OXIDATIVE DEAMINATION KINETICS IN THE PRECONVULSIVE PERIOD OF HYPERBARIC OXYGEN EPILEPSY

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UDC 616.853-092.9-02:615.835.3]-036.3-07:[616-831-008.931:577.152.143]

KEY WORDS: monoamine oxidase; hyperoxia; brain; heart.

The neurophysiological study of the course of development of experimental hyperbaric oxygen epilepsy has shown that mineralized tonicoclonic convulsions in experimental animals are preceded by at least two consecutive phases of this pathological response of the CNS: 1) compensatory (initial) and 2) preconvulsive, which constitute a period of complete well-being during observation of the animals' behavior [5, 6]. However, both electrophysiological and biochemical data are evidence of the existence of preconvulsive stages of oxygen epilepsy. Virtually all neurochemical studies have been carried out either at the height of the seizure or in its terminal phase, so that it is impossible to study the time course of adaptation of the animal to oxygen poisoning at the level of enzyme-transport processes taking place in the CNS. Thus exposure to an increased (4-6 atm) oxygen pressure for 50-60 min leads to a marked fall in the noradrenalin and serotonin levels in the brain [4, 7], which some workers consider to be the result of inhibition of activity of tyrosine hydroxylase and other pterine-dependent hydroxylases [8]. Recent investigations [2-4] also have shown a significant fall in activity of monoamine oxidase (MAO), an enzyme of oxidative deamination of neurotransmitter monoamines, under the influence of hyperbaric oxygenation (HBO) at toxic levels. The time course of changes in the properties of MAO from the first few minutes of exposure to a raised oxygen pressure until the onset of a fully developed seizure has not yet been studied.

We have studied the kinetics of deamination of some monoamines in the brain and heart of rats in the initial and preconvulsive phases of an epileptic fit induced by the action of HBO.

EXPERIMENTAL METHOD

A model of the convulsive form of oxygen poisoning was created by exposing male albino rats weighing 180-250 g in a pressure chamber to oxygen at a pressure of 6 atm for 5-15 min (the periods of compression and decompression were each 20 min). During this period of exposure, the animals did not develop convulsions, for a generalized seizure under such conditions is recorded on average after 20-30 min. The animals were decapitated at the end of decompression and the heart and brain were removed in the cold and kept in liquid nitrogen. MAO activity of a 25% brain and heart homogenate was determined by isothermic distillation of ammonia followed by nesslerization [1]. MAO substrates were used in 4-7 increasing concentrations. The results were subjected to statistical analysis with calculation of mean values and their confidence intervals at $p \leq 0.01$, $p \leq 0.02$, and $p \leq 0.05$. The results of the clinical investigations were represented graphically as Lineweaver–Burke plots.

EXPERIMENTAL RESULTS

A graph of the kinetics of serotonin deamination under normal conditions and in preconvulsive period of oxygen epilepsy, in the brain and heart of the rats, is illustrated in Fig. 1. It will be clear from Fig. 1a that both reaction parameters (K_m and V_{max}) changed in the

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0007-4888/89/0004-0465$12.50

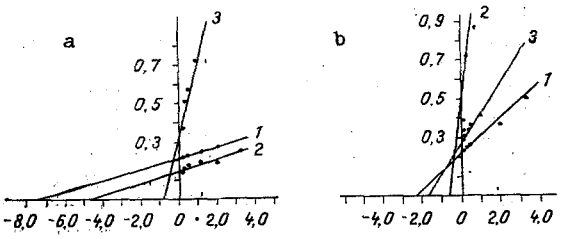

Fig. 1. Kinetics of serotonin deamination in brain (a) and heart (b) of intact rats and of rats subjected to toxic hyperoxia for 5 and 15 min, on Lineweaver–Burke plots. Abscissa, 1/[serotonin] (in mM^{-1}); ordinate, 1/V (in nmoles ammonia/mg protein/min)$^{-1}$. 1) Brain and heart of intact rats; 2) brain and heart of rats exposed to hyperoxia for 5 min; 3) brain and heart of rats exposed to hyperoxia for 15 min.

