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Mechanisms of Adaptation to Flow Reactions in Rats after Systemic Blood Arrest

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The rats survived 10- or 15-min systemic blood flow arrest were exposed to various extreme factors within the following 2 months. It was found that the processes leading to functional isolation of CNS play a protective role: they moderate behavioral response to acute stress and alleviate the degree of neuron damage induced by long-term stress during acquisition of a complex food-procuring reflex.

Key Words: *behavior; adaptation; ischemia; postresuscitation period*

Clinical data suggest that in some patients survived terminal events and resuscitation, aggravation of psycho-neurological status developed after discharge from the hospital and returning to routine life. The development of such delayed postresuscitation encephalopathy is explained by enhanced sensitivity to emotional stress and physical load [2]. However, we found no data on specific mechanisms mediating the action of functional loads on the state of the organism during postresuscitation period after recovery of the self-service ability.

Our aim was to study the mechanisms of the effects of external factors on morphological and functional changes in the brain and the whole organism at various stages of postresuscitation period.

MATERIALS AND METHODS

Experiments were carried out on male albino rats (n=95) with initial body weight of 180-230 g. The experimental rats (n=47) were narcotized with ether and subjected to 10-min (group 1) or 15-min (group 2) systemic circulatory arrest (SCA) caused by intrathoracic ligation of the vascular bundle [6] followed by resuscitation with closed-chest cardiac massage and artificial ventilation. Intact rats served as the control.

After successful cardiopulmonary resuscitation the state of the rat was assessed by the rate of elimination of neurological deficit by a 100-point scale, where 100 and 0 corresponded to cerebral death and the absence of neural deficiency, respectively. In addition, 3 various tests were used to assess behavioral activity of resuscitated rats.

Orientation and exploratory activity of some group 2 rats (n=17) was studied in the open field test (OFT) using a RODEO-2 setup. The number of rearings with and without exploring the holes in the upper cover of the chamber and the number of explored holes on the floor were automatically recorded over 3 min. Horizontal motor activity was assessed by the number of infrared beam crossings. The total behavioral activity (TBA) was determined as the sum of the above behavioral indices.

Acute stress was simulated by a single noxious transcutaneous electrical stimulation from the floor grid (30 V, 5 sec). The reaction was assessed by the

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degree of TBA inhibition during the second OFT performed 3-5 min after the end of noxious stimulation.

In all group 1 rats (n=20) and in group 2 rats (n=20) not subjected to stress stimulation, a 4-staged food-procuring conditioned reflex was developed by the method of free choice in a multi-alternative maze [8]. To solve the task completely, the rat must enter the maze from "free field", take a seed first in one, then in the second feeding-troughs, exit from the maze into the "free field", and again enter the maze to procure food. Thus, exit from the maze was a necessary condition for getting the next two seeds. Learning in groups 1 and 2 was started on postresuscitation days 10-12 and 12-14, respectively. Intact controls were examined simultaneously. Training consisted in twenty 13-min sessions with 48-hour intervals and 22-hour fasting period before each session.

After the end of learning, the rats were sacrificed under ether narcosis. The brain was isolated for morphological study of neuronal population in hippocampal field CA1, which is more sensitive to ischemia than the cerebral cortex, and which plays an important role in learning. The following indices were determined in population of CA1 hippocampal field pyramidal neurons: total density of cell population, the number and proportion of light and dark cells, morphologically altered cells, and the number of neurons with satellite glia [1].

The data were processed statistically using Mann—Whitney U test, Fisher angular transform φ test, and Student's t test.

RESULTS

The study of the effect of acute stress stimulation on rat behavior showed that in group 2 rats (n=17) the index of TBA in OF before stress stimulation was 169.1±16.9, which did not differ from the corresponding values of intact rats (176.5±13.5, n=18). On the next day, the reaction to acute stress stimulation assessed by TBA inhibition showed that single noxious electrical stimulation equally affected TBA in intact and resuscitated rats, in which these indices were 37.6±7.8 and 36.8±8.4, respectively. By contrast, in agreement with previous data [3], the rats survived 10-min SCA and tested on postresuscitation days 9-10 demonstrated higher TBA in the first OFT session in comparison with intact rats, while in the second OFT session their behavioral activity was significantly lower than that of intact rats, which attests to stronger reaction to noxious stimulation in resuscitated rats in comparison with intact controls. These data agree with previous hypothesis that the disturbances in orientation and exploratory behavior in rats exposed to 10-, 12-, or 15-min SCA, which demonstrate virtually complete elimination of external neurologic deficiency on resuscitation days 5-10, are determined by two simultaneous and opposite processes in the brain, one of which enhances orientation and exploratory activity, while the other decreases TBA [5]. The effect of the latter process is aggravated by the severity of ischemic damage, and it is directed to minimize reaction of the animal to external stimuli.

Therefore, the above tendency to decrease the behavioral response to external stimuli not only reflects the degree of ischemic damage to the brain, but also protects the organism from hyperstimulation.

