THE AMMONIA-GLUTAMIC ACID-GLUTAMINE SYSTEM OF THE BRAIN IN THE COMBINED EFFECT OF HYPOTHERMIA AND ELEVATED OXYGEN TENSION

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The effect of elevated oxygen tension on animals is accompanied by an appreciable increase in the concentration of ammonia in the brain [3-6]. Its content reaches values that have not been observed under other conditions. Convulsions ensuing in animals after a certain period of restlessness are the most severe symptom of oxygen poisoning. However, it has not been possible to note a direct relation between the concentration of ammonia in the brain and the appearance of convulsions. For example, the prolonged (90 min) effect of 3-4 atm of oxygen which does not cause the development of convulsions leads to a greater increase in the ammonia concentration in the brain (on the average up to 17.55 mg%) than the brief effect of 6 atm of oxygen, when after only 30 min the animal, after a prolonged period of convulsions, is in a terminal state. The ammonia concentration in the brain at this time is on the average 14.43 mg%. Under the effect of 3 atm of oxygen a direct relationship was noted between the time of the effect of the elevated oxygen tension and the ammonia concentration in the brain. It was proven that regardless of the behavior of the animal oxygen poisoning is accompanied by an appreciable increase in the concentration of free ammonia in the brain. The mechanism of oxygen convulsions in all probability differs from the mechanism of convulsions of a different origin.

S. I. Prikladovitskii [8] established that convulsions arising in animals exposed to an elevated oxygen tension are the result of lesions of the cerebral cortex. Urethane and ether, narcotically acting on the cerebral hemispheres, prevent the appearance of convulsions in rabbits subjected to the oxygen effect.

The experiments of Ya. I. Veksler and Z. S. Gershenovich [1], carried out in vivo and in vitro, demonstrated that the disturbance in the dynamics of the ammonia-forming systems of the brain in deep hypothermia is the consequence of processes arising in the body in connection with its cooling, and is a characteristic feature of brain metabolism in hypothermia. The restoration of normal relationships in the ammonia-glutamic acid-glutamine system upon heating after cooling occurs slowly. For example, when the temperature is dropped to 30° the ammonia content is 2.1 mg% and with subsequent heating to 30°, 4.5 mg%.

Under certain circumstances a person can be simultaneously affected by elevated oxygen tension and a low temperature. The possibility is not precluded of a combined use of hypothermia and hyperoxia in the management of certain forms of malignant tumors [12].

Taking into account the importance of the ammonia-glutamic acid-glutamine system for functioning of the brain [1, 2, 13, 4, 6, 7, 16], we studied certain aspects of its dynamics in the combined action of hypothermia and hyperoxia.

METHOD

The experiments were set up on white rats weighing 80-130 g. The rats were chilled by a cold blanket, in which water having a temperature of 4-5° was circulated. The body temperature dropped gradually (to 20-19° in 50-55 min). The temperature was measured rectally. The rats were fixed in a special stand 15-20 min before the start of cooling. When a temperature of 20-19° was reached, the experimental rats were placed together with the stand in

TABLE 1. Content of Ammonia, Glutamine, and Glutamic Acid in the Brain of Normal Rats, Rats in a State of Hypothermia, and in a Combination of Hypothermia and Hyperoxia

Index determined	Average	Limits of variation	σ±	m±
Norm				
Ammonia	0,5	0,3—0,69	0,111	0,029
Glutamine	8,02	5,75—10,8	1,87	0,122
Glutamic acid	162	131—193	20,05	6,04
Hypothermia				
Ammonia	3,16	2—4,24	0,88	0,31
Glutamine	6,27	3,96—9,86	2,206	0,74
Glutamic acid	156	130—197	23,93	8,48
Combination of hypothermia and hyperoxia				
Ammonia	3,22	2—4,58	1,06	0,375
Glutamine	6,34	5,36—9,04	1,34	0,47
Glutamic acid	150	115—200	33,34	11,11

a pressure chamber which was filled with medical oxygen to a pressure of 4 atm. The body temperature was maintained at a level of 20-19° for 30-40 min.

The control rats were left in the stand at the same temperature as the experimental animals. The control and experimental animals after exposure were removed from the stand and submerged entirely into liquid nitrogen. The brain was used entirely. The frozen brain, after careful grinding in liquid nitrogen, was extracted with 5% chilled trichloroacetic acid, centrifuged, and the ammonia was determined in the supernatant by Seligson's method as modified by Brown and Duda [13] and Silakova [9], glutamine by Silakova's method [9], glutamic acid was determined electrophoretically after Dose [14]. The results were processed statistically and are given as the average values from 9-13 experiments.*

RESULTS

Our data on the content of ammonia, glutamine, and glutamic acid in the brain of 13 control animals in a resting state (Table 1) do not differ from those in the literature [1, 5, 12, 13].