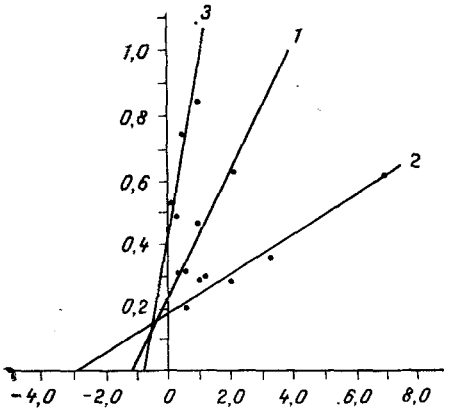

Fig. 2. Kinetics of dopamine deamination in brain of intact rats and of rats exposed to toxic hyperoxia for 5 and 15 min, on Lineweaver–Burke plot. Abscissa, 1/[dopamine] (in mM^{-1}); ordinate, 1/V (in nmoles ammonia/mg protein/min)$^{-1}$. 1) Brain of intact rats; 2) brain of rats exposed to hyperoxia for 5 min; 3) brain of rats exposed to hyperoxia for 15 min.

rat brain in the initial (compensatory) phase of epilepsy. The picture observed was similar to the effect of a noncompetitive inhibitor on serotonin deamination. In the preconvulsive phase of epilepsy, affinity of the enzyme for the substrate continued to decline, and the maximal reaction velocity also declined simultaneously. Thus the changes in serotonin deamination discovered in the first phase of poisoning later increased in intensity. In the rats' heart, in the compensatory phase of epilepsy, affinity of MAO for serotonin fell sharply compared with the normal level, but later (15th minute of exposure to oxygen) it returned to normal (Fig. 1b).

It will be clear from Fig. 2a that dopamine deamination in the rat brain in the initial stage of development of oxygen epilepsy is characterized by marked acceleration of the process, of competitive type, fllowed by a sharp decline in affinity of the enzyme for the substrate, together with reduction of the maximal reaction velocity: both these parameters came closer to the normal levels (Table 1; Fig. 2a). Dopamine deamination virtually did not take place at all in the rat heart after sessions of toxic hyperbaric oxygenation for 5 min and 15 min respectively, so that substrate saturation curves could not be obtained. Thus just as in the case of serotonin deamination, dopamine oxidation in the brain and heart of the rat with oxygen epilepsy (preconvulsive period) did not undergo a parallel change: in most cases the changes were opposite in direction in the CNS and at the periphery.

The kinetics of tyramine deamination in the rat heart in the preconvulsive period of toxic hyperbaric oxygenation is shown graphically in Fig. 3. The graph shows that the character of changes in parameters of the tyramine deamination reaction in the heart is very complex, but changes in the values of K_m from the first phase of epilepsy to the second resemble

TABLE 1. Value of Michaelis–Menten Constant (K_m, in mM, M ± m) for MAO in Rat Brain and Heart under Normal Conditions and in Toxic Hyperoxia

State of animals	Brain		Heart	
	5-HT	DA	5-HT	TYR
Intact	0,13±0,01	1,16±0,12	0,41±0,04	0,20±0,02
Hyperoxia for 5 min	0,19±0,02	0,36±0,04**	0,15±0,02***	2,55±0,30*
Hyperoxia for 15 min	1,06±0,11*	1,33±0,13	0,36±0,04	0,25±0,03

Legend. Differences between experimental parameters and those for intact control animals are shown (n = 4, where n denotes number of determinations) at: *$p \leq 0.01$, **$p \leq 0.02$, ***$p \leq 0.05$. Abbreviations: 5-HT) serotonin, DA) dopamine, TYR) tyramine.

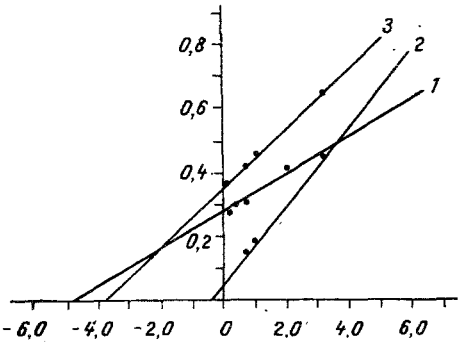

Fig. 3. Kinetics of tyramine deamination in heart of intact rats and of rats exposed to toxic hyperoxia for 5 and 15 min, on Lineweaver–Burke plot. Abscissa, 1/[tyramine] (in mM^{-1}); ordinate, 1/V (in nmoles ammonia/mg protein/min)$^{-1}$. 1) Heart of intact rats; 2) heart of rats exposed to toxic hyperoxia for 5 min; 3) heart of rats exposed to hyperoxia for 15 min.

those for serotonin oxidation in the heart: in the initial phase the affinity of MAO for the substrate fell by an order in magnitude, but in the preconvulsive period it reverted to normal. In turn, changes in parameters of the 2-phenylethylamine-deaminase reaction in the rat heart in the preconvulsive period of oxygen epilepsy were similar to those for the dopamine-deaminase reaction: we likewise did not obtain a curve for saturation of the enzyme preparation with the substrate, evidence of strong inhibition of enzymic deamination of 2-phenylethylamine.

