Effect of Indomethacin on Cerebral Blood Flow and Development of Oxygen Convulsions

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 142, No. 7, pp. 31-33, July, 2006
Original article submitted April 6, 2006

Hyperbaric oxygenation modulates cerebral blood flow affecting the development of oxygen convulsions. Before hyperbaric oxygenation-induced convulsions in rats the initial decrease in blood flow gave place to hyperemia, Po_2 increased. In rats receiving cyclooxygenase inhibitor indomethacin no convulsions were observed, blood flow and Po_2 were lower than in controls. Our results indicate that indomethacin prevents hyperemia and alleviates oxygen convulsions under conditions of hyperbaric oxygenation.

Key Words: *hyperbaric oxygenation; cerebral blood flow; indomethacin; cyclooxygenase; oxygen convulsions*

Breathing O_2 at a pressure >2 AAT (absolute atmospheres) during hyperbaric oxygenation (HBO) or underwater diving is associated with a risk of toxic action of O_2 on the central nervous system. The most severe manifestations are loss of consciousness and convulsions. The increase in cerebral blood flow (CBF) accelerating the development of seizures by supplying additional toxic doses of O_2 to the brain is the major risk factor of oxygen poisoning [5]. CO_2 and NO play a role in HBO-induced hyperemia. They exhibit high vasorelaxant activity and their production and/or accumulation in the brain increase during hyperoxia [1,6]. We hypothesized that hyperemia of cerebral vessels during HBO is also associated with the presence of arachidonic acid metabolites (prostaglandins), because they produce a strong vasorelaxant effect and molecular O_2 plays a role in cyclooxygenase (COX)-catalyzed synthesis of these compounds [8]. To test this hypothesis we studied CBF variations in rats exposed to HBO and receiving indomethacin. Indomethacin inhibits COX-1 (constitutive COX) and COX-2 (inducible COX). It should be emphasized that in endothelial cells COX-1 is inhibited more significantly than COX-2 [2].

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MATERIALS AND METHODS

Experiments were performed on 29 awake male Wistar rats weighing 220-310 g. The animals were narcotized with nembutal in a dose of 50 mg/kg. Platinum electrodes were symmetrically implanted into the striatum of the left and right hemispheres (AP=+1.0 mm, LM=±2.5 mm, V=-5.0 mm).

On days 7-10 after surgery the rats were placed in an altitude chamber (100 liters) equipped with systems for temperature, humidity, and CO_2 concentration monitoring. Basal levels of the test parameters were recorded. The chamber was ventilated with pure O_2. Compression was performed to a pressure of 5 AAT (1 AAT/min). The animals were exposed to this pressure until the appearance of seizure spikes on EEG.

The absolute level of local CBF in the striatum during HBO was measured by the method of hydrogen clearance with endogenous hydrogen generation [4]. Polarography of Po_2 and EEG recording

0007-4888/06/1421-0026

were performed in the striatum of the contralateral hemisphere. The electrodes were connected to an EEG amplifier and device for the measurement of blood flow (BF) and Po_2 (Fizioblok-03, Institute of Evolutionary Physiology and Biochemistry) through a pressure seal in the chamber wall. This device was placed outside the altitude chamber. The data were recorded, collected, stored, and analyzed on a personal computer. We used an analog-to-digital converter and WINDAQ software.

The rats were divided into 4 groups. Group 1 animals (n=6) were placed in the open altitude chamber (air breathing). Basal level of BF and Po_2 were measured in the striatum for 90 min. Group 2 rats (n=6) received intraperitoneal injection of COX inhibitor indomethacin (Sigma) in a dose of 10 mg/kg. BF, Po_2, and EEG in group 3 animals exposed to HBO (n=10) were recorded until the appearance of EEG spikes. Group 4 animals (n=7) received 10 mg/kg indomethacin intraperitoneally 30 min before HBO.

The significance of differences was estimated by Student's t test.

RESULTS

Striatal BF in group 1 rats was 67±5 ml/100 g/min. Consecutive measurements of BF over 90 min showed that its fluctuations correspond to 5-9% of the mean value. Striatal BF in control animals significantly decreased 20 min after intraperitoneal injection of 10 mg/kg indomethacin (by 23±5%) and remained unchanged over 90 min (75-85% of the basal level).

HBO modulated BF, Po_2, and EEG. Slow waves on EEG prevailed over the first 10-15 min of HBO.

TABLE 1. Striatal BF in Intact Rats and after COX Inhibition during HBO (%, $M\pm m$)

HBO duration, min	HBO	Indomethacin+HBO
10	-11.5±5.5*	-6.9±6.7
20	2.0±8.4	2.0±3.7
30	27.0±10.2*	2.0±6.4

Note. *p<0.05 compared to the basal level.

They progressively transformed into high-frequency and desynchronized activity. The appearance of seizure activity on EEG correlated with motor signs. EEG discharges reflected oxygen poisoning and were revealed after 31±3 min. To prevent the development of repeated seizures and death of animals, HBO was stopped after the appearance of seizure activity on EEG.

HBO was accompanied by biphasic changes in striatal BF (Fig. 1, *a*). BF decreased over the first 15 min. However, blood supply to the striatum returned to normal after 20 min and increased by 33±9% in the follow-up period (p<0.05). Administration of indomethacin 30 min before HBO was followed by a significant and permanent decrease in striatal BF over 80-min hyperoxic exposure.

