ROLE OF NITROGEN METABOLISM OF THE BRAIN IN THE MECHANISMS OF THE THERAPEUTIC ACTION OF HYPERBARIC OXYGEN IN HEMORRHAGIC SHOCK

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Hyperbaric oxygen therapy (3 atm O_2, 60 min) of hemorrhagic shock in cats (blood pressure 60/50 mm Hg) prevents the sharp increase, characteristic of hypoxia, in the concentration of ammonia, urea, alanine, and γ-aminobutyric acid in the brain and activates glutamine formation. In the sensomotor cortex the processes of ammonia detoxication via the pathway of increased synthesis of glutamate and glutamine take place more intensively than in the limbic structures of the brain.

KEY WORDS: *nitrogen metabolism; hyperbaric oxygenation; hemorrhagic shock; sensomotor cortex; limbic structures of the brain.*

The further development of the problem of hyperbaric oxygen therapy is linked with the need for a detailed study of the mechanism of action of oxygen under increased pressure in various pathological processes [1, 7]. The metabolic reaction of the cortical and limbic structures, responsible for the higher integrative regulation of protective and adaptive mechanisms in hypoxic states, to hyperoxia is of great interest. Investigation of the metabolic mechanisms of compensation of acute blood loss, coupled with the metabolism of nitrogen compounds in the CNS, is of great importance in this connection for the elucidation of the neurochemical basis of functional activity of the CNS in hemorrhagic shock.

In this investigation the metabolism of low-molecular-weight nitrogenous substances in the sensomotor cortex and limbic structures of the brain was studied in animals with hemorrhagic shock during hyperbaric oxygenation.

EXPERIMENTAL METHOD

Altogether six series of experiments were carried out on 84 cats weighing 2.5-4 kg. Shock was induced by fractional blood loss from the femoral artery at the rate of 10 ml/kg body weight at intervals of 10 min to a total volume of 21 ml/kg. Hyperbaric oxygenation was given with medical oxygen at a pressure of 3 atm for 60 min in a 170-liter pressure chamber, with a rate of compression and decompression of 0.4 atm/min.

In the experiments of series I nitrogen metabolism was studied in intact animals during immobilization (initial state, atmospheric pressure, blood pressure 150/140 mm Hg); in series II it was studied 10 min after the development of hypotension (blood pressure 60/50 mm Hg, compensated shock), in series III 70 min after the development of hypotension (blood pressure 40/35 mm Hg, subcompensated shock), in series IV in a state of agony, which developed in 40% of the experimental animals 70 min after the beginning of hypotension (blood pressure 10/5 mm Hg, decompensated shock), in series V 70 min after the beginning of hypotension combined with hyperbaric oxygenation for 60 min (shock + hyperbaric oxygen therapy, blood pressure 70/60 mm Hg), and in series VI immediately after hyperbaric oxygenation of healthy animals (blood pressure 140/130 mm Hg).

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TABLE 1. Changes in Content of Glutamate, GABA, and Alanine (in μmoles/g wet weight of tissue) in Cat Brain during Hemorrhagic Shock and Hyperbaric Oxygen Therapy (M ± m)

Series of experiments	Experimental conditions	Number of animals	Glutamate		GABA		Alanine	
			sensomotor cortex	limbic structures of brain	sensomotor cortex	limbic structures of brain	sensomotor cortex	limbic structures of brain
I	Initial state	13	10,17±0,21	10,66±0,23	1,26±0,08	1,67±0,08	0,46±0,03	0,49±0,03
II	Shock (blood loss of 21 ml/kg body weight) compensated	11	11,06±0,30	10,73±0,35	1,16±0,08	1,50±0,10	0,49±0,02	0,54±0,03
III	subcompensated	8	$P_I<0,05$ 10,86±0,25 $P_I=0,05$	10,75±0,41	1,29±0,12	1,66±0,13	0,58±0,03 $P_I<0,01$ $P_{II}<0,02$	0,66±0,04 $P_I=0,01$ $P_{II}<0,05$
IV	decompensated	11	9,90±0,31	10,39±0,37	1,50±0,07 $P_I<0,05$ $P_{II}<0,01$	1,93±0,11 $P_{II}<0,01$	0,59±0,02 $P_I<0,01$ $P_{II}<0,01$	0,63±0,03 $P_I<0,02$ $P_{II}<0,05$
V	hyprebaric oxygen therapy	11	10,52±0,39	10,39±0,32	1,27±0,13	1,52±0,12	0,44±0,02 $P_{III}<0,01$ $P_{IV}<0,001$	0,53±0,03 $P_{III}<0,02$ $P_{IV}<0,05$
VI	Hyperbaric oxygenation of healthy animals	10	10,13±0,38	10,80±0,29	1,32±0,10	1,54±0,10	0,52±0,02	0,54±0,02

Legend. P_I calculated relative to experiments of series I, P_{II}) series II, P_{III}) series III, and P_{IV}) series IV of experiments.

After decapitation of the animals the brain was quickly frozen in liquid nitrogen. Free amino acids were isolated from tissue of the sensomotor cortex and limbic region (hippocampus, amgydala, temporal pole, entorhinal cortex, gyrus cinguli) [10] and their content was determined by paper chromatography [6]; the ammonia content was determined by the microdiffusion method [15], glutamine by acid hydrolysis [2, 12], and urea by the diacetylmonooxime method [11].

The results were analyzed with the use of parametric statistical criteria. To analyze neurochemical relations between the cerebral cortex and the limbic structures, Wilcoxon's nonparametric criterion was used.