Only 15 of 30 intact rats (50%) acquired the conditioned food-procuring reflex during 20 sessions. In group 1 rats, 14 of 20 animals (70%) acquired this reflex, which did not significantly differ from the intact group. However, to learn the task, the intact rats needed 78.4±7.4 reinforcements, while the resuscitated rats needed only 45.5±5.8 reinforcements (p≤0.05). In group 2 rats, the percentage of trained animals increased (78%, pφ=0.06 in comparison with intact rats), although one rat of 10 died on resuscitation day 24 after 5 training sessions. At the same time, the "price" of long-term load presented in the form of maze training was different for intact and resuscitated rats. In group 1 rats, which were trained in the maze, the relative weight of the adrenals was 0.173±0.008 mg/g, which significantly surpassed the control value (0.149±0.008 mg/g, p<0.05). It should be noted that in resuscitated rats, the stress-related increase in relative weight of the adrenals was caused namely by functional load, and not by the development of primary reaction in stress-target organs to SCA, which normalizes to re-

TABLE 1. Parameters of CA1 Field Hippocampal Pyramidal Neurons in Rats with Different Ability to Acquire Food-Procuring Reflex after 15-min Systemic Circulatory Arrest (M±m)

Subgroup	Light and glia-free neurons		Morphologically altered neurons		Total
	number	proportion	number	proportion	
Rapid learning (n=4)	100.6±7.8*	0.393±0.012*	45.4±2.5	0.178±0.002	255.3±15.0
Slow learning (n=3)	132.4±1.0	0.446±0.004	45.6±3.5	0.154±0.013	297.1±4.6

Note. *p_U≤0.05 compared to slow-learning rats.

suscitation day 14 in rats exposed to 10-min SCA [7]. In group 2 rats, the body weight gain during training in the maze was 36.2±5.8 g *vs.* 56.8±5.7 g in intact rats ($p \leq 0.05$). On the whole, the data indicate limited adaptive capacity in rats during a long period (at least 2 months) after resuscitation.

Since learning is related not only to functional activity of the animals, but also to structural changes, we examined the relationships between the results of maze learning and morphology of neuronal population in CA1 hippocampal field, which is highly sensitive to ischemia and is critical for learning. The data showed that among intact rats, successfully trained animals (n=4) had 12.4% higher total density of neurons in hippocampal CA1 field in comparison with that of unsuccessfully trained rats ($p_t<0.05$). Comparison of the state of neuronal population in CA1 hippocampal field between successfully (n=5) and unsuccessfully trained group 1 rats showed that the total density of population was similar in both subgroups, but the number and proportion of morphologically altered neurons were higher in successfully trained rats (by 34.5%, $p \leq 0.01$ and by 36.7%, $p \leq 0.02$, respectively). The results of training food-procuring reflex in group 2 rats made it possible analyze the dependence of the examined indices of neuronal population state on the learning rate (U test). This analysis showed that the rapidly learning rats differed from the slowly learning animals by lower number and proportion of free light neurons (Table 1). It should be noted that the decrease in number and proportion of light free cells is a typical postresuscitation phenomenon [1].

Thus, the results of morphometric analysis showed that the typical postresuscitation alterations of neural population in hippocampal field CA1 are more pronounced in rapidly trained rats than in unsuccessfully trained rats or in rats with delayed development of food-procuring reflex tested in OFT.

We previously showed that starting from the first training session the trained intact and resuscitated rats are characterized by higher behavioral activity in comparison with unsuccessfully trained rats [4]. However, high functional activity in resuscitated and successfully trained rats requiring considerable energy and substrate resources is too high due to low potential adaptation capacity and prevents structural recovery of the brain at the level of architectonics of neuronal population. Under these conditions, the relative limitation of functional activity of resuscitated animals looks like a protective reaction preventing the development of structural damage. The processes developing during the first two weeks of postresuscitation period limit the behavioral reaction of the animal to external stimulation. They reflect a general biological regularity of functional isolation as a passive behavioral adaptive mechanism under pathological conditions [12]. Probably, the action of this mechanism is underlain by the development of desensitization of cAMP-producing system, whose degree correlates with the severity of ischemic damage to the brain [9]. The protective effect of functional isolation of CNS from the environmental stimuli is also manifested at the later postresuscitation stage as moderation of postischemic structural damages to pyramidal neurons in hippocampal field CA1.

Published data suggest that adaptation to the environment (including learning) is characterized by two opposite strategies: active strategy directed to transformation of the environment and passive strategy appearing in extreme cases and aimed at minimization of function and preservation of structures [10,11]. This inhibition of functions manifesting in various systems (respiration, circulation, and CNS) decreases energy expenditure for energy-consuming cell activity, but preserves the possibility of energy supply for biogenesis of substrate structures and their conservation under extreme conditions [10-12]. This phenomenon is widely spread in animals (unicellular organisms, insects, reptiles, and mammals) and is routinely used in clinical practice to enhance tolerance of physiological systems to anoxia and energy deficit (artificial hibernation, craniocerebral hypothermia, cardioplegia, *etc*.). In full measure, the discussed general biological regularity is also manifested in the resuscitation process, at least in those types of experiments that were reported here.

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