Nitric oxide amplifies the excitatory to inhibitory neurotransmitter imbalance accelerating oxygen seizures.

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Demchenko IT, Piantadosi CA. Nitric oxide amplifies the excitatory to inhibitory neurotransmitter imbalance accelerating oxygen seizures. Undersea Hyperb Med 2006; 33(3):169-174. CNS O_2 toxicity is manifested most profoundly by generalized motor convulsions. The hypothesis was tested that HBO_2 triggers seizures by an excitatory to inhibitory neurotransmitter imbalance produced by neuronal nitric oxide (NO) activity. Anesthetized rats were exposed to 5 ATA HBO_2 for 75 min with or without prior inhibition of nNOS. Interstitial NO and amino acids: aspartate (Asp), glutamate (Glu) and γ-aminobutyric acid (GABA) were determined in the striatum by microdialysis coupled with HPLC. Blood flow and EEG in the same striatal region were measured simultaneously. Rats treated with 7-NI showed no EEG spikes of O_2 toxicity, while seizure latency for untreated rats was 63±7 min. Significant increases in $NO^{\cdot}$ metabolites and blood flow were observed in control rats before seizures. HBO_2 did not change Glu significantly and increased Asp slightly whereas GABA decreased progressively by 37±7%. Pretreatment with 7-NI led to a significantly smaller decline in GABA. Overall, the simplified excitotoxicity index Glu/GABA increased significantly after 60 min of HBO_2 in control but fell in rats treated with 7-NI. We conclude that HBO_2-stimulated neuronal NO production promotes an imbalance between glutamatergic and GABAergic synaptic function implicated in the genesis of oxygen-induced seizures.

INTRODUCTION

Hyperbaric oxygen (HBO_2) can result in acute CNS O_2 toxicity characterized by hyperactivity, EEG spikes and motor convulsions. Oxygen seizures occur by obscure cellular mechanisms that most likely involve the HBO_2-induced overproduction of reactive oxygen and nitrogen species (ROS and RNS), directly interfering with brain cell electrical activity and synaptic discharges (1).

RNS, e.g. nitric oxide (NO), are implicated in regulating excitatory amino acid neurotransmission because NO is synthesized in postsynaptic glutamatergic neurons and its retrograde diffusion to pre-synaptic neurons can enhance Glu release and activate N-methyl-D-aspartate (NMDA) receptors leading to excitotoxicity (2,3)]. In terms of oxygen effect, it is now well known that nitric oxide synthase (NOS) inhibitors protect against HBO_2–induced convulsions (4, 5). Moreover, HBO_2 decreases total or regional content of the major inhibitory neurotransmitter in mammalian brain, i.e. GABA (6-9). Thus, GABA depletion may be effected by NO since an NO donor SNAP, can decrease GABA content (10), while NOS inhibitors increase basal GABA releases in the striatum or hippocampus of rats (10, 11). In agreement with this, and because HBO_2 stimulates brain NO production, we postulated that NO disturbances of excitatory to inhibitory neurotransmission balance leads to manifestations of CNS O_2 toxicity.

The purpose of this study was to evaluate the role of NO in the HBO_2-induced release of amino acid neurotransmitters in order

to gain information on the balance between excitatory and inhibitory neurotransmission that we suspect contributes to the development of oxygen seizures.

MATERIALS AND METHODS

Male Sprague-Dawley rats anesthetized with urethane (1.2 g/kg, i.p.) were intubated for ventilation, the femoral artery and vein catheterized to monitor blood pressure, withdraw blood samples and administer pharmacological agents. The animals were placed in a stereotaxic frame and a microdialysis probe (CMA/11, CMA/Microdialysis AB, Sweden) with a platinum electrode were inserted into the striatum (St). Rats were also equipped with EEG electrodes, connected to a respirator and enclosed in a hyperbaric chamber containing the EEG amplifier, blood pressure transducer and amplifier, heating pad, infusion pump and microdialysis setup. A microdialysis probe was perfused continuously with artificial cerebrospinal fluid (CSF) at a flow rate of 1.0 µl/min. Once arterial blood gases and body temperature were in the physiological range, control dialysate samples were collected and H_2 clearance blood flow curves recorded. The respirator was then switched from 30% to 100% O_2 and the chamber compressed with air to 5 ATA at 0.6 ATA per minute. During the HBO_2 exposures for 75 min, samples of dialysate were collected and H_2-clearance curves measured every 15 minutes. Mean arterial blood and rectal temperature were monitored continuously. EEG was recorded continuously and EEG spikes used to indicate the onset of CNS O_2 toxicity.

