MONOAMINE DEAMINATION IN THE BRAIN AND HEART OF RATS EXPOSED TO TOXIC HYPERBARIC OXYGENATION

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Biogenic amines are known to participate in the convulsant response of electrical, chemical, and hyperoxic genesis. For instance, an increase in sensitivity to convulsants was accompanied by a fall in the brain level of all biogenic amines [1]. Conversely, elevation of the noradrenalin (NA) and dopamine (DA) levels in the brain after injection of dopa and of monoamine oxidase inhibitors (MAO) followed a parallel course with elevation of the threshold of chemical and electrical convulsions [12]. In rats predisposed to audiogenic convulsions, the serotonin (5-HT) and NA levels were considerably depressed in various brain structures, whereas the DA level was raised by comparison with these parameters in animals with low excitability [9]. Activity of MAO, one of the most important enzymes of biogenic amine metabolism, has been shown [4, 6] to be considerably modified when the oxygen pressure is raised: the velocity of oxidative deamination of the principal neutotransmitter monoamines, 5-HT and NA, is reduced, whereas at the same time the enzyme acquires the property of oxidizing substrates which under normal conditions are uncharacteristic, namely GABA, putrescine, aminosugars, and other substances [4]. A similar effect, namely MAO transformation, has also been described under other extremal conditions [6], and it can be regarded as the enzyme's response to stress stimuli. However, in the publications cited above, the velocity of the deamination reaction for most MAO substrates was determined purely as an indicator of specific activity, using a single (saturating for "normal" conditions) concentration of the substrate, and a single time point, namely the 60th minute of exposure to the toxic action of oxygen. Yet it is possible that saturation curves of enzymes under stress conditions are modified, and this makes it essential to study the deamination kinetics of monoamines during exposure to hyperoxia to be investigated by the use of different concentrations. Another important aspect of the investigation is a comparative study of changes in monoamine metabolism in the presence of a raised oxygen pressure in the CNS and peripheral organs, especially those which suffer under these conditions, namely the heart.

In the investigation described below it was accordingly decided to study the deamination kinetics of the most important monoamines belonging to neurotransmitter and certain other groups in the brain and heart of rats exposed to the toxic action of hyperbaric oxygenation (HBO).

EXPERIMENTAL METHOD

The convulsive form of oxygen poisoning was simulated by exposing male albino rats weighing 180-250 g in a pressure chamber containing oxygen under a pressure of 6 atm for 60 min (the periods of compression and decompression each lasted 20 min). The time of onset of convulsions in the animals was recorded when they fell into the side position. At the end of decompression the animals were decapitated and their organs (brain and heart) were removed in the cold and kept in liquid nitrogen. MAO activity in 25% homogenates of the rat brain and heart was determined by isothermic distillation of ammonia followed by nesslerization [2]. MAO substrates were used in 4-7 increasing concentrations. The results of the kinetic investigations were represented graphically by Lineweaver–Burk plots.

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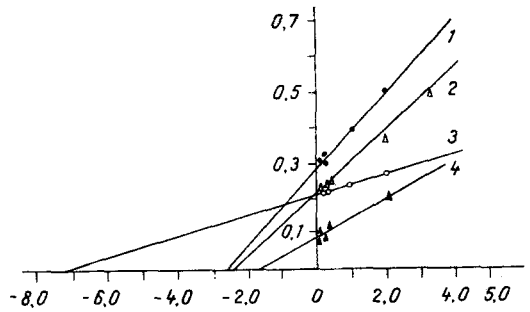

Fig. 1

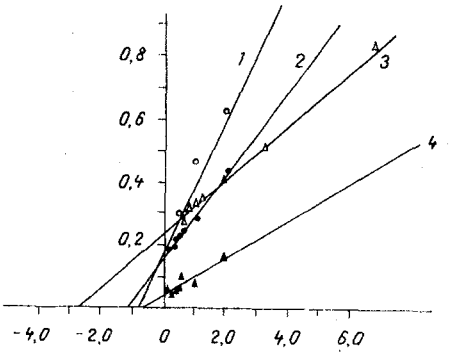

Fig. 2

Fig. 1. Deamination kinetics of 5-HT in brain (1, 3) and heart (2, 4) of intact rats (2, 3) and rats subjected to HBO (1, 4). Here and in Figs. 2 and 3: abscissa, 1/C (in mM^{-1}); ordinate, 1/V (in nmoles ammonia/mg protein/min).

Fig. 2. Deamination kinetics of DA in brain (1, 2) and heart (3, 4) of intact rats (1, 3) and of rats subjected to HBO (2, 4).

