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Neurosis of Acquired Helplessness and Role of Hypoxia in the Formation of This Disorder in Rats

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Acquisition of instrumental defense response with pain reinforcement uncertainty (25% reinforcement) induced the development of acquired helplessness in 50% rats. Acquired helplessness is characterized by the absence of responses to conditioned (light) and unconditioned stimuli (pain), minor response of plasma corticosterone to learning, gas markers of circulatory cerebral hypoxia ($\Delta A/V$ pO_2 carotid artery/jugular vein), low sensitivity to severe hypobaric conditions, and high resistance of Purkinje cells in the cerebellum. Piracetam improved learning and prevented the development of acquired helplessness. Local changes in cerebral blood flow and energy deficit in neurons responsible for emotional stress during acquired helplessness impair adaptive capacity, but reduce energy consumption and protect neuronal structures.

Key Words: *instrumental defensive response; environmental uncertainty; neurosis of acquired helplessness; hypoxia; piracetam therapy*

Exo- and endogenous conflicts, depression, and insecure future are the main causes of psychoemotional stress, neuroses, and psychosomatic diseases in humans. Apart from psychophysiological, physiological, and neurochemical factors, cerebral hypoxia plays an important role in the development of various neuroses, including neurasthenia, depression, and chronic fatigue syndrome (CFS) [2]. Emotional overload produces opposite changes in local cerebral blood flow (LCBF) or causes hypoxia and impairs energy supply to neurons with high functional activity. Energy deficit in neurons underlies their irresponsiveness to various stimuli, reduces energy consumption, and protects structures. Neurosis of acquired helplessness (AH) that serves as a model of hyposthenic neurasthenia, CFS, and depression is of particular interest in this respect. AH develops under conditions of environmental uncertainty (stochastic reinforcement) and manifests in a decrease in adaptive capacity of the organism [8].

Here we developed a model of AH in environmental uncertainty, evaluated physiological manifestations of neurosis, studied gas supply to the brain during AH, recorded the reaction of neurotic animals to stress (*e.g.*, hypoxia), and compared the efficiency of central antihypoxic agents and reference preparation in the therapy of this disorder.

MATERIALS AND METHODS

Experiments were performed on 196 male outbred albino rats weighing 250-300 g. The animals were kept in a vivarium under standard feeding conditions (5 animals per cage). Studies were performed daily (except Saturday and Sunday) in the same time and

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season. The instrumental defensive response was elicited in a special chamber. Unconditioned electric stimuli were delivered through electrified floor grid. The lamp was used to generate conditioned stimuli. The lever was positioned at a distance of 2 cm from the floor. The animals were trained to press the lever to terminate unconditioned painful stimulation. Signals and movements of rats were recorded using electrodes connected to a Commandor computer.

The light signal (2 sec) was delivered to elicit a conditioned response. This procedure was followed by 5-sec unconditioned electric stimulation. Painful stimulation was terminated when the animal exploring the chamber pressed the lever. The period between training sessions was 38 sec. Learning trials were repeated later. In case of even time distribution of search reactions, when a priori probability for their random distribution was 0.05, 25% correct responses were reinforced (light-on). The rats were subjected to 40 training sessions per day. The experiment continued until acquisition of the conditioned response (if acquired, 8 times on average). The learning was successful when the number of correct responses significantly surpassed a priori probability of accidental performance (χ^2 test, $p<0.05$). Some animals could not perform the task and had signs of neurosis. The efficiency of learning was determined by the incidence of AH and number of instrumental reactions and presentations [7].

Gas supply to the brain was estimated after intraperitoneal injection of 1000 mg/kg urethane in 3.0 ml physiological saline. To minimize blood loss, the amount of gases and content of lactic acid were measured in various animals [5]. After each training session the blood was routinely taken from the jugular vein and carotid artery to estimate the arteriovenous difference ($\Delta A/V$). It should be emphasized that brain neurons produce various effects on physiological systems and resistance to hypoxia, which depends on the degree of energy deficit. Survival of control, learned, and AH rats was determined by respiratory arrest in an altitude chamber (10,000 m).

Adrenocorticotropic hormone and glucocorticoids play an important role in conditioned learning and hypoxia-produced changes in the plasma. Corticosterone concentration in control, learned, and AH rats was measured by the radioimmune non-extraction method before and after the first and last learning sessions. The blood was taken from the caudal vein (0.5 ml).

Taking into account high sensitivity of the brain to hypoxia and the development of locomotor disturbances, the density of Purkinje cells in the cerebellum, state of surrounding glia, and qualitative composition of cells were determined by morphological indexes after learning (dark, light, and degenerative cells). The animals were killed under ether anesthesia. The cerebellum was removed and fixed in Carnoy's fluid. Slices (5 μ) were embedded in paraffin and stained with cresyl violet by the method of Nissl. The data were processed using special software (Institute of General Reanimatology) [1].

Experimental therapy included intraperitoneal injections of central nootropic antihypoxant piracetam in a dose of 200 mg/kg 40 min before and during learning. The anxiolytic afobazole in a dose of 5 mg/kg served as the reference preparation.

