ACTA MED OKAYAMA 2000; 54(1): 15-20

Increase in Cerebral Blood Flow as a Predictor of Hyperbaric Oxygen-Induced Convulsion in Artificially Ventilated Rats

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In spontaneously breathing rats, a transient increase in cerebral blood flow (CBF) has been shown to be a predictor of hyperbaric oxygen (HBO)-induced convulsion. In the present study, we evaluated whether artificially ventilated animals also show an increase in CBF prior to the onset of HBO-induced convulsion. Rats were ventilated with 100% oxygen in 5 atmospheres. CBF, blood pressure, and an electroencephalogram were monitored continuously. Convulsion was observed at 41 ± 12 min after the initiation of HBO treatment. A single abrupt increase in CBF, reaching 223 ± 39% of the control level, was observed at 29 ± 13 min after the initiation of HBO exposure and lasted until the onset of convulsion 12 ± 2 min later. The time of the increase in CBF correlated strongly with the onset of convulsion ($r = 0.99$, $P < 0.001$). Further, the logistic regression curve demonstrated a close relationship between the duration of increased CBF and percentage of epileptiform electrical-discharge incidence ($r = 0.92$, $P < 0.006$). The durations of increased CBF causing convulsion in 10%, 50%, and 90% of the rats were 8.4 min, 11.7 min, and 15.1 min, respectively. These results indicate that an increase in CBF is a predictor of HBO-induced convulsion in artificially ventilated rats. The increase in CBF may be involved in the pathogenesis of HBO-induced convulsion.

Key words: oxygen toxicity, laser-Doppler flowmetry, seizures, electroencephalogram, artificial ventilation

Hyperbaric oxygen (HBO) has been used in treatment for carbon monoxide poisoning (1), decompression sickness (2), cerebral ischemia (3), and so on. However, oxygen has a degree of toxicity to the central nervous system, manifest in the appearance of tonic clonic convulsion (4, 5). Consequently, the clinical use of HBO has been severely curtailed to avoid oxygen toxicity at high pressure.

In spontaneously breathing rats, HBO causes several transient increases in cerebral blood flow (CBF) prior to the appearance of the first epileptiform electrical-discharge (6, 7). Recently, Chavko *et al.* (6) observed that the onset time of the first transient increase in CBF (5 ± 2 min after the initiation of HBO administration) closely relates to, and is about 25-30% of, the onset time of convulsion (19 ± 6 min). Thus, CBF could be monitored and used as a predictor of HBO-induced convulsion, thereby improving the safety of HBO administration in a clinical setting.

HBO has complex effects on the respiratory system, causing CO_2 to accumulate in peripheral tissue due to reduced CO_2 transport capacity (8) (Haldane effect), and causing CO_2 production to increase in peripheral tissue by increasing the work of breathing to compensate for the high viscosity of oxygen. Consequently, during HBO administration, decrease in end tidal CO_2 pressure due to hyperventilation has been observed in spontaneously breathing humans (5). In contrast, artificially ventilated animals do not assume the increased work to breathe, and their $PaCO_2$ levels are maintained within the normal range. Because CBF is greatly influenced by arterial carbon dioxide pressure ($PaCO_2$), it remains unclear whether the preconvulsive changes in CBF also occur in artificially ventilated animals. In the clinical setting, unconscious patients are sometimes ventilated during HBO administration. Because these patients cannot manifest preconvulsive phenomena, such as progressive contraction of the visual field (9) and muscle twitching (5), a

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reliable predictor of convulsion is especially important to ensure safe HBO administration.

The present study was designed to test whether artificially ventilated animals show predictable changes in CBF prior to the onset of convulsion. To this end, continuous electroencephalography (EEG) and monitoring of CBF and blood pressure were performed in rats during HBO administration.