EXPERIMENTAL RESULTS

Experimental results are given in Table 1 and Fig. 1, which show that in compensated shock an increase in the reserves of free ammonia and a rise in the glutamine level were observed in the cortical and limbic structures. In the cortex the glutamine content also was increased. In subcompensated shock the ammonia content in the CNS also was raised. Compensated and subcompensated shock were characterized by a higher level of ammonia in the limbic structures of the brain than in the sensomotor cortex ($P < 0.05$). At the same time, there was a sharp increase in the urea content and an increase in the alanine content in the CNS. In decompensated shock the rise in the ammonia level in the CNS was accompanied by an increase in the content of urea, alanine, and γ-aminobutyric acid (GABA). Under these circumstances ammonia formation was much more intensive in the cerebral cortex than in the limbic structures ($P < 0.05$).

The neurochemical mechanisms of protection of the CNS against the toxic effect of ammonia operate through intensification of the synthesis of glutamine and glutamate with the participation of glutamate dehydrogenase and glutamine synthetase, the activity of which is increased at the beginning of the shock and in the postshock periods [3, 8]. Glutamate stimulates excitation in cerebral cortical neurons [13], and this is increased in the initial stage of shock. In severe degrees of hypoxia glutamine synthetase activity is depressed [4], and consequently a deficiency of glutamine synthesis relative to the increased reserves of ammonia develops in sub- and decompensated shock. The rise in the urea level in the CNS accompanying ATP deficiency [5] can be explained not by the synthesis, but by the liberation of urea from its bound forms. The urea content in the CNS, moreover, rises particularly in decompensated shock, when the breakdown of macromolecules under the conditions of severe hypoxia cannot be ruled out. Under these circumstances urea possibly plays a protective role in the CNS.

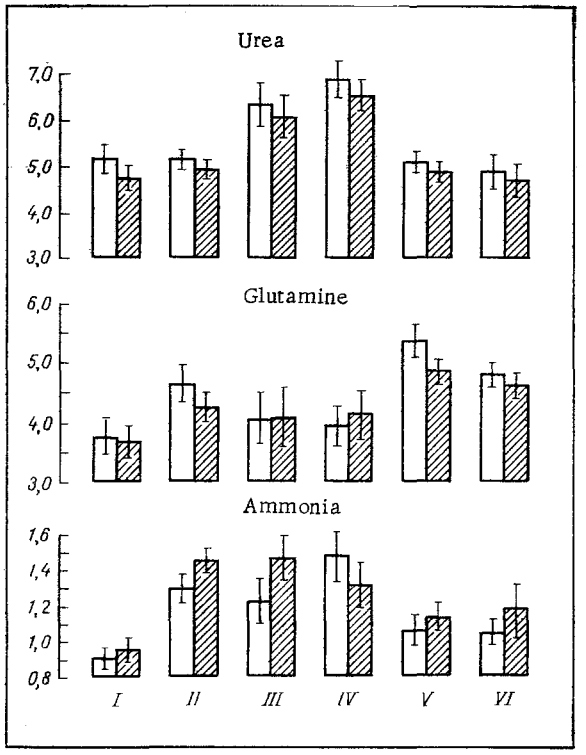

Fig. 1. Changes in content of ammonia, glutamine, and urea in cat brain during hemorrhagic shock (blood loss of 21 ml/kg body weight) and hyperbaric oxygenation (3 atm. O_2, 60 min). Ordinate, quantity of ammonia, glutamine, and urea (in μmoles/g wet weight of tissue): I) initial state; II) compensated shock; III) subcompensated shock; IV) decompensated shock; V) shock + hyperbaric oxygen therapy; VI) hyperbaric oxygenation of healthy animals. Unshaded columns: sensomotor cortex; shaded columns: limbic structures.

The process of excessive alanine formation during increasing hypoxia is of great importance as a mechanism of compensation of metabolic acidosis, for some of the pyruvate is converted into alanine as a result of transamination with glutamate.

Not only glutamate decarboxylase, the activity of which is increased in acidosis [14], but also, evidently, the blocking of the path of entry of GABA into the Krebs' cycle at the succinate level during hypoxia, is responsible for the increase in the GABA level, intensifying inhibition in the CNS [9]. During hyperbaric oxygenation of animals in a state of hemorrhagic shock stimulation of glutamine synthesis in the CNS was observed. In the sensomotor cortex the processes of reductive animation of α-ketoglutarate with the formation of glutamate and the reaction of amidation of glutamate with its conversion into glutamine took place more intensively in the sensomotor cortex than in the limbic structures of the brain. Under these circumstances the ammonia level in the sensomotor cortex was restored to normal and in the limbic structure it was lower than in the untreated animals. Hyperbaric oxygen therapy maintained the free urea and alanine concentrations at their initial level and also prevented disturbance of the ratio between excitatory and inhibitory mediators in the glutamate–GABA system. The intact brain responds to hyperoxia by stimulation of glutamine synthesis.

Hyperbaric oxygenation not only abolished the hypoxia but also stimulated the metabolic mechanisms of protection of the CNS in shock. The ammonia–glutamate–glutamine system occupies the leading position in this mechanism. Other metabolic mechanisms with a

protective-adaptive role (glutamate—GABA, pyruvate—alanine, bound urea—free urea) reflect the extreme degree of their mobilization in hypoxia, and the need for their mobilization during hyperbaric oxygen therapy does not arise, for real conditions are created for activation of the Krebs' cycle.

Low-molecular-weight nitrogen compounds thus occupy an important place in the metabolic mechanisms of the therapeutic action of hyperbaric oxygen for they are responsible both for prevention of disturbances and for restoration of the biochemical processes in the CNS in hemorrhagic shock.

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