The results were analyzed by Student's *t* test and χ^2 test.

RESULTS

After acquisition of the instrumental defense response under conditions of environmental uncertainty the rats were divided into 3 groups: learned animals (52.7%, group 1), not learned animals with AH (42.3%, group 2), and resistant not learned animals without AH (5%, group 3). Group 3 rats were excluded from observations.

As differentiated from learned rats, group 2 animals demonstrated the absence of appetite, body weight loss, hypothermia, and local hair loss. These rats displayed orientation and exploratory activity only at the initial stages of learning. Then this behavior in group 2 rats became irregular and less pronounced than in group 1 animals (Table 1). Group 2 rats crowded together, hid in a corner, and vocalized in response to conditioned and unconditioned stimulation. As differentiated from learned rats, handling of not learned animals induced avoidance and paradoxical reactions (escape, clawing, and biting). Conditioned light and unconditioned electrocutaneous stimulation did not induce the avoidance response in group 2 rats (as differentiated from group 1 animals). The conditions of learning were inadequate for resistant rats of group 3. Taking into account hypodynamia, irresponsiveness to conditioned and unconditioned stimuli, paradoxical reactions to manipulations, and vegetotrophic disorders, the rats with AH were characterized by neurosis that resembled hyposthenic neurasthenia, CFS, or depression.

Considerable changes in gas supply to the brain were found in animals with AH, but not in group 1 rats. These changes were manifested in a considerable increase in $\Delta A/V\ pO_2$ and reflected the development of circulatory (microcirculatory) hypoxia in brain structures. $\Delta A/V\ pO_2$ was measured in the jugular vein and carotid artery. This parameter characterizes oxygen supply to the whole brain, but not to individual structures. Under conditions of sufficient LCBF, individual brain structures could maintain other test parameters at a normal level (pH, BE, and lactate).

TABLE 1. Search Behavior in Learned Rats and Animals with Acquired Helplessness (*M*±*m*)

Group	Period of learning				
	1	2	3	4	5
Learned rats (*n*=51)					
number of correct responses	0.36±0.27	1.18±0.25	2.27±0.56	3.45±1.40	6.82±1.02
number of incorrect responses	14.82±5.15	18.64±4.23	12.55±3.20	13.55±2.97	15.36±3.88
Animals with AH (*n*=47)					
number of correct responses	0.25±0.07	0	0	0	0
number of incorrect responses	19.00±3.56	7.81±1.99	5.75±2.32	7.94±0.24	6.79±1.90

Hypoxia and energy deficit resulted in low reactivity of brain structures in animals with AH. Reactivity of the adrenal cortex to learning sharply decreased. Plasma corticosterone level before (*n*=30) and after the first learning session was 20.25±3.70 and 158.8±12.7 μg/ml, respectively. After the last learning session plasma corticosterone concentration in learned rats (119.4±8.0 μg/ml, *n*=20) significantly differed from that in not learned animals with AH (56.4±6.3 μg/ml, *n*=10). Emotional stress and low energy supply to cortical structures are probably accompanied by irresponsiveness not only to conditioned and unconditioned stimuli, but also to the most important steroid component of stress.

Oxygen deficiency in CNS and circulatory and respiratory systems results in their irresponsiveness to stimulatory and inhibitory factors (severe energy deficit) and increases their resistance to low O_2 supply. It was observed in neuronal structures of the brain [11], whole heart [12] and its individual fragments (hibernation and stunning) [10], and neurons of the respiratory center [4]. This method is widely used in brain and heart surgery (*e.g.*, lytic mixtures, hibernation, and cardioplegia). "Isolation" of central neurons and stress-realizing endocrine organs from factors causing energy deficit maintains their survival and resistance of cellular and subcellular structures. Passive adaptation is the only mechanism that preserves vital activity of brain cells [9]. This explains maximum preservation of the structure of Purkinje cells and glia in rats with AH and weak search reaction.

Our results show that AH is accompanied by irresponsiveness of brain structures to conditioned and unconditioned stimuli (decrease in energy consumption), low reactivity of the steroid regulatory mechanism mobilizing energy and plastic resources in the organism, reduction of LCBF, and increase in the resistance of cerebellar neurons to severe hypoxia. The data indicate that this system plays an important role in the protection of neurons. We found that the antihypoxant piracetam (but nor afobazole) normalizes energy supply, increases the efficiency of substrate consumption in the brain, optimizes enzyme-catalyzed tissue respiration, reduces the incidence of ineffective search reactions, actualizes skill acquisition, and prevents the development of AH. By contrast, afobazole produces opposite effects [3].

Acquisition of the instrumental defensive response with pain reinforcement uncertainty (stochastic electrostimulation) induced the development of AH in 50% rats. Environmental and subjective uncertainty is the main cause of serious neurotic disorders [8]. AH is manifested in reactive, locomotor, and trophic disturbances typical of neuroses. It can be hypothesized that AH impairs adaptation of the organism to environmental conditions and causes a variety of endogenous disorders. It should be emphasized that AH should be considered as a passive form of brain adaptation to conditions exceeding the reserve capacities during learning [6,12].

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