Thus the first (compensatory) phase of oxygen epilepsy is characterized by activation of oxidative deamination of dopamine in the rat brain, with a very small decrease in the affinity of MAO for serotonin and with marked shifts of the parameters of monoamine-oxidase reactions in the heart; oxidation of dopamine and of 2-phenylethylamine, moreover, was recorded only in response to high substrate concentrations, whereas tyramine deamination took place with reduced affinity of MAO for the serotonin and normalization of values of K_m for dopamine deamination were observed in the brain, whereas in the heart, oxidation of dopamine and 2-phenylethylamine remained blocked, and values of K_m for the serotonin- and tyramine-deaminase reactions returned to normal. It can be concluded from these results that the preconvulsive period of oxygen epilepsy ends (for after exposure to oxygen for 20-30 min under the pressures indicated above, a generalized convulsion is recorded) the time of significant worsening of the serotonin-deaminase activity of the brain and of complete blockade of the deamination of dopamine and 2-phenylethylamine in the rat heart. Under those conditions, accumulation of serotonin in the brain and of dopamine and 2-phenylethylamine in the cardiovascular system ought evidently to be observed, and may be a factor in protection of the animal against the toxic action of oxygen. Whatever the case, the most impressive disturbances of oxidative deamination of monoamines during toxic hyperbaric oxygenation take place in the rat's heart and not in its brain, which evidently is subjected to the less harmful action of oxygen in the initial period of epilepsy.

The kinetics of monoamine deamination in the heart and brain of rats thus changes after an exposure of 5 min to hyperbaric oxygen. The kinetics of monoamine deamination in the compensatory and preconvulsive phases of oxygen epilepsy differs considerably; in some cases the disturbances observed in the first phase disappear in the second phase or are reversed. The most marked changes in catalytic properties of MAO due to hyperbaric oxygenation in the preconvulsive period are observed in the rat's heart and not in its brain, evidence of the important role of the cardiovascular system in the genesis of oxygen poisoning, and they lead to the conclusion that, as is reflected also in the electrophysiological data, in the preconvulsive period of oxygen epilepsy definite changes are observed also in neurotransmitter metabolism and, in particular, at the level of their oxidative deamination.

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ACTION OF A HOMOLOGOUS SERIES OF UBIQUINOLS ON LIPID PEROXIDATION IN BRAIN MITOCHONDRIAL AND SYNAPTOSOMAL MEMBRANES

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UDC 616-018.1-008.939.15-039-07

KEY WORDS: ubiquinols; synaptosomal membranes; mitochondrial membranes.

Radicals of oxygen and lipids and molecular products of lipid peroxidation (LPO), which can damage the membrane apparatus of nerve cells, are nowadays regarded as pathogenetic factors in a whole range of mental and organic diseases of the CNS (schizophrenia, Down's syndrome, senile dementia, Parkinson's disease, etc.), and also in drug-induced diseases caused by the action of antidepressants and neuroleptics [3, 5, 12]. These ideas are based on experimental data which indicate an exceptionally strong damaging action of free-radical reactions on such important neuronal functions as reception and transmembrane transmission of impulses, and also on uptake and release of neurotransmitters [6, 10]. The following factors have been named as possible causes of excessive accumulation of LPO products: hyperproduction of active forms of oxygen, deficiency of enzymic and nonenzymic antioxidative systems and a combination of these two factors. The two most important systems generating active forms of oxygen are the electron-transport chains of the endoplasmic reticulum and mitochondria respectively. Oxygen radicals are generated in the endoplasmic reticulum as a result of NADPH-dependent regeneration of components of the NADPH electron transport chain: cytochrome P-450 reductase and cytochrome P-450 itself. Oxygen reduction products in mitochondria are firmly bound with the active center of cytochrome oxidase, but they can "leak" to other components of the electron-transport chain, from ubiquinone for example.

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(Presented by Academician of the Academy of Medical Sciences of the USSR S. E. Severin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 107, No. 4, pp. 420-422, April, 1989. Original article submitted June 23, 1988.

0007-4888/89/0004-0468$12.50