Materials and Methods

Anesthesia and general surgical procedures. The animals were housed and handled in accordance with the guidelines set out by the Council of the American Physiological Society. Ten adult male Wistar rats weighing 210–370 g (Charles River, Yokohama, Japan) were used. Animals had *ad libitum* access to commercial laboratory animal food and water. Anesthesia was induced with 3% halothane in oxygen, and surgical fields were shaved. Following tracheal intubation, anesthesia was maintained using 1% halothane in a mixture of 70% nitrogen and 30% oxygen under mechanical ventilation (SN-480-7, Shinano, Tokyo, Japan). Catheters (PE-50) were inserted into the tail artery, femoral artery, and femoral vein for purposes of blood sampling, blood pressure monitoring, and continuous infusion of analgesics, respectively.

Monitoring. After placement in a stereotaxic apparatus (Narishige, Japan), each rat's skull was exposed, and a burr hole was drilled 3 mm posterior and 3 mm lateral to the bregma. A laser Doppler flowmeter probe (ALF2100, Advance, Japan) was placed on the surface of the dura through the burr hole for continuous measurement of regional CBF. A laser Doppler flowmeter does not measure absolute CBF, rather, it accurately measures relative changes in absolute CBF (10). EEGs were recorded *via* needle electrodes placed subcutaneously in the right frontal region. All data were gathered and analyzed using an analog-digital system (AxoScope and Digidata1200B, Axon Instruments, USA). The changes in EEG were evaluated by power spectrum analysis in each frequency band; *i.e.*, Δ(1–4 Hz), θ(4–8 Hz), α(8–13 Hz), and β(13–30 Hz). Following preparation for monitoring, the exposed skull was covered with paraffin oil to reduce gas exchange between the exposed cortical area and the ambient atmosphere (11). Pre-compression values were used as control data.

HBO procedure. An HBO 300-liter chamber (PHC-special products, TABAI, Japan) was used for all exposures. After the rats had been placed individually in the HBO chamber, halothane anesthesia was changed to fentanyl analgesia (the ED50 for preventing pain in rats is 13 μg/kg (12)). Following a single injection of fentanyl (15 μg/kg) and pancuronium (1 mg/kg) through the femoral vein, fentanyl and pancuronium were continuously infused at rates of 5 μg/kg/h and 1 mg/kg/h, respectively. The animals were ventilated in pure oxygen. After a 30-min equilibration period, each animal was compressed with 100% oxygen to 5 atmospheres absolute (atm abs) at the rate of 1 atm abs/min, and kept in oxygen at 5 atm abs for 60 min.

Physiological variables. During preparative surgery and the experimental period, arterial blood pressure was continuously recorded. Arterial blood gases, pH (ABL4/0, Radiometer, Denmark), blood glucose, and hemoglobin were measured before and at 20 min after the onset of compression. Body temperature was kept at 37.0 ± 0.5 °C using a rectal thermocouple connected to a heated water blanket in the HBO chamber.

Statistical analysis. Values are expressed as mean $\pm$ SD. Changes in physiological variables before and at 20 min after HBO onset were evaluated using the paired Student's t-test. Linear and logistic regression analysis were used to evaluate the correlation of the onset time of increased CBF with that of the first electrical discharge, and the correlation of duration of increased CBF with the percentage of discharge incidence, respectively. A level of $P < 0.05$ was considered to be significant in all statistical tests.

Results

No differences in physiological variables, except PaO_2, were observed between before and at 20 min after the onset of HBO (Table 1). The controlled ventilation successfully kept $PaCO_2$ stable under hyperbaric conditions.

A set of representative results is shown in Fig. 1. In the present study, the first epileptiform electrical-discharge, which was distinguished by paroxysmal spiking-activity with an amplitude of more than 100 μV, was observed at 41 ± 12 min after the onset of HBO. Using a laser Doppler flowmeter, a single abrupt increase in CBF was observed at 29 ± 13 min after the onset of HBO, and this increased level lasted until the appearance of the first electrical discharge 12 ± 2 min later. The

Table 1 Values of physiological variables before and during hyperbaric oxygen (HBO) exposure

	pH	$PaCO_2$ (mmHg)	PaO_2 (mmHg)	Glucose (mg/dl)	Hemoglobin (g/dl)
Before HBO exposure	7.42 ± 0.04	37 ± 4	371 ± 63	119 ± 29	16 ± 1
During HBO exposure*	7.41 ± 0.04	35 ± 3	not measured	120 ± 30	16 ± 1