Interstitial amino acid concentrations were measured using HPLC with electrochemical detection as described (13) with slight modifications. Briefly, amino acids derivatives were formed by reaction with O-phthaldialdehyde for 2 min at room temperature. The amino acids derivatives were immediately applied to the HPLC system and separated on a 3-µm C18 column operated at a constant flow rate of 1.2 ml/min. Mobile phase was 0.1 M Na_2HPO_4, 0.13 mM Na_2EDTA and 28% methanol (pH 6.4). Amino acid levels were quantified with a Coulochem electrochemical detector (ESA model 5100A) with the first detector set at -0.4 V and second detector set at +0.6 V. The guard cell was set at potential +0.65 V. The amino acids were identified and quantified by comparing retention times and peak areas with those of external standards.

Cerebral blood flow (CBF) was measured by a hydrogen clearance method modified for hyperbaric conditions (11). Extracellular NO levels were estimated from the yield of nitrite and nitrate (NO_X) in dialysate samples using catalytic reduction of NOx to NO gas followed by chemiluminescence detection in ozone (NO Analyzer, Cervitex, MA).

To evaluate the role of NO in the HBO_2-induced release of amino acid neurotransmitter measurements were done with or without nNOS inhibition with 7-nitroindozol (7-NI). Grouped data are reported as means ± SEM. Amino acid concentrations, CBF, NOx and physiological variables over time were compared using ANOVA and Fisher's post hoc analysis. Paired t-tests were used to compare changes in amino acids, NO or CBF in HBO_2. Differences were considered significant when the *P* value was < 0.05.

RESULTS

Average basal striatal blood flow (all rats) was 76±7 ml/100g/min (n=48). At 5 ATA, CBF decreased over the first 15-30 min, then gradually increased, and over 75 min rose by 73±14%. Paroxysmal EEG spikes were observed in 12 of 14 rats at a mean time of 63±5 min after the onset of HBO_2. Rats treated with 7-NI and exposed to 5 ATA O_2 exhibited

less decrease in CBF over the first 30 min and a moderate increase by 25±5% after 75 min. EEG spikes were observed only in 3 of 14 rats treated with 7-NI.

The time course of NOx showed a tendency to change in parallel to CBF at 5 ATA of oxygen. The average basal NOx concentration recovered in dialysate from striatum was 3.7± 0.7 μM. HBO_2 decreased NOx over the first 15 min but within next 60 min NOx gradually increased to above control level by 43±7% ($P<0.05$).

Striatal amino acid content in control rats exposed to 5 ATA HBO_2 changed over time. Glu decreased within 45 min, returned to pre-exposure levels at 60 min and then fell rapidly at 75 min of the beginning HBO_2. Return of Glu to pre-exposure levels correlated with EEG spikes and CBF elevations after vasoconstriction. Aspartate concentrations rose and significant elevations were detected after 45 min HBO_2 when EEG seizures appeared. Unlike Glu and Asp, extracellular GABA decreased gradually during HBO_2 and after 75 min was 37±7% below pre-exposure baseline ($P<0.05$).

In rats treated with 7-NI and exposed to HBO_2 at 5 ATA, Glu decreased gradually beginning at 30 min of exposure and at 75 min had decreased by 37±8% ($P<0.01$). Inhibition of NOS led to a slight and non-significant decrease in Asp throughout the exposure but GABA concentration did not change significantly over 60 min and after 75 min only decreased by 20±6% ($P<0.05$). The balance between excitatory and inhibitory brain neurotransmitter was defined qualitatively by an "excitotoxicity index" of [extracellular glutamate] / [extracellular GABA]. The rationale for this index is that glutamate is essential for activation of NMDA receptors. In contrast, GABA is mainly inhibitory, and therefore should oppose glutamatergic neurotoxicity. The calculated excitotoxicity index rose progressively during HBO_2 exposure while nNOS inhibition attenuated affect of hyperoxia and prevented oxygen seizures (see Figure 1).

