 ml/kg) with a mixture of oxygen (50 to 100 ml/min) and room air, following paralysis with pancuronium bromide (an initial dose of 0.3 mg/kg and a continuous dose of 0.3 to 0.4 mg/kg/h, I.V., Organon, Teknika, Oss, The Netherlands). The depth of the anesthesia was monitored by the probe on vessels with a diameter of larger than 0.1 mm. Tilting the body caused a significant artifact resulting from tissue movement caused by the tilting. The hole in the dura matter was closed with chemical glue to minimize the leakage of cerebrospinal fluid (CSF). Moreover, the probe was fixed with dental cement to prevent its dislocation.

In this series of experiments, we inserted a laser Doppler flow probe (ALF21 type NSC, Advance) into the cerebral cortex approximately 1 mm from the surface by using a micromanipulator to avoid a possible artifact resulting from tissue movement caused by the tilting. The apparatus emits a continuous semiconductor laser beam with a wavelength of 780 nm and an available power of 2.0 mW. The sample volume covers a radius of about 1.0 mm. The time constant for signal processing was set to 1.0 s. Data logging was performed digitally with the MacLab system (ML401, AD Instruments, Castle Hill, NSW, Australia). After baseline measurements were taken with the rabbits in the horizontal prone position, the animals were tilted head-down at 45° for 5 min and then at 75° for 5 min. The output of the CBF recording was averaged for 5 min. The relative value of CBF was expressed as a percentage of the pre-HDT baseline value in each animal.

Experiment 1. In eight rabbits, the acute responses of CBF to HDT were examined. The CBF was continuously measured by means of a laser Doppler flowmeter (ALF21, Advance, Tokyo, Japan). A tiny hole was made in the dura mater with a 26-G needle. In this series of experiments, we inserted a laser Doppler flow probe (ALF21 type NSC, Advance) into the cerebral cortex approximately 1 mm from the surface by using a micromanipulator to avoid a possible artifact resulting from tissue movement caused by the tilting. The hole in the dura matter was closed with chemical glue to minimize the leakage of cerebrospinal fluid (CSF). Moreover, the probe was fixed with dental cement to prevent its dislocation.

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Experiment 2. In the next series of experiments, the time course of changes in CBF and SEPs were observed in rabbits exposed to a 45° HDT or a 75° HDT for 1 h. Animals were randomly assigned to one of the following groups: control group (n = 8); 45° HDT group (n = 8); and 75° HDT group (n = 8). Animals in the control group were kept in the horizontal prone position throughout the experiment.

In this series of experiments, the dura was left intact to prevent any leakage of CSF and to minimize injury to the brain. A different type of laser Doppler probe (ALF21 type P, Advance) was positioned on the dura 5 mm lateral to bregma on the right side by the use of a micromanipulator. Care was taken to avoid placing the probe on vessels with a diameter of larger than 0.1 mm. Tilting the body caused a significant artifact on the output of the CBF recording, which was probably due to slight tissue movement. Therefore in this series of experiments, we started to record the
CBF signal immediately after the onset of HDT. The output of the CBF recording was averaged every 5 min, and the relative value of CBF was expressed as a percentage of the first 5-min–averaged value. For the measurement of SEPs, a hole 3 mm in diameter was drilled 5 mm lateral to bregma on the contralateral side. A silver–silver chloride ball electrode with a diameter of 0.7 mm was placed on the dural surface and served as a recording electrode for SEPs. A reference electrode was fixed at 15 mm anterior to bregma. Bipolar stimulating electrodes were placed on the skin surface above the median nerve, contralateral to the recording electrode. Square-wave stimuli with a 0.1-ms duration and 10–30 V, which were strong enough to cause a maximum response, were delivered via an isolator (SS-102J, Nihon Kohden, Tokyo, Japan) to the skin over the nerve at a rate of 1 Hz. Signals from the recording electrodes were amplified with a bandwidth of 5–3,000 Hz. An averager (QC-111J Nihon Kohden) was used to average 128 responses over a 50-ms period gated to the time of 5 ms before stimulation. The waveforms captured on a memory oscilloscope (VC-11, Nihon Kohden) were recorded by means of a data acquisition system (MacLab/400, AD Instruments, Castle Hill, NSW, Australia). Median nerve SEPs consisted of a main positive component P wave. The P wave could be recorded only on the contralateral sensory area. This positive component was considered to be the primary response of the cortex to median nerve stimulation [12]. The amplitude and latency of the main component were measured from each SEP recording. The amplitude was measured from the baseline to the peak of the component (Fig. 1) and expressed as a percentage of the value during the baseline period. The SEP was measured 30 and 15 min before and 5, 30, and 60 min after the onset of HDT. After 30 min in the horizontal prone position (baseline period), the rabbits were tilted head-down for 1 h. Before and 1 h after the onset of HDT, 0.3 ml of arterial blood was drawn through a three-way stopcock to measure $P_{\text{aCO}_2}$. The same amount of physiological salt solution was injected to replace the blood each time. To confirm the hydrostatic effect of tilting on the systemic circulation, the arterial pressure in the abdominal aorta was measured by a pressure transducer (TP-400T, Nihon Kohden), which was secured at the same height as the tip of the arterial catheter and recorded on a thermal array recorder (RTA-400, Nihon Kohden).