TABLE 1. Michaelis–Menten Constant (K_m, in nM) for Brain and Heart MAO of Normal Rats and Rats Exposed to HBO for 60 min (M ± m)

Experimental conditions	Brain				Heart			
	5-HT	2-PEA	DA	TYR	5-HT	2-PEA	DA	TYR
Control	0,13±0,01	1,07±0,11	1,16±0,12	1,10±0,02	0,41±0,04	1,0±0,10	0,36±0,04	0,2±0,02
HBO	0,38±0,04**	1,56±0,16	0,87±0,09**	2,63±0,30	0,57±0,06	1,05±0,10	1,42±0,02*	4,0±0,4*

Legend. *$p \leq 0.01$, **$p \leq 0.05$ compared with control. Number of determinations 4-8.

EXPERIMENTAL RESULTS

HBO in toxic doses considerably modified the kinetic parameters of deamination of 5-HT in the rat brain (Fig. 1, Table 1). For instance, judging from the graph, affinity of the enzyme for the substrate was very considerably reduced, evidence of profound disturbances in the active center of MAO, responsible for deamination of this transmitter. These data are in agreement with results obtained in [5]. Conversely, no significant changes in the kinetic characteristics of 5-HT deamination were found in the rat heart (Fig. 1, Table 1). Thus the toxic action of HBO was accompanied by a sharp fall in affinity of MAO for 5-HT in the rats' CNS, but not in their heart.

By contrast with 5-HT, the value of the Michaelis constant (K_m) of DA deamination in the brain of rats with toxic hyperoxia did not differ from that in animals of the control group (Table 1, Fig. 2). In the heart, a marked decrease of affinity of MAO for DA was found. Just as in the study of 5-HT deamination, opposite changes in the brain and heart also were observed for DA during oxygen convulsions.

In the brain of rats with toxic hyperoxia, the maximal velocity of the tyramine-deaminase reaction was increased whereas affinity for the substrate was unchanged, but in the heart the value of K_m showed a marked increase, accompanied by a small increase in the maximal reaction velocity (V_{max}, see Table 1 and Fig. 3). Values of the Michaelis–Menten constant for deamination of 2-phenylethylamine (2-PEA) in the brain and heart of the rats with HBO were virtually unchanged (Table 1).

Considerable changes in the kinetic characteristics of deamination of different types of MAO substrates were thus discovered in the organs of rats exposed to the toxic action of oxygen. The degree of severity of these changes differed for the different substrates studied. The important fact is the disparity between results obtained for the brain and heart. The principal pathological responses to an increased oxygen pressure are known to develop in the CNS and the cardiovascular and respiratory systems. Consequently differences in disturbances of monoamine degradation in the brain and heart reflect the organ specificity of the effects of monoamines and help to identify the transmitters which are important for these two organs in this particular situation. For example, a sharp fall in the affinity of MAO for 5-HT (Table 1) is characteristic of the response of this transmitter to hyperoxia in the brain, but not in the heart. According to data in the literature, 5-HT raises the threshold of convulsions

TABLE 2. Specific MAO Activity in Brain and Heart of Rats (in nmoles ammonia/mg protein/min) Exposed for 27 and 60 min to HBO (M±m)

Experimental conditions	Brain				Heart			
	5-HT	2-PEA	DA	TYR	5-HT	2-PEA	DA	TYR
Control	3,5±0,34	0,92±0,08	5,03±0,04	4,51±0,13	2,72±0,11	0,97±0,06	1,7±0,01	1,83±0,12
27-min HBO	3,32±0,05	1,58±0,02*	3,95±0,32***	4,01±0,41	3,33±0,36	0,35±0,01**	2,24±0,29	3,54±0,35*
Control	4,8±0,34	2,45±0,52	5,19±0,6	5,93±0,37	6,5±0,35	1,77±0,26	4,54±0,16	7,12±0,42
60-min HBO	5,12±0,19	1,51±0,3	5,39±0,18	7,01±0,24	2,4±0,53**	1,3±0,03	2,43±0,29**	3,65±0,1**

Legend. *$p \leq 0.001$, **$p \leq 0.01$, ***$p \leq 0.05$ compared with control. Number of determinations 4.

in rats and mice in response to injection of metrazol, strychnine, and picrotoxin [3], and it also delays the onset of oxygen convulsions [7]. It can accordingly be postulated that inhibition of 5-HT deamination in the brain by reduction of affinity of MAO for it leads to its accumulation, and to some extent this counteracts the toxic effects of oxygen. The mechanism of protection of the cardiovascular system against oxygen convulsions is evidently not as closely connected with serotoninergic transmission as it is in the CNS. According to data in the literature, NA, another very important neurotransmitter, which like 5-HT, is oxidized by type A MAO, on the contrary accelerates the onset of convulsions during toxic hyperoxia [11], and destruction of noradrenalin-rich brain structures such as the globus pallidus or red nucleus prevents the development of oxygen-induced convulsions in general [10]. Thus the onset and development of oxygen epilepsy may be due to correlation between activity of the serotoninergic (protective) and noradrenergic (proconvulsive) systems, which can perhaps be most expediently judged by the velocity of oxidative deamination of NA and 5-HT in the CNS.