*20 min after the onset of oxygen compression
All values are means ± SD. (n = 10)

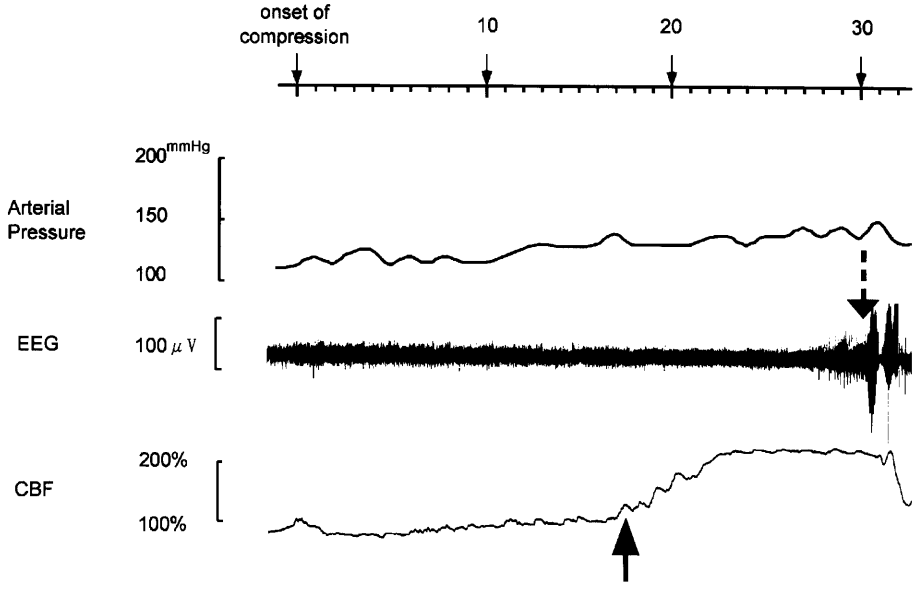

Fig. 1 A set of representative results showing the changes in mean arterial blood pressure, electroencephalogram (EEG), and laser-Doppler flowmetry during 60 min of hyperbaric oxgen (HBO) (5 atmospheres absolute). Mean arterial blood pressure gradually increased with the duration of exposure to HBO. The laser-Doppler flowmeter detected an abrupt increase in cerebral blood flow (CBF) at 17 min after the commencement of HBO (arrow). The increase in CBF reached 280% of the control level, and this level was sustained until the appearance of the first electrical discharge. Following 3 min of spiking activity, EEG reading showed the first electrical discharge at 31 min after the commencement of HBO (dotted arrow). The EEG did not show any activation preceding the increase in CBF.

increase in CBF reached 223 ± 39% of the control level at the time of the appearance of the discharge. With the administration of HBO, mean arterial blood pressure steadily increased from a pre-HBO value of 121 ± 10 mmHg to 164 ± 13 mmHg at the onset of discharge. EEG readings did not show remarkable changes prior to the increase in CBF. Preceding the appearance of the first electrical discharge, sporadic spikes were observed for 2–3 min (Fig. 2).

As shown in Fig. 3, a close relationship was found between the onset time of increased CBF (29 ± 13 min) and that of the first electrical discharge (41 ± 12 min) ($R = 0.99$, $P < 0.001$). Fig. 4 shows the relationship between the duration of increased CBF and the percentage of discharge incidence ($R = 0.92$, $P = 0.006$). By using the regression curve, the durations of increased CBF (with a 95% confidence interval) causing discharge in 10%, 50%, and 90% of the animals were estimated to be 8.4 min (5.4–9.7 min), 11.7 min (11.0–12.4 min), and 15.1 min (13.9–17.6 min), respectively.

