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Abstract

To elucidate the mechanism by which hyperbaric oxygen (HBO2) induces electrical discharge, changes in the extracellular concentrations of GABA and glutamate were measured every 5 min using a microdialysis technique in rats during a period of exposure to HBO2 (5 atm abs). Electrical discharge was observed at 28 +/- 4 min after the onset of exposure. Though the extracellular concentrations of glutamate remained unchanged, the extracellular GABA concentrations (pre-exposure level, 0.026 +/- 0.005 microM in dialysate) began to decrease 15 min after the onset of exposure and reached their lowest level (74 +/- 14%, 0.019 +/- 0.004 microM) at the time of appearance of the discharge. There was a close logistic relationship between extracellular GABA concentrations and the discharge incidence, and the extracellular concentrations of GABA causing electrical discharge in 50% of the animals were estimated to be 80% of the pre-exposure level. These results suggest a possible mechanism that HBO2 exposure-induced discharge is caused by the decrease in extracellular concentration of GABA.

KEYWORDS: glutamic acid, hyperbaric oxygenation, gamma-aminobutyricacid, microdialysis, seizures

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Original Article

The Close Relationship between Decreases in Extracellular GABA Concentrations and Increases in the Incidence of Hyperbaric Oxygen-Induced Electrical Discharge

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To elucidate the mechanism by which hyperbaric oxygen (HBO_2) induces electrical discharge, changes in the extracellular concentrations of GABA and glutamate were measured every 5 min using a microdialysis technique in rats during a period of exposure to HBO_2 (5 atm abs). Electrical discharge was observed at 28 ± 4 min after the onset of exposure. Though the extracellular concentrations of glutamate remained unchanged, the extracellular GABA concentrations (pre-exposure level, 0.026 ± 0.005 μM in dialysate) began to decrease 15 min after the onset of exposure and reached their lowest level (74 ± 14%, 0.019 ± 0.004 μM) at the time of appearance of the discharge. There was a close logistic relationship between extracellular GABA concentrations and the discharge incidence, and the extracellular concentrations of GABA causing electrical discharge in 50% of the animals were estimated to be 80% of the pre-exposure level. These results suggest a possible mechanism that HBO_2 exposure-induced discharge is caused by the decrease in extracellular concentration of GABA.

Key words: glutamic acid, hyperbaric oxygenation, gamma-aminobutyric acid, microdialysis, seizures

Despite the beneficial effects of exposure to hyperbaric oxygen (HBO_2) for treatment of carbon monoxide poisoning [1-3], gas gangrene [4-6], and cerebral ischemia [7-9], the clinical use of HBO_2 has been severely curtailed due to oxygen toxicity in the central nervous system under hyperbaric conditions, which are manifested by the appearance of convulsions and electrical discharge in an electroencephalogram.

GABA is a major inhibitory amino acid in the brain. Because the total GABA content in cortical tissue is reduced during HBO_2 exposure [10, 11], decreases in the levels of GABA activity may play an important role in the mechanism by which HBO_2 induces discharge. However, because the total GABA content was measured in these previous studies at a single time point (10.5 min and 20 min after the onset of HBO_2 exposure, 6 atm abs) without monitoring an electroencephalogram in mice and rats, respectively, the time course of GABA activity until the appearance of HBO_2-induced electrical discharge has not been shown, nor has the relationship between GABA activity and the incidence of electrical discharge been analyzed.

To examine the possible involvement of decreases in GABA activity in the mechanism of the induction of electrical discharge by HBO_2, dynamic changes in the extracellular concentrations of GABA and of glutamate were observed (every 5 min) until the appearance of electrical discharge using a microdialysis technique in the rat cerebral cortex under hyperbaric conditions (5 atm abs). The relationship between the discharge incidence

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and extracellular GABA concentrations was logistically analyzed.

Materials and Methods

Twenty-eight male Wistar rats weighing 348 ± 30 g (Charles River Japan, Yokohama, Japan) were used. All experiments were performed in accordance with the National Institutes of Health animal care guidelines and were approved by the Animal Research Control Committee of Okayama University Medical School.

