Postresuscitation Recovery of Functional Activity of Central Nervous System in Rats during Combination Treatment with Mexidol and Neuropeptides Delta Sleep-Inducing Peptide and Oxytocin

N. A. Gorenkova, I. V. Nazarenko, and A. V. Volkov

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In experiments on rats we studied the effects of antioxidant and membrane-protecting agent mexidol and neuropeptides delta sleep-inducing peptide and oxytocin administered during resuscitation after 12-min clinical death. Individual and combination treatment with these substances accelerated recovery of the neurological status and partially or completely corrected behavioral disorders associated with changes in the emotional and motivational status. Combined administration of mexidol and oxytocin most significantly promoted postresuscitation recovery of functional activity in the central nervous system.

Key Words: *clinical death; neurological status; behavior; antioxidants; regulatory peptides*

Free radical processes, structural and functional changes in membranes, and imbalance between excitation and inhibition in the central nervous system (CNS) are the basis mechanisms of the pathogenesis of postresuscitation diseases and require early correction [6]. Previous studies demonstrated a positive effect of various drugs with membrane-protecting and antioxidant properties [3]. Regulatory peptides accelerate the recovery of functional activity in CNS and prevent the development of encephalopathies after ischemia of different etiology [1,2,5].

Here we studied the postresuscitation effects of mexidol possessing membrane-protecting, antioxidant, and nootropic properties and combinations of mexidol with delta sleep-inducing peptide (DSIP) or oxytocin (OXT). DSIP exhibit antihypoxic, antistress, and antioxidant activity, modulates neurotransmitter systems in the brain, and activates the GABAergic system. OXT affects wakefulness, emotional reactivity, mnestic functions, accelerates recovery of the neurological status, and normalizes the state of neuronal populations in the brain cortex and cerebellum after clinical death (CD) [1,2]. We hypothesized that membrane protection will enhance the positive effect of neuropeptides.

MATERIALS AND METHODS

Experiments were performed on 140 adult outbred albino rats; 12-min CD was modeled by the method of V. G. Korpachev *et al*. [4] under ether anesthesia. Mexidol was injected subcutaneously in a dose of 50 mg/kg during standard resuscitation that included external cardiac massage, intratracheal administration of 0.1 mg/kg epinephrine, and artificial ventilation with air. The rats additionally received intraperitoneal injection of DSIP (120 μg/kg) or OXT (10 U/kg) 30 min after successful cardiopulmonary resuscitation. DSIP, OXT, or mexidol was administered to animals of the reference group. Control rats received equivalent volumes of physiological saline. The dynamics of neurological deficit and mortality rate were recorded over 2 weeks after resuscitation.

Institute of General Reanimatology, Russian Academy of Medical Sciences, Moscow. ***Address for correspondence:*** niiorramm@mediann.ru. Gorenkova N. A.

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Behavioral activity of animals was studied in various tests for 4 months. Anxious-phobic reactions were assayed in the multiparametric test [7]. Spontaneous behavior of rats was studied under stress conditions in the open-field test. The open field was a round area (80 cm in diameter) with wooden floor divided by 8 diametrical lines and 2 concentric circles and surrounded by 40-cm walls. The rats were placed into the center of the arena. Horizontal and vertical activity, number of movements away from the wall and entires into the center of the arena, time of grooming, and defecation rate were estimated visually. The behavior was studied for 5 min. A red lamp (15 W) was turned on instead of the standard incandescent lamp (150 W) 3 min after the start of testing. The initial conditions of illumination were restored after 1 min.

Active avoidance response was trained in a special chamber with conducting grid floor. A plastic platform was localized in a corner at a distance of 15 cm from the floor. Four training sessions (each session consisted 10 presentations of conditioned and unconditioned stimuli) were performed at 24-h intervals. Acoustic stimulation served as the conditioned stimulus. Unconditioned stimulus (electric current) was delivered 2 sec after termination of conditioned stimulation. The strength of current was selected individually. The period between stimulations was 15-25 sec. We recorded the number of reactions (jumps on the platform after conditioned stimulation and before unconditioned stimulation), intersignal reactions (jumps on the platform between unconditioned and conditioned stimulation), and short-latency reactions (jumps on the platform over a period of 1-2 sec after unconditioned stimulation).

