Behavioral Reactions of Rats in the Early Postresuscitation Period after Systemic Circulatory Arrest of Different Duration

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Rats were exposed to systemic circulatory arrest for 10, 12, and 15 min. During the first 10 days after resuscitation exploratory activity of animals depended on the duration of systemic circulatory arrest and was determined by two opposite factors: high reactivity of the central nervous system contributing to intensification of exploratory activity, and impaired general state of survivors (inhibition of animal behavior).

Key Words: *behavior; ischemia; postresuscitation period*

Postresuscitation disease is a dynamic and multistage process. The recovery of functional activity after circulatory arrest (CA) depends on the severity of brain injuries and adequate correction of disturbances [7]. Factors that modulate functional state of the central nervous system (CNS) in the early period after resuscitation should be evaluated to perform adequate pathogenetic therapy of patients with postresuscitation disease. Here we studied the state and behavioral reactions as integral parameters of functional activity of CNS in rats survived CA of different duration in the early postresuscitation period.

MATERIALS AND METHODS

Experiments were performed on 158 male albino rats weighing 180-230 g. In 69 animals systemic CA was produced by ligation of coronary vessels for 10, 12, and 15 min [4] and followed by closed-chest cardiac massage and artificial ventilation. The control group included 89 intact rats.

After cardiopulmonary resuscitation the state of animals was evaluated by the dynamics of neurological deficiency. Neurological deficiency was scored using a 100-point scale, which included 19 characteristics (hearing, vision, limb muscle tone, pain sensitivity, external appearance, *etc.*). Brain death corresponded to 100 points, while the absence of neurological deficiency corresponded to 0 points [5]. The total neurological deficiency (TND) was scored starting from its appearance to its complete disappearance. Rat behavior was studied in the elevated plus-maze (EPM) [8] and open-field tests [2] for 5 and 3 min, respectively. Exploratory activity in the open field was automatically recorded using a RODEO-2 device. The number of rearing postures with exploration of holes in the top of the chamber (upper-level activity), rearing postures without exploratory activity (intermediate-level activity), horizontal locomotor activity (crossed infrared beams), and explored holes in the bottom of the chamber (hole-board activity). The general behavioral activity (GBA) was calculated as the sum of recorded parameters. Behavioral reactions of rats were studied after the disappearance of neurological deficiency.

The results were analyzed by Student's t test and nonparametric tests.

RESULTS

In rats survived 10-min CA, TND on days 9-10 after resuscitation was 25.8±2.5 points, while GBA in these animals surpassed the control (205.4±9.9 *vs.* 175.4± 15.1, $p<0.05$). High GBA in resuscitated rats was associated with increased horizontal and

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upper-level activities over the first 30 sec of the open-field test. In this period horizontal activities of resuscitated and intact rats were 91.3±4.8 and 76.8±4.7, respectively ($p<0.05$). Upper-level activities of these animals were 4.4±0.6 and 3.1±0.5, respectively ($p<0.05$). Therefore, resuscitated rats display more pronounced reactions to exogenous stimulation compared to control animals. This was manifested in high GBA and horizontal and upper-level activities of resuscitated rats in the initial period of behavioral tests.

In some rats exposed to CA for 15 min TND was 43.1±6.9 points, *i.e.* was higher than after 10-min CA ($p<0.05$). GBA in these rats did not differ from the control (146.1±24.5 and 176.5±13.5, respectively). However, on the 3rd minute of the open-field test the number of explored holes in this group was below the control (1.7±0.5 and 6.3±1.1, respectively, $p<0.05$). In other rats exposed to CA for 15 min TND was 66.8± 7.6 points ($p<0.05$ compared to the previous group). These rats had lower GBA than intact animals (173.0±19.5 and 230.7±15.6, respectively, $p<0.05$). The decrease in GBA was associated with low number of explored holes on the 3rd minute of observation (4.5± 1.0 and 9±1 in resuscitated and intact rats, respectively, $p<0.05$) and reduced horizontal activity in the open field (134.4±15.6 and 181.9±13.1 in resuscitated and intact rats, respectively, $p<0.05$).