Anesthesia was induced with a mixture of 3% halothane in oxygen. Following oral tracheal intubation, anesthesia was maintained by artificial ventilation (SN-480-7; Shinano, Tokyo, Japan) with 1% halothane in 40 % oxygen balanced with nitrogen. Polyethylene catheters (PE-50) were inserted into the tail artery, right femoral artery, and right femoral vein for the purposes of blood sampling, blood pressure monitoring, and continuous infusion of anesthetics, respectively. After catheterization, halothane anesthesia was changed to fentanyl anesthesia, as volatile anesthetics cannot be used under hyperbaric conditions and because fentanyl has little effect on CBF [12] or on the cerebral metabolic rate of oxygen [13]. Following a single injection of fentanyl (15 μg/kg) and pancuronium (1 mg/kg) through the right femoral vein, fentanyl and pancuronium were continuously infused at rates of 5 μg/kg/h and 1 mg/kg/h, respectively, to maintain constant anesthesia levels. After each rat had been placed in a stereotaxic apparatus (Narishige, Tokyo, Japan), microdialysis probes (A-I-4-02; membrane length of 2 mm, molecular weight cutoff of 50,000, outer diameter of 0.22 mm; Eikom, Kyoto, Japan) were implanted in the left parietal cortex according to the reported brain atlas [14] (shown in Fig. 1; 3 mm posterior to and 3 mm left of the bregma). An EEG (S1516, Nihon Koden, Tokyo, Japan) was recorded via needle electrodes placed subcutaneously in the right frontal region. All data were collected and analyzed using an analog-digital system (AxoScope and Digidata 1200B; Axon Instruments, Foster, CA, USA).

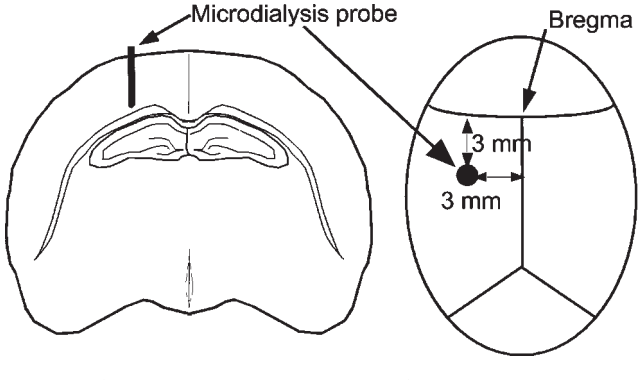

Fig. 1 Coronal and dorsal coordinates of the microdialysis probe site of implantation. The microdialysis probes were implanted in the left parietal cortex (3 mm posterior to and 3 mm left of the bregma).

Exposure to HBO_2. A 300-liter chamber was used (PHC-special products, TABAI, Tokyo, Japan). Fourteen animals were pressurized to 5 atm abs with pure oxygen until the appearance of electrical discharge and were used as an HBO_2 group. Another 14 animals were pressurized to 5 atm abs with 8% oxygen for 60 min to show normal PaO_2 levels and were used as a control group. Rectal temperature was monitored and maintained at 37.0 ± 0.5 °C with a heated water-blanket during the period of exposure to HBO_2. The animals were ventilated using a mechanical ventilator. HBO_2 exposure (5 atm abs) was undertaken at a rate of 1 atm abs/min until the appearance of electrical discharge or for 60 min. Decompression was carried out at a rate of 0.2 atm abs/min. After the termination of HBO_2 exposure, animals were sacrificed to confirm the location of the dialysis probe. The probe tip was found in the subcortical white matter in the parietal cortex, and it was confirmed that no probe had penetrated to the subcortical gray matter.

Sample collection. The microdialysis probes were perfused with Ringer's solution at a flow rate of 2 μl/min using an infusion pump (ESP-32, Eikom, Kyoto, Japan), and the dialysate was collected every 5 min. For the assay of GABA concentrations, the lower limit of the HPLC system used in the present study was 60 femto mol. Because the amount of GABA in the dialysate (10 μl) was too small (260 ± 50 femto mol in the control sample) to assay the GABA and glutamate concentrations in the same sample, the extracellular concentrations of GABA and glutamate were measured from different animals (n = 7 in each group).