Data analysis. Data shown in the text, figures, and tables are expressed as mean ± standard error of the mean (SEM). The results were analyzed statistically by the use of a paired $t$-test between two groups or repeated-measures analysis of variance (ANOVA) followed by a post hoc Fisher’s test for multiple comparisons. A $p$ value of less than 0.05 was considered significant. All analyses were done by means of STATISTICA (StatSoft, Tokyo, Japan).

RESULTS

Experiment 1

Typical recordings of the mean arterial pressure and CBF during the acute phase of HDT are shown in Fig. 2. The mean arterial pressure in the abdominal aorta in eight rabbits was 104.9 ± 5.7 mmHg at horizontal prone, 95.4 ± 5.0 mmHg at 45° HDT, and 87.5 ± 5.0 mmHg at 75° HDT. Although the mean arterial pressure in the abdominal aorta decreased at the onset of HDT as a result of the hydrostatic effect, CBF did not change significantly throughout the experiment. The CBF in eight rabbits was 100.8 ± 1.5% at 45° HDT and 102.4 ± 2.9% at 75° HDT.

Experiment 2

Physiological variables. Table 1 shows changes in mean arterial pressure, arterial CO₂ ten-
tion, and pH in each group. The mean arterial blood pressure in the abdominal aorta decreased significantly during 75° HDT but not during 45° HDT. \( P_{aCO_2} \) and pH did not change significantly throughout the experiment in any group.

**Cerebral blood flow.** In the control group, CBF did not change significantly throughout the experiment. In the 45° HDT group, CBF did not change significantly after the onset of HDT. At 1 h after 45° HDT, the CBF was 95 ± 6% of the baseline value. However, in the 75° HDT group, CBF decreased gradually to 79 ± 5% of the baseline value at 1 h after the onset of HDT, which was significantly \( p<0.05 \) lower than the corresponding value in the control group (Fig. 3).

**Somatosensory-evoked potentials**

Figure 4 shows a typical recording of SEPs before and during HDT in the 45° HDT group and in the 75° HDT group. In the control group, the baseline value of the latency was 14.8 ± 0.1 ms and did not change significantly throughout the experiment. No significant differences were found in the baseline value of latency between any two groups. Neither did SEP latency change significantly in the 45° HDT group or the 75° HDT group throughout the experiment.

In the control group, the SEP amplitude did not change significantly throughout the experiment. The SEP amplitude was 104 ± 7% at 1 h after 45° HDT and 106 ± 5% at 1 h after 75° HDT. These values were not significantly different from the corresponding value obtained in the control group.

**DISCUSSION**

The major findings in the present study are as follows: (1) Contrary to our hypothesis, CBF did not increase significantly in the cerebral cortex of rabbits within 5 min after the onset of 45° or 75° HDT; (2) CBF decreased to 79% of the pre-HDT baseline during 1 h of 75° HDT, but did not change in the 45° HDT group; and (3) exposure to 45° or 75° HDT for 1 h did not affect the amplitude and latency of SEP.