Thus, we found a negative correlation between GBA and TND reflecting the severity of ischemia-induced changes. It should be emphasized that behavioral activity was suppressed in rats exposed to 10-min CA. This was confirmed by a negative correlation between the number of explored holes on the 2nd minute of the open-filed test and TND 9-10 days after resuscitation ($r=-0.65$, $p\leq0.05$).

Behavioral parameters depended on the general state of experimental animals survived 10-min CA, which was confirmed by studies of exploratory activity in EPM and open field 6-8 days after resuscitation. As differentiated from the open-field test, the rats in EPM can escape from exogenous stimulation in dark arms that are more preferential for hollowed animals. In EPM behavioral activity of rats survived 10-min CA was lower than that of intact controls (Table 1). However, exploratory activity of resuscitated animals on the 1st minute of the open-field test surpassed the control (upper-level and horizontal activities, Fig. 1). Behavioral characteristics of rats after 12-min CA were similar. Five-six days after resuscitation upper-level and horizontal open-field activities did not differ from the control. However, on the 3rd minute of the open-field test resuscitated rats explored a lower number of holes compared to intact animals (6.0±0.9 and 9.9±1.3, respectively, $p<0.05$). We found a negative correlation between the number of explored holes on the 2nd minute of the open-field test and TND. Seven-eight days after resuscitation behavioral activity of animals in EPM was lower than in the control. Resuscitated rats demonstrated a lower number of rearing postures (7.0±0.2 *vs.* 12.5±1.9 in the control, $p<0.05$) and shorter duration of horizontal activity (11.6±2.5 *vs.* 21.9±3.8 sec, respectively, $p<0.05$). Therefore, the state of animals determines their behavioral activity in the early postresuscitation period. Our previous experiments and published data show that exploratory activity of rats exposed to CA for 12 and 15 min increases in the late, but not in the early postresuscitation period (by the end of the 2nd week after resuscitation) [3,6]. High reactivity of CNS to exogenous stimulation (activation of behavior) is typical of the postresuscitation period irrespective on the duration of CA [3,6]. Thus, exploratory activity over the first 10 days of the postresuscitation period is determined by the ratio between 2 opposite factors: high reactivity of CNS and severity of pathological changes inhibiting behavioral reactions.

High reactivity of CNS in the early postresuscitation period indicates that the inhibition of reactions to exogenous stimulation plays a protective role in patients with severe hypoxic brain injuries. The development of delayed encephalopathies not related to somatic complications in resuscitated patients with the rapid recovery of CNS activity (speech contact) is associated with inconsistency between inadequately high reactions to exogenous stimulation and inability of systems to respond to functional loads [7]. Clinical observations showed that administration of various metabolic activators, including piracetam, instenon, and actovegin, to patients with severe hypoxic injuries can be followed by the development of paroxysmal EEG activity and aggravation of psychoneurological state [1]. Thus, the brain remains unprotected during direct

TABLE 1. EPM Behavior of Intact Animals and Rats Survived 10-Min CA (6-7 Days after Resuscitation, $M\pm m$)

Parameter	Intact (n=32)	Resuscitated (n=35)
Number of open arm entries	3.3±0.5	1.1±0.7
Number of closed arm entries	3.5±0.5	0.8±0.2
Number of overhangs	2.6±0.5	0.7±0.2
Number of rearing postures	15.8±1.5	10.6±1.0
Time of horizontal locomotor activity	24.0±2.3	10.50±1.57

Note. $p\leq0.01$ compared to intact animals.