Assays of GABA and glutamate. Extracellular levels of GABA and glutamate were assayed using microbore HPLC with computerized control (Nanospace Syscon 21, Shiseido, Tokyo, Japan). With the use of a refrigerated microsampler (Nanospace SI-2 3023, Shiseido, Tokyo, Japan), the samples (each 8 μl) were automatically precolumn-derivatized for 2 min with a mixture of o-phthalaldehyde and b-mercaptoethanol (2 μl)

[15]. The samples were separated isocratically using a C_{18} reverse-phase column (1.5 × 150 mm, 3 μm, UG120, Shiseido, Tokyo, Japan). For electrochemical detection, oxidative potential was applied to a glassy carbon working electrode at 700 mV *vs.* an Ag/AgCl reference electrode. For the assay of GABA, 25% acetonitrile in 100 mM sodium acetate buffer (pH 6.0) was used as the mobile phase and was pumped using a delivery unit equipped with a micro volume double-plunger system (Nanospace SI-2 3001, Shiseido, Tokyo, Japan) at a rate of 200 μl/min. For the assay of glutamate, 15% acetonitrile in 100 mM sodium acetate buffer (pH 6.0) was used as the mobile phase and was pumped using a delivery unit equipped with a micro volume double-plunger system (Nanospace SI-2 3001, Shiseido, Tokyo, Japan) at a rate of 150 μl/min.

Statistical analysis. Values are expressed as means ± SDs. Changes in the extracellular concentrations of GABA and glutamate were evaluated by repeated measurement analysis of variance (ANOVA). If the F value was significant, Scheffé's F test was performed. A logistic regression curve (probit curve) was used to evaluate the probability of discharge incidence with decreases in the extracellular GABA concentrations. Because it is supposed that the probability of discharge incidence converges to 100% with very severe attenuation of extracellular GABA concentrations, linear regression was not used to evaluate the correlation. Instead, a probit curve, which expresses the probability of occurrence and is generally used to search for LD50 in toxicology, was used. A probit curve was drawn using personal computer software (Microcal Origin 5.0, Microcal Software, Northampton, MA, USA). A level of $P < 0.05$ was considered to be significant in all statistical tests.

Results

The values of physiological parameters, obtained before and at 20 min after the onset of HBO_2 exposure, are shown in Table 1. The values of all parameters were maintained within normal limits. PaO_2 in the control group was maintained at the same level before and during the period of exposure to HBO_2 by pressurization to 5 atm abs with 8% oxygen.

In the HBO_2 group, electrical discharge was observed at 28 ± 4 min after the onset of exposure, whereas no discharge was observed in the control group.

For the assays of GABA and glutamate concentrations, the linearity of the HPLC system used in the present study was confirmed in the range 0.006–0.100 μM (60–1000 femto mol in 10 μl of sample) and 0.1–10.0 μM (1–100 pico mol in 10 μl of sample), respectively. The recovery rates of the microdialysis probe were 10.8 ± 2% for GABA and 12.7 ± 2% for glutamate. The concentrations of GABA and glutamate in dialysate before initiation of HBO_2 exposure were 0.026 ± 0.005 μM and 0.89 ± 0.55 μM, respectively, and were within analytical limits in our HPLC system. The percent changes in extracellular GABA concentrations during HBO_2 exposure are shown in Fig. 2. The animals in the HBO_2 group showed a significant decrease in GABA concentrations from 15 min after the onset of HBO_2 exposure ($P = 0.006$). The GABA concentrations were maximally decreased at the time of appearance of the electrical discharge (mean, 0.019 ± 0.004 μM, 74 ± 14% of pre-exposure level; range, 85–47%). After the appearance of electrical discharge, the GABA concentrations were temporally increased, probably due to the membrane depolarization of neuronal cells. In contrast to these changes, the extracellular glutamate concentrations (Fig. 3) were found to be stable and were not affected by HBO_2 exposure. As shown in Fig. 4, there was a very close logistic relationship (probit curve, $P < 0.008$, $R^2 = 0.86$) between the probability of discharge incidence and percent changes in the extracellular GABA concentrations with regard to the appearance of the first electrical discharge. By using the logistic regression curve, the extracellular concentrations of GABA (with a 95%