Fig. 5 shows regional CBF, mean arterial blood pressure, and power spectrum analysis of EEG for the 20 min preceding the first electrical discharge. Mean arterial blood pressure increased continuously after the onset of HBO and reached 145 ± 12 mmHg at the time at which CBF started to increase. In the power spectrum analysis

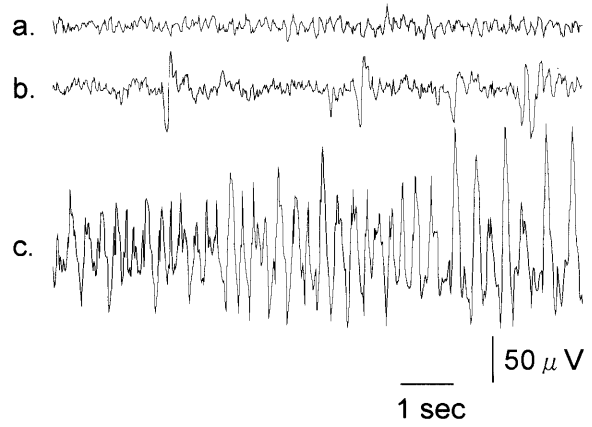

Fig. 2 Representative EEG during HBO administration. (a) 5 min after the commencement of HBO. (b) 2 min before the onset of the first electrical discharge. Sporadic spikes are observed. (c) The first electrical discharge. EEG; HBO, see legend to Fig. 1.

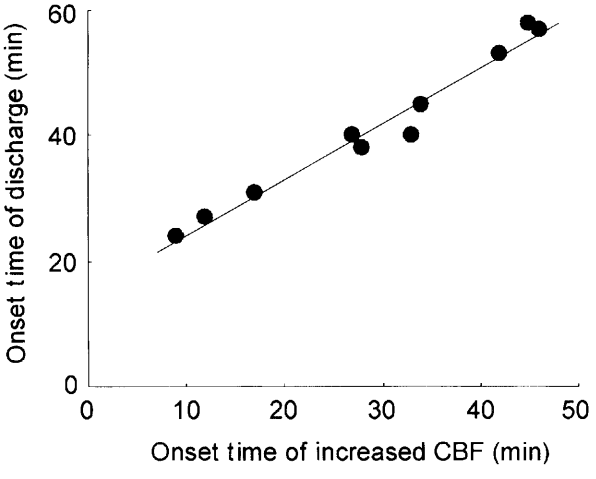

Fig. 3 Relationship between onset time of increased CBF and that of the first electrical discharge in artificially ventilated animals (n = 10) during HBO administration (5 atmospheres absolute). In spite of the difference in onset times of discharge among animals, the onset of increased CBF was overall closely related to discharge ($R = 0.99$, $P < 0.001$). CBF; HBO, see legend to Fig. 1.

of EEG, all bands indicated a gradual decrease in amplitude during HBO administration and showed no activation at the onset of increased CBF.

Discussion

We observed an abrupt but steady increase in CBF at

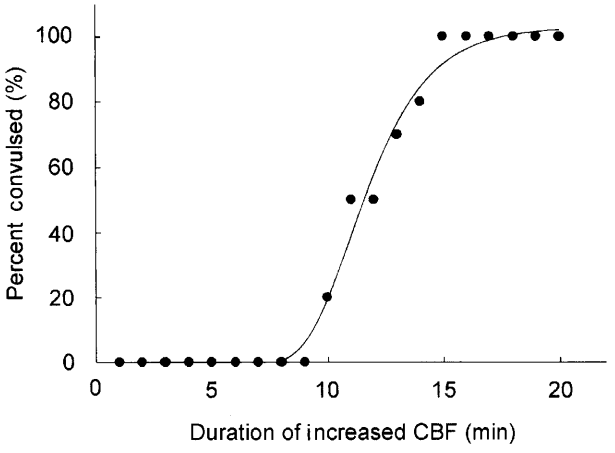

Fig. 4 Relationship between the duration of increased CBF and the percentage of discharge incidence in artificially ventilated animals (n = 10) during HBO administration (5 atmospheres absolute) ($R = 0.92$, $P = 0.006$). Points respectively represent the percentage of discharge incidence at every minute after the onset of increased CBF. The duration of increased CBF causing discharge in 50% of rats was estimated to be 11.7 min (11.0–12.4 min, 95% confidence interval). CBF; HBO, see legend to Fig. 1.