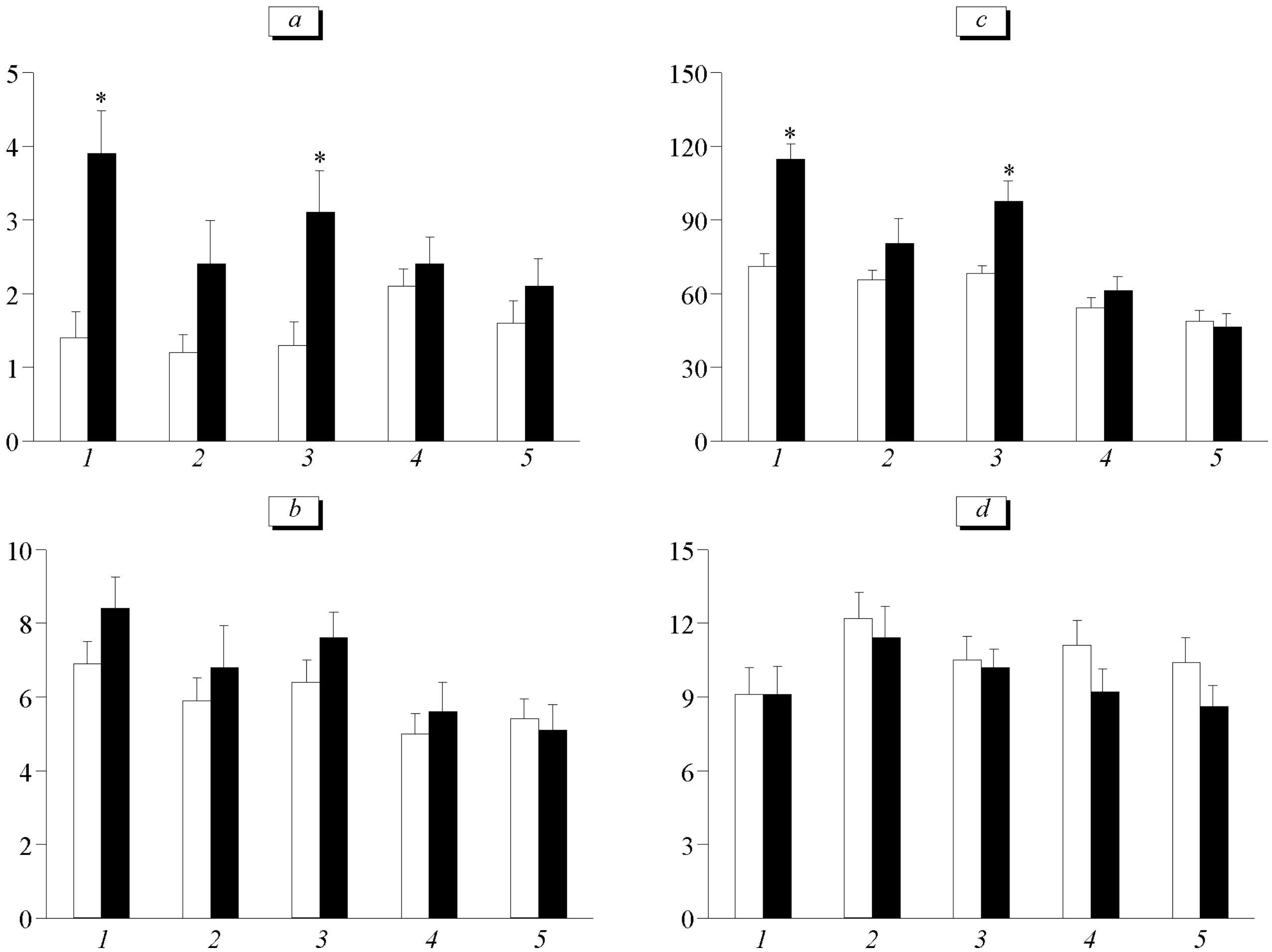

Fig. 1. Upper- (*a*) and intermediate-level activities (*b*), horizontal locomotor activity (*c*), and number of explored holes (*d*) in the open-field test (calculated per 1 min) in intact (light bars) and resuscitated rats exposed to 10-min circulatory arrest (7-8 days after resuscitation, dark bars). Time: 0-30 sec (*1*), 30-60 sec (*2*), 1 min (*3*), 2 min (*4*), and *3* min (5). **p*≤0.05 compared to intact animals.

pharmacological treatment of the nervous tissue. Clinical observations and our results demonstrate the mechanisms of functional disturbances in CNS during the early postresuscitation period. These data promote adequate pathogenetic therapy of patients with brain anoxia and increase the possibility of complete rehabilitation.

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