Table 1 Physiological parameters before and during the period of exposure to hyperbaric oxygen

Group		pH	$PaCO_2$ (mmHg)	PaO_2 (mmHg)	Bloodsugar (mg/dl)	Hemoglobin (g/dl)
Control	Before	7.42 ± 0.02	37 ± 2	201 ± 27	110 ± 12	16 ± 1
(n = 14)	During	7.41 ± 0.02	40 ± 2	215 ± 33	112 ± 16	16 ± 1
HBO_2	Before	7.42 ± 0.03	38 ± 3	189 ± 30	122 ± 28	16 ± 1
(n = 14)	During	7.40 ± 0.03	37 ± 2	–	123 ± 20	16 ± 1

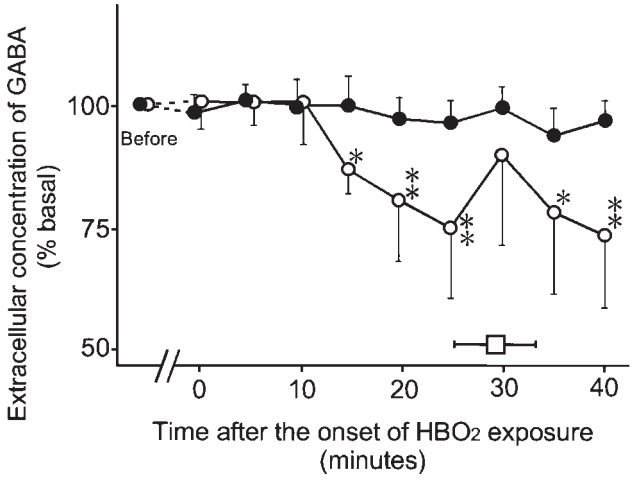

Fig. 2 Changes in extracellular GABA concentrations in the parietal cortex during the period of exposure to hyperbaric oxygen (HBO_2). Open and filled circles represent the HBO_2 group (pressurized to 5 atm abs with pure oxygen) and the control group (pressurized to 5 atm abs with 8% oxygen to show a normal PaO_2 level), respectively. The extracellular GABA concentrations were measured every 5 min. Decreases in GABA concentrations were observed in the HBO_2 group from 15 min after the onset of HBO_2 exposure. The open square indicates the time of onset of electrical discharge. Values are means ± SDs, * $P < 0.05$, ** $P < 0.01$.

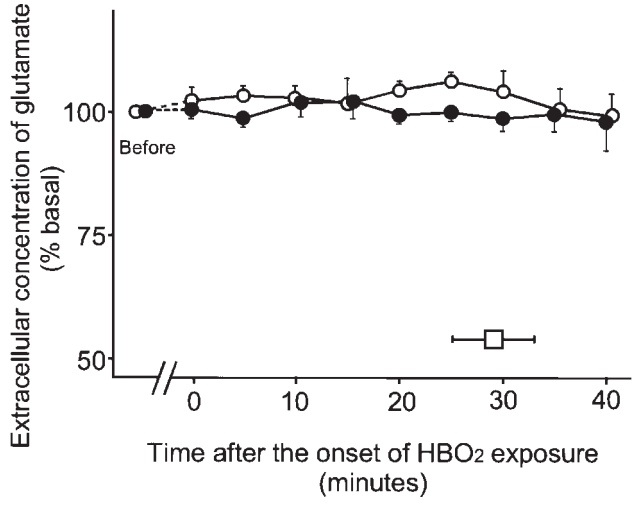

Fig. 3 Changes in extracellular concentrations of glutamate in the parietal cortex during the period of exposure to hyperbaric oxygen (HBO_2). Open and filled circles represent the HBO_2 group (pressurized to 5 atm abs with pure oxygen) and the control group (pressurized to 5 atm abs with 8% oxygen to show a normal PaO_2 level), respectively. The extracellular glutamate concentrations were measured every 5 min and remained stable during the exposure. The open square indicates the time of onset of electrical discharge. Values are means ± SDs.