29 ± 13 min after the onset of HBO, which preceded convulsion by 12 ± 2 min. The onset time of the increased CBF was closely related to that of the first electrical discharge (Fig. 3; $R = 0.99$, $P < 0.001$), which finding demonstrates that the increase in CBF observed in artificially ventilated animals is a predictor of the first electrical discharge at approximately 12 ± 2 min before onset. Moreover, as Fig. 4 shows, the dose-response relationship revealed a high correlation between duration of increased CBF and the percentage of discharge incidence ($R = 0.92$, $P = 0.006$). Using this regression curve, the duration of increased CBF causing discharge in 50% of rats was estimated to be 11.7 min (11.0–12.4 min, 95% confidence interval). This close relationship suggests that the duration of increased CBF may play a part in the pathogenesis of HBO-induced discharge. Thus, monitoring changes in CBF appears to provide a useful method for predicting the onset of convulsion in artificially ventilated subjects during HBO administration.

Fentanyl is an opioid and has little effect on CBF or the cerebral metabolic rate of oxygen (13, 14). Furthermore, it does not induce seizures by itself, even in response to massive doses (15, 16). In the present study, we used fentanyl to achieve an analgesic state in rats, at

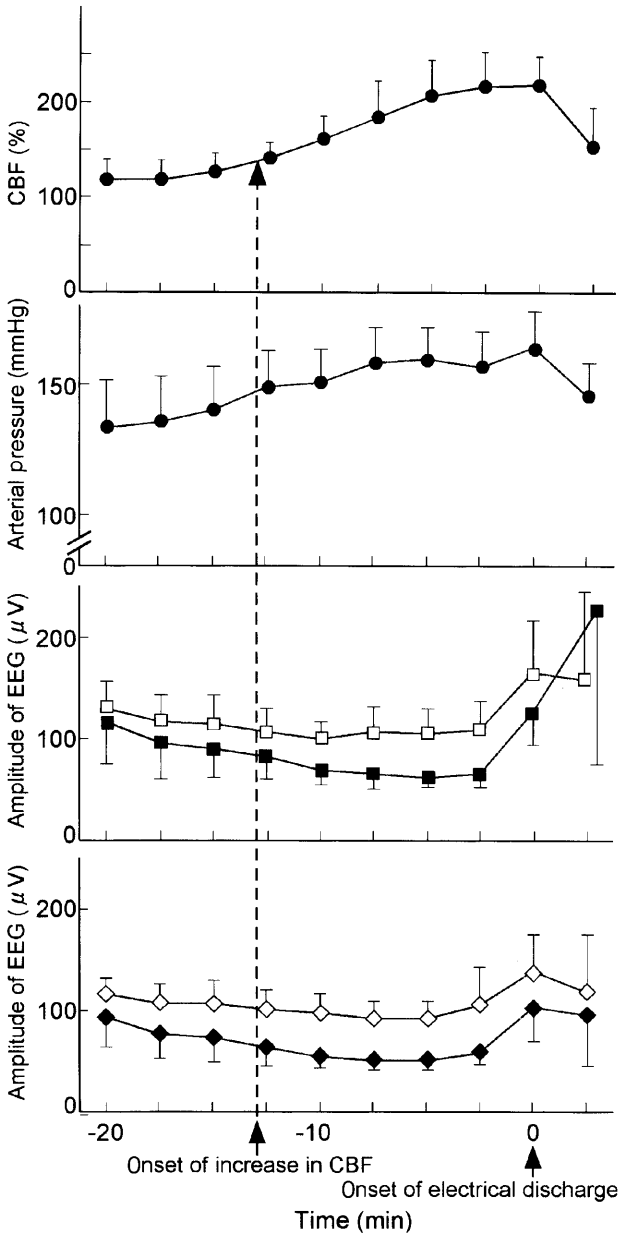

Fig. 5 Open square, filled square, open diamond, and filled diamond represent amplitudes of θ, Δ, β, and α bands, respectively. The figure shows changes in CBF, mean arterial blood pressure, and power spectrum analysis of EEG during the 20 min preceding the appearance of the first electrical discharge. Points respectively represent the mean of changes every 2.5 min. The dotted line represents the onset of the increase in CBF (increase of 10% of control level per min). The power spectrum analysis of EEG in all bands showed no changes at the onset of the increase in CBF.
CBF; EEG, see legend to Fig. 1.

a dose level (initial dose of 15 μg/kg + a continuous dose of 5 μg/kg/h, intravenously) that exceeded the ED_{50} for preventing pain in rats (13 μg/kg (12)). In addition, we confirmed in preliminary studies that animals were mildly sedated and adapted to the stereotaxic apparatus at this dose of fentanyl for 60 min.