The results were analyzed by parametric Student's *t* test and nonparametric Mann—Whitney and Fischer's test.

RESULTS

Postresuscitation mortality did not differ between control and experimental groups (22 and 10-19%, respectively). Mexidol significantly accelerated recovery of corneal reflexes and reduced neurological (1 day) and general deficit (8 days) compared to the placebo group ($p<0.05$). Combination treatment with mexidol and DSIP or OXT promoted recovery of neurological status after CD. On day 4 neurological deficit completely disappeared in 66.7% rats receiving mexidol+DSIP, in 88.9% rats receiving mexidol+OXT, and in only 5% rats receiving placebo ($p<0.05$). It should be emphasized that on day 5 after individual administration of mexidol, DSIP, and OXT neurological deficit disappeared in 73.0, 80.0, and 71.4% animals, respectively. Therefore, combination treatment with test preparations potentiated their effects in the initial recovery period.

Behavioral activity of rats was studied 2 weeks after resuscitation. It was found that phobic anxiety after CD was 43% lower than in intact animals ($p<0.001$). In mexidol-treated rats anxious-phobic status tended to normal and by 31% surpassed the corresponding parameter in placebo-treated animals ($p=0.06$), but was 24% lower than in intact rats ($p<0.05$). Administration of DSIP alone or in combination with mexidol completely normalized the degree of anxiety ($p<0.05$). OXT had no effect on anxious-phobic reactions in resuscitated rats. However, combination treatment with OXT and mexidol increased anxiety to a level observed in intact animals (Fig. 1).

Inhibition of passive defense in rats after CD led to an increase in exploratory and locomotor activity. Resuscitated animals displayed increased locomotor and exploratory activities, and changes in emotional reactivity under stress conditions in the open field. Horizontal and vertical activity, number of crossed central squares, and number of entries into the center increased by 2, 1.8, 3.4, and 4 times, respectively, compared to intact animals ($p<0.009$, Fig. 2). The time of grooming increased by 64% on the 4th minute during a sharp change in the illumination conditions ($p<0.03$). Mexidol normalized locomotor activity and emotional reactivity of resuscitated rats, which manifested in decreased horizontal activity and decreased number of crossed central squares (by 59 and 53%, respectively, $p<0.04$ compared to placebo).

Treatment with DSIP alone or in combination with mexidol reduced horizontal activity of resuscita-

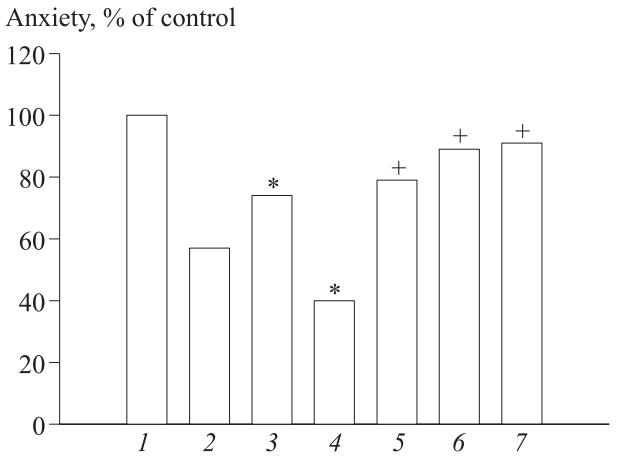

Fig. 1. Effect of mexidol, oxytocin (OXT), and combination mexidol+OXT on the degree of anxiety in albino rats after 12-min cardiac arrest. *1*) intact rats ($n=32$), *2*) resuscitated rats receiving placebo ($n=31$), *3*) mexidol ($n=29$), *4*) OXT ($n=14$), *5*) mexidol+OXT ($n=9$), *6*) delta sleep-inducing peptide (DSIP) ($n=10$), and *7*) mexidol+DSIP ($n=9$). Here and Figs. 2 and 3: $p<0.05$: *compared to intact rats; +compared to placebo. Combined results after testing for 3 and 5 min.