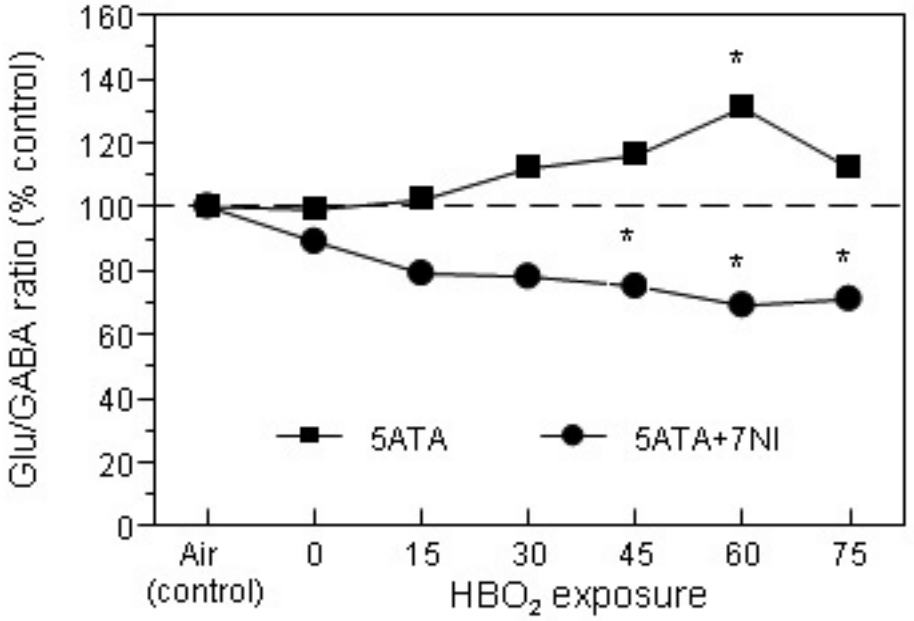

Fig. 1. Glutamate/GABA ratio in the brain of rats exposed to 5 ATA HBO_2 with and without inhibition of neuronal NOS. Glutamate/GABA ratio, defined as an excitotoxicity index, was calculated after measurement of these amino acids in interstitial fluid in the rat striatum. Glutamate and GABA concentrations measured in HBO_2 were compared to control values measured in the same rats breathing air. * $P<0.05$ compared with control Glu/GABA ratio.

DISCUSSION

In this study, basal amino acid concentrations in striatum in the rat were similar to those measured by several authors using microdialysis and HPLC (14-18). Oxygen under pressure (5 ATA) did not significantly change Glu and increased Asp slightly, while the main inhibitory neurotransmitter, GABA, decreased progressively in the striatum by approximately 30% before the appearance of EEG spikes**.** In general, these data are consistent with our earlier (19) and other studies that have shown minor glutamate changes but significant GABA depletion in whole rat brain or in the striatum after HBO_2 (6-8). A similar decrease in GABA content that correlated with the appearance of EEG discharges was recently reported in rat cortex during HBO_2 exposure at 5 ATA (20).

Significant differences were found in the temporal efflux of Glu and Asp compared to GABA during HBO_2 exposure. While Glu and Asp release were elevated before the onset of EEG spikes, GABA release was inhibited. This HBO_2-induced perturbation of Glu-Asp/GABA equilibrium in favor of glutamate and aspartate could produce a relative increase in excitation in interconnected striatal neurons. Within such circuitry, the result would be an increase in burst firing with the consequent generation and propagation of EEG spikes and convulsions. On this basis, an HBO_2-induced imbalance in excitatory versus inhibitory transmission can be implicated as an etiological factor in oxygen seizures. Glutamate and GABA regulate the excitability of virtually all neurons in brain and are important mediators of many critical physiological and pathophysiological events including epilepsy (21,22). This general observation prompted us to apply a simple excitotoxicity index (Glu x Asp/GABA) to the timing of oxygen seizures. As expected, this index progressively increased over HBO_2 exposure time and attained statistical significance from controls at the appearance of EEG spikes.

The biochemistry has not been elucidated by which HBO_2 favors excitatory amino acids leading to EEG seizures. But this study demonstrates for the first time that EEG spikes linked to the imbalance of excitatory/ inhibitory transmission could involve neuronal NO production. Striatal NO production increases and correlates with the appearance of HBO_2-related EEG spikes in this and earlier studies (21,22). Pre-treatment with 7-NI was able to delay or prevent the expression of EEG seizures corresponding with alterations in temporal amino acid-release profiles. Selective or non-selective NOS inhibitors did not change basal Glu levels significantly in our studies (19) but in rats pre-treated with 7-NI and exposed to 5 ATA O_2 both Glu and Asp rapidly decreased and achieved lower levels than in animals with intact nNOS. In contrast, 7-NI significantly delayed seizures and attenuated a decrease in GABA release**.** These results suggest that NO plays opposing roles in HBO_2-induced Glu, Asp and GABA release, enhancing excitatory and depressing inhibitory neurotransmission. This agrees with *in vivo* studies in which NO amplified NMDA-induced release of Glu in striatum (25) but the NO inhibitors, L-NAME and L-NMMA, caused a concentration-dependent inhibition of Glu responses to NMDA (26). In contrast, non-selective NOS inhibitors increase basal or NMDA-induced GABA releases in the striatum or hippocampus of rats (10,11), while NO donor SNAP, decreases GABA content (10). Because NO is synthesized in postsynaptic neurons after NMDA stimulation it may act as a retrograde messenger on pre-synaptic glutamatergic terminals to facilitate Glu release. This may mean that increased NO production following NMDA receptor activation mediates the increase in release of Glu triggered by activation of striatal NMDA receptor. This may be attributed

to the possibility that the facilitation in Glu and suppression in GABA release may initiate HBO_2-induced seizures and the trigger may be related to nNOS.

In summary, we provide new neurochemical evidence for the participation of glutamate, aspartate, and GABA in the convulsive action of HBO_2. The data demonstrate that maintenance of excitability within the striatum is accompanied by inhibition of GABAergic transmission in HBO_2. This impairment of GABAergic inhibition and/or even unchanged in Glu-Asp excitation appears to be critical for triggering the HBO_2–induced burst firing occurring in the striatum stimulated by the neuronal production of nitric oxide.

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