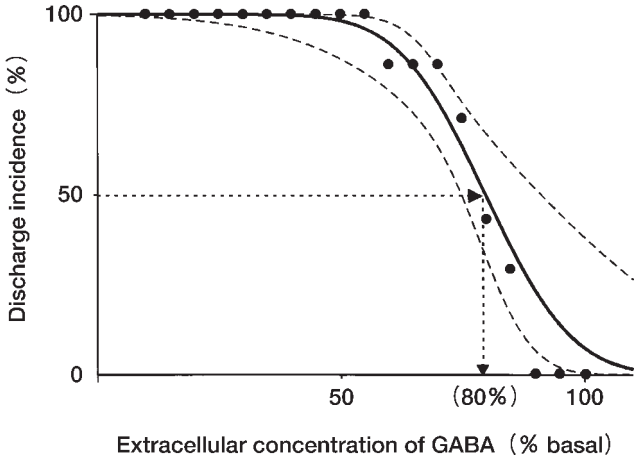

Fig. 4 Relationship between the probability of discharge incidence and percent changes in extracellular GABA concentrations regarding the appearance of the first electrical discharge ($P < 0.008$, $R^2 = 0.86$). Closed circles respectively represent the percentage of discharge incidence at every 5% of the extracellular concentration of GABA. The dashed lines represent the upper and lower limits of the 95% confidence interval. The extracellular GABA concentrations causing discharge in 50% of rats were estimated to be 80% of pre-exposure levels (arrow).

confidence interval) causing electrical discharge in 30%, 50%, and 70% of the animals were estimated to be 87 % (107–80%), 80% (91–75%), and 72% (78–66%) of the pre-exposure levels, respectively.

Discussion

The extracellular concentrations of GABA and glutamate during the period of exposure to HBO_2 until the appearance of electrical discharge were measured using a microdialysis technique and an HPLC system. Decreases in extracellular GABA concentrations were detected from 15 min after the onset of exposure, and GABA levels reached a minimum (74 ± 14%) at the time of the appearance of discharge (28 ± 4 min). These results indicate a possibility that the decrease in extracellular GABA concentrations is the cause of HBO_2 exposure-induced discharge.

As shown in Fig. 4, a logistic relationship was observed between the incidence of electrical discharge and decreases in extracellular GABA concentrations. By using this logistic regression curve, the decrease in extracellular GABA concentrations causing electrical

discharge in 50% of the animals was estimated to be 80 % of the pre-exposure level (95% confidence interval, 91–75%). To the best of our knowledge, this is the first study showing the threshold of extracellular GABA concentrations causing electrical discharge in a mammalian brain. This very close relationship between decreases in extracellular GABA concentrations and the percentage of discharge incidence ($P < 0.008$, $R^2 = 0.86$) indicates a possibility that controlling extracellular GABA levels may prevent HBO_2 exposure-induced discharge, although further study will be required to clarify the mechanism. In the present study, the extracellular GABA concentrations ranged from 85% to 47% of the pre-exposure levels in each animal at the time of appearance of the electrical discharge. In agreement with this finding, Hu *et al.* [16], who measured extracellular GABA concentrations in the rat during the appearance of spike-wave discharge (4–6 Hz) in response to injection of γ-hydroxybutyric acid, have reported that the spike-wave discharge is observed when extracellular GABA concentrations are lower than 87% of the control level (as indicated by a figure in the report by Hu *et al.*).

Extracellular GABA concentrations are modulated by GABA production, GABA release, and GABA uptake.

GABA is produced by decarboxylation of glutamate with the enzymatic activity of glutamic acid decarboxylase (GAD). It is unlikely that a decrease in GABA production is caused by a shortage of substrate, *i.e.*, glutamate, as the extracellular concentrations of glutamate in the present study remained unchanged (Fig. 3). It is more likely that the activity of GAD is decreased by HBO_2 exposure. It has been reported that oxygen (GAD65, Ki = 0.46 mM; GAD67, Ki = 0.29 mM) has inhibitory effects on GAD activity [10, 17]. Because the estimated turnover time of GABA in rat caudate nuclei is only 2.5 min [18], suppression of GAD activity due to increases in oxygen tension may reduce the GABA pool to a critical level, thus affecting extracellular GABA concentrations.

