The Influence of One Hyperbaric Oxygen-Induced Seizure on Brain Eicosanoid Content

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ABSTRACT

The levels of prostaglandin F_1 (6-keto-PGF $_{1\alpha}$), thromboxane B_2 (11-dehydro-TxB $_2$), and peptidoleukotriene C_4 (LTC $_4$) were measured (acetylcholinesterase immunoassay) in the frontal cortex (FC) and the striatum (SA) of the rat brain to study the possible role of eicosanoids in seizures induced by hyperbaric oxygen (HBO). The rats were exposed to (1) hyperbaric oxygen (HBO, 6 ATA O $_2$) up to the first seizure (2) compressed air (6 ATA air, i.e., $\cong$ 1.25 ATA O $_2$) or (3) atmospheric pressure (1 ATA air, i.e., 0.21 ATA O $_2$); there was no seizure in groups 2 and 3. Transition from 6 ATA to atmospheric pressure was obtained in 15 min; the rats were then decapitated and their heads frozen in liquid nitrogen before extraction and analysis of prostanoids. Whatever the conditions, cortical levels of 6-keto-PGF $_{1\alpha}$ and 11-dehydro-Tx B_2 are higher than striatal levels; considering the same area, 11-dehydro-Tx B_2 and LTC $_4$ concentrations were not significantly different whatever the condition, but there is a trend for lower 6-keto-PGF $_{1\alpha}$ levels in FC after HBO seizure. Biochemical mechanisms are discussed. Eicosanoids do not seem to play a major role in HBO seizures, although some modifications of their metabolism may take place.

Index Entries: Brain; eicosanoids; enzyme immunoassay; free radicals; hyperbaric oxygen toxicity; rat; seizure.

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INTRODUCTION

Prostaglandins (PG) and leukotrienes (LT) are normally present in the rat brain in very small amounts (Lindgren et al., 1984; Wolfe and Coceani, 1979). Electrically or chemically induced convulsions markedly increase free fatty acid content (Bazan, 1970; Siesjö et al., 1982) and subsequently PG content (Försterman et al., 1983) and LT (Simmet et al., 1988); this increase is not the only consequence of cerebral ischemia, a well-known stimulus of eicosanoid synthesis (Försterman et al., 1983), which may take place during some convulsions (Siesjö et al., 1982). Ionizing radiations damage tissues and increase PG content (Dubois et al., 1987); normobaric hyperoxia also damages tissues, but PG content varies with the considered tissue: it may decrease in vascular endothelium (an important target for both ionizing radiation and hyperoxia [Setty et al., 1984]) or increase in lung bronchoalveolar lavage fluid (PG and LT content, Smith et al., 1988). Free radicals have been suggested to play an important part in the initiation of HBO-induced central nervous system (CNS) toxicity (Yusa et al., 1984) as, in general, O₂ and ionizing-radiation toxicities (Gerschman et al., 1954). Nevertheless there are contradictory reports on the exact role of free radicals in HBO CNS toxicity according to in vitro (Faiman et al., 1981, 1982) and in vivo models (Yusa et al., 1984). The results of pharmacological trials of PG synthesis inhibitor (Ruszczewski et al., 1979) suggest a role for PG in development of HBO seizure. Nevertheless, definitive interpretation of such pharmacological data is difficult, owing to the multiplicity of sites of action of drugs, eicosanoids, and O₂. Many mechanisms may lead to generation of eicosanoids: during the first hyperoxic seizure there is no hypoxia (Mayevsky, 1984) at variance with electrically or chemically induced seizure; nevertheless, lipoperoxidation of cell membranes (Noda et al., 1983) and convulsion itself via increased neuronal activity (Försterman et al., 1983) may modulate prostanoid brain contents. However, in vitro lipid peroxidation induced by normobaric hyperoxia decreases arachidonic acid level (Rehncrona et al., 1980), so the final results upon eicosanoid brain content after HBO seizure is difficult to assess. To our knowledge, no data on brain eicosanoids content after the first HBO seizure are available. A study was therefore carried out to try to provide elements of answers to the following questions:

1. Is there a change in eicosanoid content of rat brain after the first HBO-induced seizure? (It might play a part in mechanisms of seizures.)
2. Is there any difference in cortical and striatal eicosanoid content after the first HBO-induced seizure? (As striatum is possibly inducing the HBO epileptic activity [Bertharion and Barthelemy, 1964; Mayevsky, 1984; Mialon et al., 1990], a great difference in levels in these two areas could be of interest.)