By contrast with 5-HT, the kinetic parameters of deamination of DA were definitely modified in the rats' heart during toxic hyperoxia, but not in the brain (Table 1). Slowing of DA degradation in the heart during toxic hyperoxia may be evidence of a possible protective function of the neurotransmitter under these conditions at the periphery, but not in the CNS.

Analysis of the results (Figs. 2 and 3; Table 1) shows similar changes in the Michaelis–Menten constants for deamination of two mixed substrates of MAO of types A and B, namely DA and tyramine (TYR). Data on the effect of TYR on oxygen convulsions in animals are not to be found in the literature. However, constriction of blood vessels, leading to raising of the blood pressure, is one of the defensive-adaptive responses of the body to hyperoxia given for therapeutic purposes [8]. Unlike 5-HT and DA, TYR is not a neurotransmitter; it enters the body with the food, but has a strong indirect pressor effect. Accordingly, the slowing of its metabolism which we found in the rat heart during hyperoxia is evidently partially responsible for the vasoconstrictor action of oxygen.

Our kinetic analysis of oxidative deamination of monoamines in the brain and heart of rats exposed to oxygen poisoning broadens our ideas on changes in neurotransmitter metabolism during long-term exposure of animals to hyperbaric oxygen. However, at these times of hyperoxia, what we observe is the terminal stage of a convulsion which begins on the average at the 27th minute of exposure. Thus the 27th minute of exposure to oxygen under toxic conditions is the critical time, the moment of collapse, of exhaustion of the reserves of the protective forces of the body in the fight against oxygen poisoning. This is the end of the adaptation stage and the beginning of convulsive activity. We considered it interesting to compare changes in specific MAO activity at the 60th and 27th minutes of exposure of rats to oxygen.

At the 60th minute of exposure, with saturating concentrations of substrates, specific MAO activity in the rat brain relative to all substrates studied was unchanged, but in the heart its activity relative to 5-HT, DA, and TYR fell sharply (Table 2). At the 27th minute, deamination of 2-PEA increased in the brain and decreased in the heart; a sharp increase of deamination of TYR in the heart and some decrease of DA oxidation in the brain also were observed. The results are evidence, first, of differences in monoamine deamination at different stages of exposure to oxygen and, second, of the need to undertake kinetic studies to characterize changes in the enzyme during toxic hyperoxia.

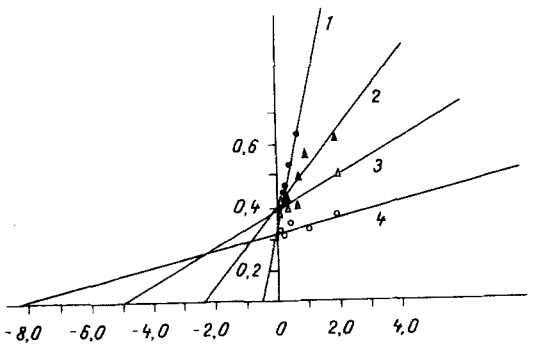

Fig. 3. Deamination kinetics of tyramine in brain (1, 4) and heart (2, 3) of intact rats (3, 4) subjected to HBO (1, 2).

In this paper we have in fact dealt with only the last stage of delayed adaptation of the animal to hyperbaric oxygen. It will be perfectly evident that to understand the mechanism of onset of oxygen epilepsy, it is important to have some idea of changes in monoamine metabolism in the preconvulsion stage, characterized on the basis of the EEG by alternation of three states: transitional, compensated, and preconvulsive [8].

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SELECTIVE ANTICONVULSANT ACTION OF N-SUBSTITUTED IMIDAZOLE-4,5-DICARBOXYLIC ACIDS AGAINST QUINOLINIC ACID

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A new trend in research into convulsive states in recent years has been the study of neuroactivity of tryptophan metabolites — kynurenine (KYN) and quinolinic acid (QUA) — as endogenous convulsants [7]. Their convulsant effect, established initially in mice, sexually immature rats, and frogs [7], has also been observed in experiments on rats when injected into the cerebral ventricles (KYN) [12] and into the dorsal hippocampus (QUA) [13]. Most information about the antagonists of the convulsant action of KYN and QUA has been obtained in mice. Four groups of antagonists were distinguished in experiments on these animals: 1) KYN metabolites (kynurenins) — kynurenic, picolinic, xanthurenic, and nicotinic acids; 2) inhibitory amino acids — taurine and glycine; 3) GABA derivatives — fenibut, baclofen, and sodium hydroxybutyrate; 4) standard anticonvulsants — phenobarbital, phenytoin sodium, and methindione. However, virtually all these antagonists were effective only against KYN and not against QUA. The only antagonists of QUA, namely fenibut, baclofen, and sodium hydroxybutyrate, proved to be ineffective in experiments on rats [3]. Because of this state of affairs, the search for antagonists predominantly of QUA is an urgent problem. The investigation described below was undertaken to solve this problem.

N-substituted derivatives of imidazole-4,5-dicarboxylic acid, which can be regarded as QUA analogs (Table 1), were chosen for testing. It was shown previously [8] that to counter-

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