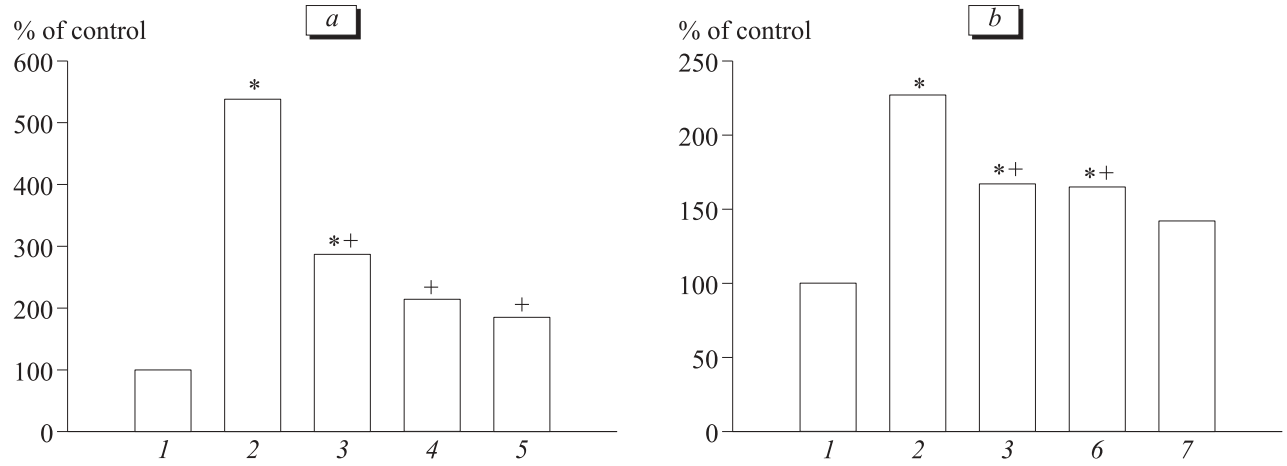

Fig. 2. Effect of mexidol, oxytocin, or delta sleep-inducing peptide and their combinations on open-field behavior in rats after 12-min cardiac arrest. Number of entires into the center (*a*) and horizontal activity (*b*).

ted rats (Fig. 2). However, in the group receiving mexidol+DSIP these changes were insignificant. Combination treatment with mexidol and OXT more significantly normalized exploratory activity of animals in the open field. In these rats the number of entires into the center and movements away from the wall was lower than in resuscitated animals by 58.7 and 65.6%, respectively ($p<0.04$). OXT produced a similar, but less pronounced effect (Fig. 2). Our results indicate that administration of OXT and DSIP alone or in combination with mexidol normalized some parameters of behavioral activity reflecting emotional reactivity of rats under stress conditions. Hence, the test compounds possess antistress properties. Potentiation of the effects was observed only for combination mexidol+OXT.

Active avoidance response was studied in the late recovery period (2 months after CD). Resuscitated animals demonstrated impaired learning. On days 1, 3, and 4 the number of correct responses in these rats was lower than in intact animals by 46.5, 62, and 64%, respectively ($p<0.001$). However, the number of short-latency escape responses in resuscitated rats surpassed that in intact animals (Fig. 3). The decrease in the number of correct reactions and increase in the number of short-latency reactions suggest that the animals learned to escape electrical stimulation, but could not avoid it in response to the conditioned stimulus. These data show that the ability of rats to recognize significant stimulus and associate it with negative reinforcement was impaired after CD.