No previous data was available; moreover, since the hyperbaric environment prevented access to animals in real time, only stable eicosanoid metabolites could be used, in order to reflect global changes in metabolism; LTC₄ was selected from among all leukotrienes because of its potential role in seizure (Simmet et al., 1988). Analyses were performed after the first seizure because only this seizure is HBO-specific; continuous exposition leads to status epilepticus and death, at which time the specificity of the initiating agent is lost among common and deep modifications in brain biochemistry, including prostanoids. Controls on rats at atmospheric pressure and at the same absolute pressure (6 ATA) as the HBO group, but in air ($\cong$ 1.25 ATA PIO₂), were added: at this oxygen partial pressure there is no nervous toxicity in rats (Clark, 1974, for review), but, as an effect of pressure might be possible, it should be assessed.

MATERIALS AND METHODS

Sprague-Dawley mature male rats (weight 200–250 g) were received from the supplier (Charles Rivers, France) 1 wk prior to use, housed in a quiet room (temperature 19–23°C, natural day/night cycle) and maintained on same diet (Extralabo Ets Pietrements, Provins, France) and water ad libidum except for 4 h before the experiments, when food was withdrawn. The care and treatment of the animals were managed according to the Principles of Care and Use of Experimental Animals of the French Agricultural Department.

Every experiment took place between 10 and 12 AM. The rats were divided into three groups: one exposed to 6 ATA O₂ until the first seizure, one exposed to 6 ATA air, and another exposed to 1 ATA air for an equivalent time (the sum of the compression time and the time elapsed between the end of compression and the beginning of the first seizure, taking into account previous work [Mialon et al., 1990]).

Hyperbaric Oxygen Exposure

HBO Group

The HBO group was composed of seven rats, exposed individually to 6 ATA O₂ in a small hyperbaric tank (45 L), up to the first clinical seizure. The tank was free of CO₂ (soda lime absorber); internal temperature was $22 \pm 1^\circ\text{C}$. The tank was fitted with two portholes, allowing constant visual monitoring of the animals. It was flushed with O₂ until the oxygen concentration (FIO₂) was >0.95 (O₂ analyzer Beckman D2). The pressure was then increased to 6 ATA with pure medical oxygen (compression time, 5 min). Immediately after the onset of the first clinical seizure (11.2 ± 1.1 min after the start of compression), the chamber was flushed with compressed air (6 ATA, 2 min) in order to lessen FIO₂ to $\neq 30\%$ and avoid iterative seizure. Decompression lasted 15 min, under

constant visual control without any risk of overpressure of the lung. If a rat convulsed more than once, it was not used for the study.

Pressure Air (PA) Group

The "pressure air group" (PA) was composed of seven rats, exposed individually to 6 ATA air. Compression time was 5 min, exposure was maintained for 10 min, and the chamber was then flushed at 6 ATA with air for 2 min (to mimic auditive stimulations of the HBO group when ventilated with air after the seizure). Decompression lasted 15 min. In simulated dives, for these durations and pressure, rats did not develop decompression sickness.

Atmospheric Control (AC) Group

The "atmospheric control group" (AC) was composed of seven rats, left individually for 35 min in the chamber with atmospheric pressure. A low air flow (5 L/min) was applied during the first 5 min, and then from the 15th to the 35th min, to mimic the auditive stimulations of both HBO and PA groups.

After its removal from the tank, each animal was instantly decapitated (<1 min). The heads were immediately frozen in liquid nitrogen and, 5 min later, transferred into ice-cold methanol, where they were allowed to thaw gradually. The brains were then removed *in toto*. Three samples were taken from the right FC, and three more from the left SA. Each one was weighed in liquid nitrogen and transferred to -80°C .