In the brain of intact rats Po_2 estimated by polarographic current increased by 597-658% during compression and then increased by 1334±221% 30 min after HBO (Fig. 1, *b*). The increase in BF and Po_2 preceded the appearance of spike activity on EEG. O_2 concentration in the brain of indomethacin-receiving animals increased during compression. However, oxygenation of the striatum did not increase in rats of the HBO group and

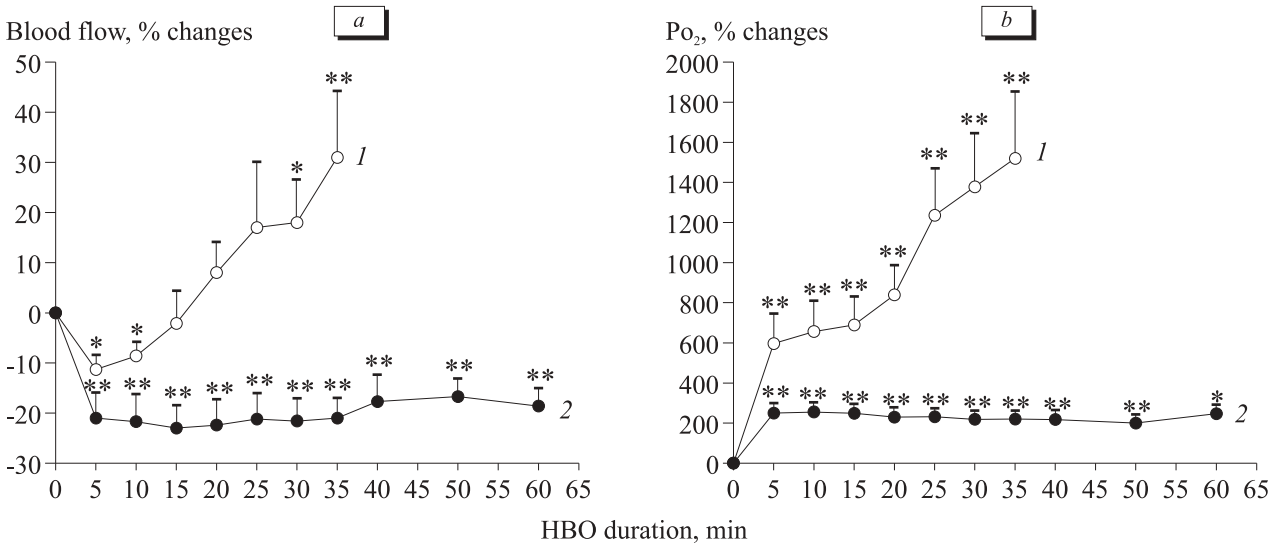

Fig. 1. Effect of HBO on BF (*a*) and Po_2 in intact rats (*1*) and after COX inhibition (*2*). *p<0.05 and **p<0.01 compared to the basal level.

was much lower compared to intact animals. Spike activity on EEG was not found in rats with COX inhibition.

The HBO-induced decrease in BF in rats with COX inhibition results from the combined vasoconstrictor effect of indomethacin and HBO. Striatal BF decreased by 23±5% 30 min after indomethacin administration. BF under these conditions was taken as the basal level. HBO for 10 min significantly decreased BF in intact animals (Table 1). However, indomethacin decreased the degree of HBO-induced vasoconstriction. Differences in variations of BF in animals with COX inhibition and intact rats were insignificant by the 20th minute (Table 1). After 30 min, HBO-induced changes in BF significantly differed in group 3 and 4 animals. Hyperoxic hyperemia was observed in group 3 rats, while BF remained unchanged in group 4 animals (Table 1).

Our results show that BF decreases in indomethacin-treated rats under conditions of air breathing. Therefore, products of arachidonic acid metabolism contribute to basal vasorelaxation (similarly to nitric oxide). Hyperoxic vasoconstriction decreases striatal BF and is probably associated with the inhibition of prostaglandin synthesis. Previous studies showed that prostaglandin synthase is inhibited by peroxynitrite [9]. Production of peroxynitrite increases under conditions of HBO [3]. Moreover, hyperoxia is accompanied by a decrease in the concentrations of prostaglandins E and 6-keto-β-PGF_{1A} in the striatum and cortex [6], but has no effect on thromboxane level. 6-Keto-β-PGF_{1A} is the product of prostacyclin hydrolysis [8]. It can be hypothesized that prostaglandins are involved in the mechanisms of cerebral vasoconstriction during HBO.

HBO-induced hyperemia was not observed after the inhibition of prostaglandin synthesis with indomethacin. Probably, arachidonic acid derivatives play a role in vasodilation. Since O_2 serves as a cosubstrate in prostaglandin synthesis [8], these specific features can be related to variations in COX during hyperoxia.

HBO probably results in the following changes. HBO stimulates production of free oxygen and nitrogen radicals in the brain. These changes are accompanied by inhibition of prostaglandin synthesis and decrease in BF during the 1st stage of hyperoxia. The neurotoxic effect of prolonged HBO involves arachidonic acid products. HBO initiates production of vascular prostaglandins. These compounds exhibit high vasomotor activity, play a role in hyperemia and, therefore, accelerate the development of seizures by supplying the toxic dose of O_2 to the brain.

This work was supported by the Russian Foundation for Basic Research (grants No. 03-04-49715 and 03-04-49697).

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