Administration of mexidol or OXT did not improve learning in animals survived CD. However, combination treatment with OXT and mexidol considerably increased the number of correct responses

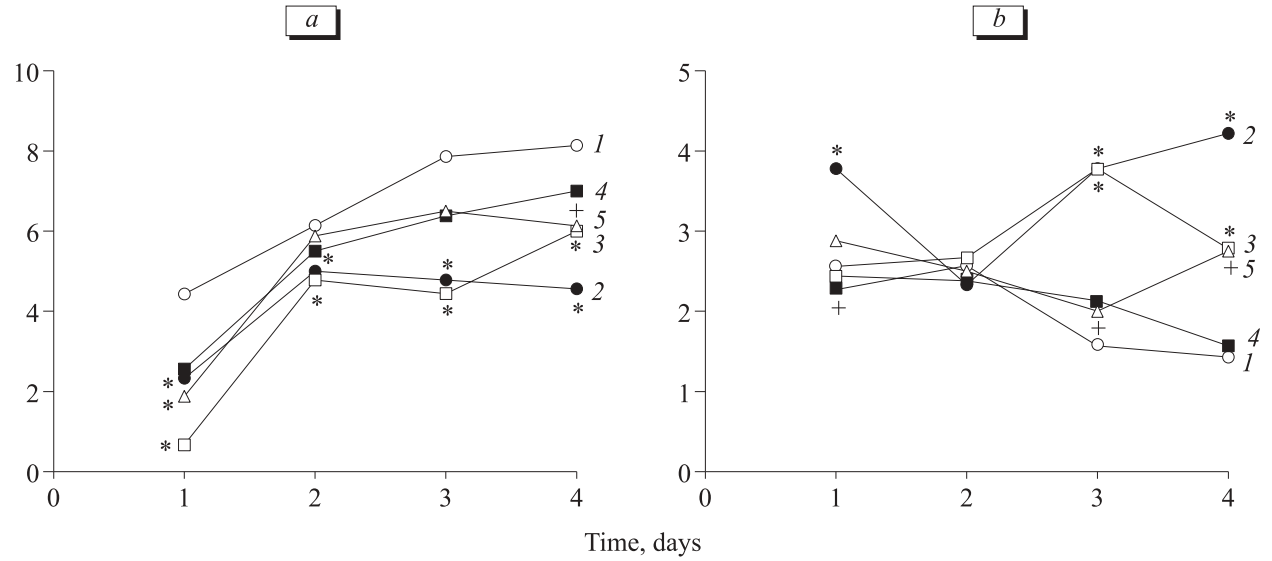

Fig. 3. Effect of individual and combination treatment with mexidol and delta sleep-inducing peptide (DSIP) on active avoidance conditioning in albino rats after 12-min cardiac arrest. Intact rats (n=32, *1*) and resuscitated rats receiving placebo (n=31, *2*), mexidol (n=29, *3*), DSIP (n=10, *4*), and mexidol+DSIP (n=9, *5*).

(day 1) and slightly reduced the number of short-latency reactions. DSIP improved learning in resuscitated rats, increased the number of correct reactions (day 4), and decreased the number of short-latency reactions (days 1 and 4). The observed changes were similar, but not statistically significant after combination treatment with mexidol and DSIP. The number of intersignal reactions progressively decreased in animals of both groups from day 1 to the end of observations. In these rats the number of intersignal reactions on day 4 was lower than on day 1 (by 65.8 and 84%, respectively, $p<0.004$). Therefore, combination treatment with test compounds stabilized behavioral activity of rats (Fig. 3).

Our findings indicate that combination treatment with mexidol and DSIP or OXT produces an antihypoxic effect in the early posthypoxic period, which manifested in early disappearance of neurological deficit. We observed potentiation of the effects produced by mexidol and regulatory peptides.

It should be emphasized that individual and combined administration of peptides and mexidol produced an antistress effect, which differed in animals of various groups. Mexidol and OXT potentiated the effects of each other, which increased their antistress activity. Combination treatment with mexidol and peptides normalized emotional and motivational status of resuscitated rats and affected conditioned active avoidance behavior.

Our results indicate that combination treatment with test compounds in the resuscitation period produces different early and delayed consequences (disappearance of the previously observed effects and appearance of new effects). These data should be taken into account in the development of new approaches to combination pharmacological therapy. Combined administration of the membrane-protecting agent and neuropeptides holds promise for preventing dysregulation pathology after CD. Combination treatment with mexidol and OXT most significantly restored integrative activity of the brain in the postresuscitation period.

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