Analytical Technique

One of the three pieces of brain tissue was sonicated in 1 mL of methanol/water (10/90% v/v). After centrifugation (15 min, 2000g, $+4^{\circ}\text{C}$), the supernatant was injected into a C18 J. T. Baker (Phillipsburg, NJ, USA) column (1mL) prewashed successively with 5 mL of methanol/EDTA 0.5% (50%/50% v/v), 5 mL of methanol, and 5 mL of water. The whole sample went through the column, which was then washed with 5 mL of hexane. Elution used 5 mL of ethanol. These procedures are derived from Luderer et al., 1983, and Salari and Steffenrud, 1986, being a compromise between optimal conditions for all products. Eluates were lyophilized. Residues were resuspended in 400 μL of enzyme immunoassay (EIA) buffer and 50 μL was used for each analysis. Assessment of extraction efficiency used labeled synthetic PG and LT (New England Nuclear, Boston, MA, USA). As only a single piece from each sample was used for analysis, the two remaining pieces were labeled before sonication with 0.01 μCi tritiated 6-keto-PGF_{1 α} , (120–180 Ci/mmol) and with tritiated LC₄ (20–60 Ci/mmol), respectively, and then went through the previously described procedures. Residue was resuspended in 1 mL of methanol/water (90/10% v/v) with 4 mL of scintillation cocktail (Insta Gel, Packard Instrument Corporation, Downers Grove, IL, USA). Radioactivity was determined by using a liquid scintillation β counter (Rack

Beta 1214, LKB, Bromma, Sweden). For each labeled piece the ratio of actual count to added count was calculated, giving extraction efficiency.

Analysis of prostanoids used solid-phase EIA; this analytical technique of eicosanoids is equivalent and simpler than standard RIAA (Pradelles et al., 1985). All these EIA kits may be purchased from Cayman Chemical (Ann Arbor, MI, USA). To each well of a 96-well microtiter plate previously coated with IgG antibody were added first 50 μ L of each sample, then 50 μ L of enzymatic tracer (acetylcholinesterase), and last 50 μ L of specific rabbit antiserum (6-keto-PGF_{1 α} or 11-dehydro-TxB₂ or LTC₄). After 18 h of incubation (4°C) the plates were washed two times with a mixture of phosphate-buffered saline and Tween 20 (0.05%), and then filled with 200 μ L of Ellman's reagent. After a while, optic density of the yellow product was read with a Multiscan (Titertek, Flow Laboratories, Pureaux, France). Each sample was assayed in duplicate at two different dilutions. Standards were assayed on each plate at concentrations ranging from 0.25 to 100 pg/well. Nonspecific binding was determined by using an incubation mixture in which the specific antibody was replaced by 50 μ L of assay buffer. The amount of tracer bound in the absence of prostaglandin or leukotriene (B₀) was determined by replacing standard or sample with 50 μ L of assay buffer. A standard curve was obtained by plotting B/B₀% (the absorbance measured on the bound fraction with prostanoid divided by the absorbance in the absence of competitor) vs picograms of standard, by well. Fitting the standard curve and calculating the quantity of prostanoid in samples were performed with a nonlinear curve-fitting program (Pradelles et al., 1985). Sensitivities were, respectively, 0.45, 2, and 3 pg/well for 11-dehydro-TxB₂, 6 keto-PGF_{1 α} , and LTC₄; 11-dehydro-TxB₂ was selected because its reliability is better than that of TxB₂, as it cannot be released from activated platelets (Granström and Kumlin, 1987); indeed, conversion from glial TxB₂ to 11-dehydro-TxB₂ is mostly extraneural, but Grandström and Kumlin (1987) have shown that in some tissue this conversion takes place only when NAD is added. During the first HBO seizure the oxidation of nucleotides results in very high levels of NAD (Mayevsky, 1984), so we suspected the conversion was possible. Simultaneous analysis of TxB₂, though logical, has not been performed, as major coagulation interference takes place when TxB₂ from brain samples is analyzed without microwave irradiation (20-fold increase in TxB₂ level, Anton et al., 1983), precluding any interpretation from such data. The final results obtained from EIA-plate data were corrected for volume and extraction efficiency. Tritiated 11-dehydro-TxB₂ was not available; nevertheless, recovery rates for 6-keto-PGF_{1 α} and TxB₂ do not differ much (5%, Luderer et al., 1983), so the same value was taken for both compounds.