In summary, a decrease in the extracellular concentrations of GABA to 74 ± 14% of control levels was observed before the appearance of electrical discharge. There was a close logistic relationship between decreases in GABA concentrations and the discharge incidence. These results indicate a possible mechanism that HBO_2 exposure-induced discharge is caused by the decrease in extracellular concentration of GABA.

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References

1. Durmaz E, Laurence S, Roden P and Carruthers S: Carbon monoxide poisoning and hyperbaric oxygen therapy. Br J Nurs (1999) 8: 1067–1072.
2. Thom SR: Hyperbaric-oxygen therapy for acute carbon monoxide poisoning. N Engl J Med (2002) 347: 1105–1106.
3. Weaver LK, Hopkins RO, Chan KJ, Churchill S, Elliott CG, Clemmer TP, Orme JF Jr, Thomas FO and Morris AH: Hyperbaric oxygen for acute carbon monoxide poisoning. N Engl J Med (2002) 347: 1057–1067.
4. Korhonen K, Klossner J, Hirn M and Niinikoski J: Management of clostridial gas gangrene and the role of hyperbaric oxygen. Ann Chir Gynaecol (1999) 88: 139–142.
5. Lindsey D: Hyperbaric oxygen and gas gangrene. Br J Hosp Med (1996) 55: 147.
6. Morgan MS, Lytle J and Bryson PJ: The place of hyperbaric oxygen in the treatment of gas gangrene. Br J Hosp Med (1995) 53: 424–426.
7. Sunami K, Takeda Y, Hashimoto M and Hirakawa M: Hyperbaric oxygen reduces infarct volume in rats by increasing oxygen supply to the ischemic periphery. Crit Care Med (2000) 28: 2831–2836.
8. Hart GB and Thompson RE: The treatment of cerebral ischemia with hyperbaric oxygen (OHP). Stroke (1971) 2: 247–250.
9. Neubauer RA and End E: Hyperbaric oxygenation as an adjunct therapy in strokes due to thrombosis. A review of 122 patients. Stroke (1980) 11: 297–300.
10. Faiman MD, Nolan RJ, Baxter CF and Dodd DE: Brain gamma-aminobutyric acid, glutamic acid decarboxylase, glutamate, and ammonia in mice during hyperbaric oxygenation. J Neurochem (1977) 28: 861–865.
11. Zhang J, Su Y, Oury TD and Piantadosi CA: Cerebral amino acid, norepinephrine and nitric oxide metabolism in CNS oxygen toxicity. Brain Res (1993) 606: 56–62.
12. Vernhiet J, Macrez P, Renou AM, Constant P, Billerey J and Caille JM: Effects of high doses of morphinomimetics (fentanyl and fentathienyl) on the cerebral circulation in normal subjects. Ann Anesthesiol Fr (1977) 18: 803–810.
13. Yaster M, Koehler RC and Traystman RJ: Effects of fentanyl on peripheral and cerebral hemodynamics in neonatal lambs. Anesthesiology (1987) 66: 524–530.
14. Loskota WJ, Lomax P and Verity MA: A stereotaxic atlas of the mongolian gerbil brain. Ann Arbor Science Publishers, Ann Arbor (1974).
15. Smolders I, Sarre S, Michotte Y and Ebinger G: The analysis of excitatory, inhibitory and other amino acids in rat brain microdialysates using microbore liquid chromatography. J Neurosci Methods (1995) 57: 47–53.
16. Hu RQ, Banerjee PK and Snead OC IIIrd: Regulation of gamma-aminobutyric acid (GABA) release in cerebral cortex in the gamma-hydroxybutyric acid (GHB) model of absence seizures in rat. Neuropharmacology (2000) 39: 427–439.
17. Davis K, Foos T, Wu JY and Schloss JV: Oxygen-induced seizures and inhibition of human glutamate decarboxylase and porcine cysteine sulfinic acid decarboxylase by oxygen and nitric oxide. J Biomed Sci (2001) 8: 359–364.
18. Bertilsson L, Mao CC and Costa E: Application of principles of steady-state kinetics to the estimation of gamma-aminobutyric acid turnover rate in nuclei of rat brain. J Pharmacol Exp Ther (1977) 200: 277–284.