The formation of ammonia is activated in the rat brain in deep hypothermia (see Table 1). This corresponds to the data of Ya. I. Veksler and Z. S. Gershenovich [1], who demonstrated that at the end stage of cooling, the brain of young rats (weighing 80-100 g) contains 3.9 mg% of ammonia and of old rats (weighing 250-350 g), 5.91 mg%.

In the animals subjected to the joint effect of hypothermia and hyperoxia (see Table 1) we did not find substantial differences in comparison with those in a state of hypothermia. The characteristic oxygen convulsions in animals in a state of hypothermia were not noted.

In another series of experiments the control and experimental animals, in contrast to the preceding series, were cooled by lowering the body temperature to 20-19° in 15-20 min (Table 2).

We established that the high ammonia content in the brain arising in hypothermia is not changed under the subsequent effect of an elevated oxygen tension. The concentration of free ammonia in the brain which was established in hypothermia is sufficiently high, and its further increase proves to be impossible. We can expect that in hypothermia the sources of ammonia release in the brain are exhausted and the subsequent effect of hyperoxia does not cause its further liberation. The rate of development of hypothermia plays a definite role here: in the rapidly chilled animals the ammonia content of the brain did not reach values which were observed at the same stage of chilling with

* The values of the content of ammonia and glutamine are presented as converted to nitrogen and of glutamic acid, to the entire molecule.

TABLE 2. Content of Ammonia, Glutamine, and Glutamic Acid in the Brain Under the Joint Effect of Hypothermia and Hyperoxia (in mg%)

Animal group	Ammonia				Glutamine				Glutamic acid			
	Average	Limits of variation	±	±	Average	Limits of variation	±	±	Average	Limits of variation	±	±
Control	3,61	2,9—4,76	0,62	0,194	6,79	4,24—9,8	1,72	0,57	169	95—206	31,21	9,42
Experimental	3,74	2,92—5,21	0,725	0,26	6,75	4,29—9,75	1,9	0,223	170	109—227	34,33	10,84

a gradual lowering of body temperature.

In spite of the lability of ammonia formation, a new level of the relation between the release and binding of ammonia is established.

It is known that in hyperoxia the level of free glutamine drops simultaneously with an increase in the ammonia content in the brain [5]. However, Z. S. Gershenovich and A. A. Krichevskaya [5] established that free glutamine can be a source of the formation of ammonia only under certain conditions, when the enzyme systems retain their activity. In the opinion of Ya. I. Veksler and Z. S. Gershenovich [1], free ammonia can be considered as an active source of ammonia only in the first stages of chilling the animal. When the body temperature is dropped to 25-20°, deamidization of glutamine almost entirely ceases. Apparently this is due to the almost identical level of the glutamine content in the brain in the experiments and in the control.

Glutamic acid plays an important role in the system of ammonia formation and elimination. It is subjected to many conversions in the brain: amidation, deamination, decarboxylation, etc. However, with a high content of ammonia in the brain, its further participation in ammonia formation amounts to nothing [16, 17]. At various periods of oxygen poisoning the quantity of glutamic acid in the brain increases [4]. An important source of the accumulation of this acid is direct synthesis by reductive amidation of ketoglutaric acid under conditions of both hyperoxia [5] and hypothermia [1]. But in hypothermia (25-20°) the accumulation of glutamic acid in the brain ceases. At the end of supercooling the animal, its content somewhat drops [1]. At this period the glutamic acid is possibly subjected to decarboxylation with the formation of γ-aminobutyric acid. This premise is also confirmed in our experiments. Thus, in a series of experiments in which the time after the start of chilling of the animals until their decapitation is longer, the content of glutamic acid in the brain drops (to 150-156 mg%).

The results of a study of the combined action of hypothermia and hyperoxia confirm that ammonia is not the cause of various functional states of the brain, but a consequence of chemical processes occurring in the dicarboxylic acid and protein system of the brain [4].

SUMMARY

A study was made of the ammonia—glutamic acid—brain glutamine system in combined action of hypothermia (body temperature—20-19°C) and hyperoxia (4 atmospheres). The system studied showed no significant differences from that in animals in the state of hypothermia: the ammonia content was 3.22 and 3.16 mg percent, glutamine 6.37-6.27 mg percent glutamic acid—150-156 mg percent, respectively. There were no characteristic oxygen convulsions in hypothermic animals.

Investigation of combined action of hypothermia and hyperoxia demonstrated that ammonia was not the cause of various functional states of the brain, but the result of chemical processes in the dicarboxylic acid—brain protein system.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