Statistics

Statistical analysis of data was carried out at the 5% level by analysis of variance (ANOVA).

Table 1
Eicosanoid Levels in Rat Brain^a

	Atmospheric Control Group <i>n</i> = 7			Air-Pressure Group, <i>n</i> = 7			HBO Group, <i>n</i> = 7		
	6K	dTxB ₂	LTC ₄	6K	dTxB ₂	LTC ₄	6K	dTxB ₂	LTC ₄
Cortex	59.2 ± 9.2	1.2 ± 0.3	7.5 ± 1.3	68.4 ± 11.4	2.0 ± 0.3	9.5 ± 1.1	33.1 ± 5.4	1.1 ± 0.1	8.3 ± 0.4
Striatum	25.9 ± 4.4	0.4 ± 0.1	9.3 ± 1.1	13.4 ± 2.1	0.3 ± 0.1	8.7 ± 0.6	15.3 ± 5.9	0.4 ± 0.1	10.2 ± 2.8

^aMean values (ng/g wet tissue) ± SEM of 6-keto-PGF_{1α} (6K), 11-dehydro-TxB₂ (dTxB₂), and leukotriene C₄ (LTC₄) in rat brain.

RESULTS

The results are given in Table 1. Extraction efficiency was roughly 50% for PG and 20% for LTC₄. The eicosanoid values are high, but in keeping with the method of killing (Anton et al., 1983). Whatever the environmental conditions, regional differences between FC and SA are found for 6-keto-PGF_{1α} and 11-dehydro-TxB₂ (FC > SA) at variance with LTC₄. Regional differences could be the result of decapitation, as suggested by the results of Anton et al. (1983); nevertheless, the duration of dissection was quite similar, FC always taken first, and, moreover, congelation proceeds from cortex to striatum: thus it may seem doubtful that the constancy of the gradient between FC and SA is totally explained by sampling maneuvers. Neither the HBO nor the PA group differs significantly (at the 5% level) from the AC group, though there is a nonsignificant trend for HBO to lower 6-keto-PGF_{1α} levels (HBO vs AC in FC.) Hence, neither pressure, PIO₂, tissue PO₂ nor convulsion seems to have a major impact on eicosanoid metabolism under our experimental conditions, though a possible effect of HBO on 6-keto-PGF_{1α} content cannot be precluded, because of the small size of our samples.

DISCUSSION

Eicosanoid brain levels in the HBO group were not higher than in the AC group, despite some reports of PG and LT elevation during seizures of various origins (Försterman et al., 1983; Simmet et al., 1988), or during free-radical generation (Dubois et al., 1987; Smith et al., 1988). One could object that sampling conditions may have modified the results: decapitation and immediate immersion in liquid nitrogen, though imperfect, is classical (Bazan, 1970; Försterman et al., 1983; Lindgren et al., 1984; Simmet et al., 1988). If the brain remains in the head for 1 min, there is little difference between microwave irradiation and decapitation in terms of levels of free fatty acid (Cenedella et al., 1975). There is always a possibility that prostanoid tissue levels may have been modified by the killing procedures, whatever they may be; however, as timing of procedures is very similar for all experiments (80 ± 10 s), differences between groups would stay unchanged. Another factor of variation is time of sampling: analyses in the HBO and air-pressure group were delayed after seizure because of flushing with compressed air and decompression (≅ 17 min). This delay cannot be shortened further: explosive decompression kills the animal and strongly interferes with any action of HBO on prostanoid metabolism, and too short a decompression induces formation of silent bubbles in vessels with membrane activation and possible hidden ischemia (air-pressure group, not HBO). However, as the analyses were delayed, it may be possible to speculate that brain eicosanoid content may have increased during hyperbaric seizure and quickly de-