The effects of microgravity on the cerebral circulation have been investigated in humans using noninvasive techniques such as TCD ultrasound [5, 6], near-infrared spectroscopy (NIRS) [7], and SPECT [8], as well as in animals [14–18]. No study, however, has ex-
examined the time course of CBF in animal experiments. In this study, we employed LDF to measure CBF continuously, and since surgery is necessary to apply this technique, animal experiments were carried out instead of human studies. Although rat tail suspension has been extensively used as an animal model for simulated microgravity, we used rabbits: the bigger the size of the animal, the greater the hydrostatic effect produced by HDT. For example, the increase in intracranial pressure caused by exposure to 45° HDT is approximately 2 mmHg in rats [15] and 9 mmHg in rabbits [16].

Two types of Doppler probes were used in the present study. Preliminary studies demonstrated that tilting an animal caused an artifact on the output of the signal obtained from a Doppler probe placed on the dura, which prohibited the comparison of CBF measurements obtained before and during HDT. Thus in the first series of experiments, the probe was inserted into the cerebral cortex to avoid its dislocation during tilting. As shown in Fig. 2, no artifact was observed in these recordings. In the second series, the probe was positioned on the dura. It was important to minimize the tissue damage in these experiments, since SEPs were measured simultaneously. Although the artifact was produced during the tilt, the CBF measurement did not begin until after completion of the tilt. The CBF during the first 5 min of HDT was taken as the control. Therefore the artifact resulting from the dislocation of the probe did not affect the results.

To our surprise, CBF did not change in the rabbit parietal cortex during 1 h of 45° HDT. There are several factors in our experimental design that differ from those of the human studies, including species, tilt angle, anesthesia, and technique for measuring CBF. It is worthwhile, however, to compare our results with those obtained in human studies. Kawai and colleagues [6] demonstrated by means of TCD ultrasound that mean CBF velocity increased to 61.5 cm/s at 30 min after the onset of HDT in humans, from a baseline value of 55.5 cm/s. The CBF velocity was measured in the middle cerebral artery. Therefore there are two possibilities to explain these observations. First, the cross-sectional area of the middle cerebral artery may have decreased while the flow velocity increased, keeping the CBF constant during HDT. This is possible because HDT increases the intraluminal pressure in cerebral arteries, which in turn causes myogenic contraction of smooth muscles in the arteries. Second, CBF actually increased in some parts of the cerebral tissue fed by the middle cerebral artery, but not in the cortex. This is consistent with the finding obtained by a SPECT study in which CBF increased at 5 min after the onset of HDT in the basal ganglia, but not in the cerebral hemispheres [8].

Various tilt angles have been tested in previous experiments. Thus in the present study, we examined the effects of 75° HDT on CBF as well as those of 45° HDT. Although CBF did not change within 5 min after the onset of 75° HDT, it decreased gradually to 79% of the baseline value at the end of 1 h of HDT. These findings suggest that the regulation of CBF is well tolerated against HDT up to 45°, but not to 75°.

The latency and amplitude of SEPs did not change during 1 h of HDT in rabbits. Yamanaka and colleagues [19] showed that motor-evoked potentials were not altered after 20° d of HDT in human subjects. On the other hand, Vaitl and co-workers [20] demonstrated that the fluid shift induced by HDT resulted in increases in δ and θ frequency bands in electroencephalogram and slow reaction time of task, which implied cortical inhibition. The inhibitory effects, however, were observed during 23 h of HDT. Wei and colleagues [11] reported that exposure to 15° HDT for 10 min decreased the amplitude of the N100 component, but the peak-to-peak amplitude did not change significantly. These findings suggest that a considerable change in SEP amplitude does not occur during the early phase of HDT, but it may change after prolonged exposure to HDT.

In conclusion, CBF and SEP do not change significantly during 1 h of 45° HDT. Exposure to 75° HDT disturbs the regulation of the cerebral circulation, but it does not affect cortical function, at least for 1 h.

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