Chavko *et al.* (6) observed that several transient increases in CBF preceded the appearance of the first electrical discharge in spontaneously breathing animals at 5 atm abs. In their experiment, the first increase in CBF and the first electrical discharge were observed 5 $\pm$ 2 min and 19 $\pm$ 6 min after the commencement of HBO, respectively. In the present study, however, artificially ventilated animals showed a single abrupt increase in CBF 29 $\pm$ 13 min after HBO administration, which was sustained until the appearance of discharge 12 $\pm$ 2 min later. Given that the animals in these experiments were exposed to HBO at the same pressure (5 atm abs), it is unlikely that an increase in PaO_2 and/or increased oxygen radicals can account for the disparate results. In humans, the work of breathing in a resting period is 3 J/min under ambient pressure. If the work of breathing increases to 3 times (8–10 J/min) that in a resting period, fatigue of the diaphragm becomes apparent (17). In rats, airway resistance is much higher than it is in humans (airway resistance is inversely proportional to the fourth power of the airway radius (18)), and is further increased by the high viscosity of compressed oxygen. Moreover, respiration is interfered with by the turbulent flow corresponding to an increase in Reynold's number, which increase is the result of high oxygen pressure (18). Although the current experiment did not provide data that would account for discrepancies in CBF behavior, the increased work of breathing during HBO administration may transiently increase the $PaCO_2$ level and CBF in spontaneously breathing animals. Since CBF is one of the determining factors of tissue oxygen-pressure, the repetitive increases in CBF elevate the oxygen-pressure in cerebral tissue and might exacerbate the oxygen toxicity in spontaneously breathing animals.

In the present study, power spectrum analysis of the EEGs did not detect any activation in responce to the onset of increased CBF (Fig. 5), indicating that the mechanism of increased CBF is not directly related to an electrophysiological event. In contrast, mean arterial blood pressure increased from a pre-HBO value of 121 $\pm$ 10 mmHg to 145 $\pm$ 12 mmHg at the onset of increased CBF. This value is close to the upper limit of the

autoregulatory range in CBF (50–150 mmHg) (19). Once blood pressure exceeds this range, increased vascular resistance decreases due to pathological dilatation of short segments of cerebral vessels (20). Given that blood pressure steadily increased until the appearance of convulsion (164 ± 13 mmHg), dilatation of cerebral vessels due to high blood pressure might be involved in the increase in CBF. Acidosis in cerebral tissue is another factor that might contribute to the increase in CBF. Since accumulation of lactate is observed in cortical tissue preceding the appearance of convulsion (21), it is possible that lactic acidosis decreases the pH in cerebral vessels. However, the gradual accumulation of lactate may not occur simultaneously with the abrupt increase seen in CBF (Fig. 1). The endothelium-derived relaxing factor, nitric oxide, also has a strong effect on the dilatation of cerebral vessels. However, its depressor action is inhibited by superoxide radicals (22, 23). Increases in free radicals have been observed during HBO administration (24, 25); the as yet unknown contribution of nitric oxide to the increase in CBF remains to be elucidated.

In conclusion, in the present study, artificially ventilated animals showed an abrupt increase in CBF 29 ± 13 min after the commencement of HBO (5 atm abs), and this increased level was sustained for 12 ± 2 min, at which time the first electrical discharge occurred. The duration of increased CBF causing discharge in 50% of rats was estimated to be 11.7 min (11.0–12.4 min, 95 % confidence interval.) These results demonstrate that monitoring changes in CBF appears to provide a useful method for predicting the onset of convulsion in artificially ventilated subjects during HBO administration.

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Received October 1, 1999; accepted November 11, 1999.