creased during decompression to control levels. But the assayed products have different and slow turn-over: 20 min after revascularization following partial ischemia of the rat brain, concentrations of TxB_2 , 6-keto- $\text{PGF}_{1\alpha}$, and LTC_4 are still, respectively, 4, 2, and 3 times higher than control levels; furthermore, LTC_4 increases up to the 45th min (Minamisawa et al., 1988). The results of Shohami et al., (1982) and Jacobs et al., (1987) are also consistent with stability of produced eicosanoids for delays longer than 15 min. During HBO exposure there is an immediate cerebral vasoconstriction, with only transient vasodilation during the seizure and with return to initial tone at least at the end of the first seizure (Mayevsky, 1984). During most of the decompression cerebral vasoconstriction takes place, as the animals are in hyperoxic ambience. Therefore a total washout of a putative transient cerebral elevation of prostanoids is very unlikely. Moreover, after one HBO seizure, there is a tendency for 6-keto- $\text{PGF}_{1\alpha}$ levels to decrease (at least in FC). How then is one to explain the results? If lipid peroxidation of cell membranes occurs during the first HBO seizure (convulsion and membrane modifications), a rapid increase in the levels of free fatty acids, including arachidonic acid (AAc), should occur. As the usual biosynthesis of eicosanoids is regulated via substrate availability, subsequent accumulation of stable final products is expected. Our results suggest that available AAc does not increase and/or that AAc and other fatty acid changes are balanced as some unsaturated fatty acids compete with AAc (Lands and Kulmacz, 1984). Because Rehncrona et al. (1980) found a slight decrease in AAc and decohexaenoic acid levels in parallel with *in vitro* lipid peroxidation (1 ATA O_2), the first hypothesis seems more likely. One should also consider that the two major enzymatic pathways (cyclooxygenase for PG and 5-lipoxygenase and LTC_4 synthase for LT) might also be inactivated by HBO exposure. Setty et al. (1984) found a 30% decrease in $V_{\max}$ of vascular tissue cyclooxygenase exposed 15 min to 1 ATA O_2 , so greater inactivation when exposed to 6 ATA O_2 is likely (endothelium is a "first-line" target). The same conclusions regarding cyclooxygenase inactivation by reactive oxygen species can be drawn from the work of Hempel et al. (1990). We have no data on 5-lipoxygenase activity in HBO, but LTC_4 synthase is dependent on the intracellular concentration of glutathione (GSH; Shimizu, 1988), which could change during HBO (oxydation of GSH to GSSG in relation to the metabolism of free radicals, McCord and Fridovitch, 1978). Local ionic variations in the brain during seizure (Mayevsky, 1984) could lead to a greater enzyme inactivation. However, as eicosanoid levels are high within both groups, one has to admit that the synthesis of enzymes in brain is normal at the time of decapitation; this is suggestive of minimal HBO enzyme inactivation, for it is usually slow to reverse (Faiman et al., 1981). Moreover, after the first hyperoxic seizure there are no great histological, neuronal, astrocytic, or vascular abnormalities (Balentine, 1982). Considering that, a major role of enzyme HBO transient inactivation seems unlikely, but it is a possible additive factor in

conditions of restricted availability of substrate, and this could explain the trend to lower 6-keto-PGF_{1 α} levels in the HBO group. Considering that point of view our results seem more consistent with those of Yusa et al. (1984), which implicate the formation of free radicals in rat brain at the beginning of the first HBO seizure, than with those of Faiman and Dirks (1982), who found no formation of free radicals in the same in vivo conditions. A direct evaluation of AAc levels and activity of enzymes in HBO conditions, however, is necessary to give more detailed information on all steps of prostanoid metabolism, but the observed preliminary results do not support the idea that eicosanoids could play a major role in initiation of HBO seizures, although some modifications in the whole metabolism could occur after one HBO seizure.

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