GENERAL PATHOLOGY AND PATHOLOGICAL PHYSIOLOGY

Effect of MK-801 on Sensitivity of the Respiratory System to Oxygen Deficiency and Organism's Resistance to Hypoxia

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We studied changes in organism's sensitivity and resistance to hypoxic hypoxia under conditions of NMDA receptor blockade with MK-801. Breathing hypoxic gas mixture after administration of MK-801 sharply decreased the mean blood pressure in the systemic circulation and slightly improved lung ventilation in animals. Respiratory arrest was observed in 40% animals. NMDA receptor blockade decreased organism's sensitivity and resistance to hypoxic hypoxia.

Key Words: *hypoxia; MK-801; NMDA receptors; regulation of respiration; rat*

Ischemia and hypoxia are accompanied by glutamate accumulation in the extracellular medium in the brain, which is the initial stage of nerve cell destruction. *In vitro* experiments showed that glutamate neurotoxicity can be prevented by blockade of glutamate receptors [10,14]. NMDA receptors play a key role in the realization of glutamate neurotoxicity [6,13]. These receptors mediate intracellular Ca^{2+} influx and calcium overload in nerve cells [6]. A large body of evidence indicates that glutamate receptors (*e.g.*, NMDA receptors) are involved in the central regulation of respiration and transmission of afferent impulses from carotid chemoreceptors sensitive to oxygen deficiency to respiratory neurons of the brain stem [4,5,7,9]. Blockade of glutamate receptors can cause dysfunction of the respiratory system, which is particularly sensitive to cerebral ischemia and hypoxia.

Here we studied changes in sensitivity of the respiratory system and organism's resistance to hypoxic hypoxia under conditions of NMDA receptor blockade.

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MATERIALS AND METHODS

Experiments were performed on 11 male outbred albino rats weighing 350-500 g. The animals were intraperitoneally narcotized with sodium pentobarbital in a dose of 40-50 mg/kg (Spofa); then anesthesia was maintained by intravenous injection of pentobarbital in low doses. (+)-MK-801 (Dizocilpine, Sigma) in a dose of 3 mg/kg was injected intravenously to block NMDA receptors [12]. Body temperature was maintained at 37.0-38.5°C using an infrared lamp. Tracheotomy was performed in the upper third of the trachea and a plastic tube of the corresponding diameter was inserted. This tracheal cannula was connected to a transducer for monitoring of external respiration. A valve for delivering air and gas mixture (10% O_2 in nitrogen, 5 min) was connected to the distal end of a transducer. Minute ventilation (MV), respiratory rate, and pneumotachogram were recorded on an MKh-01 polygraph equipped with a respiration monitoring unit (BTPS conditions). Systemic blood pressure (BP) and heart rate (HR) were determined using a catheter introduced into the femoral artery and connected to a tensiometric transducer. Intraesophageal pressure was recorded with a catheter with an elastic balloon filled with water introduced into the esophagus and con-

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nected to an MKh-01 transducer. The pressure in the system was so adjusted that during passive inspiration it was 0 mm Hg. Experimental data were recorded on a H3031-6 ink automatic recorder. The results were analyzed statistically and expressed as arithmetic means and standard errors. The significance of differences was estimated by Student's t test ($p<0.05$).

RESULTS

The respiratory system exhibited variable response to hypoxia. This reaction is usually biphasic [1,2]. The initial phase of increased ventilation is mediated by the glutamatergic mechanism [15], while subsequent inhibition of respiration is related to the development of secondary hypocapnia and accumulation of inhibitory neurotransmitters in the brain (*e.g.* GABA and adenosine) [8,11,15]. Phase I (increased ventilation) developed after delivery of the hypoxic mixture and lasting until the secondary mechanisms inhibiting respiration were triggered was considered as a criterion of the sensitivity of the respiratory system to oxygen deficiency. Phase II (stabilization of respiration parameters after 4 min) developing against the background of low BP reflected organism's resistance to hypoxia. This biphasic reaction of the respiratory system to hypoxia was observed before and after administration of MK-801 (Fig. 1).

Under control conditions lung ventilation rapidly increased and reached maximum after breathing hypoxic mixture for 1 min (Fig. 1). This parameter increased by 1.5-2 times (Table 2). MV decreased on the 4th minute of breathing hypoxic mixture (compared to the maximum value observed by the end of the 1st

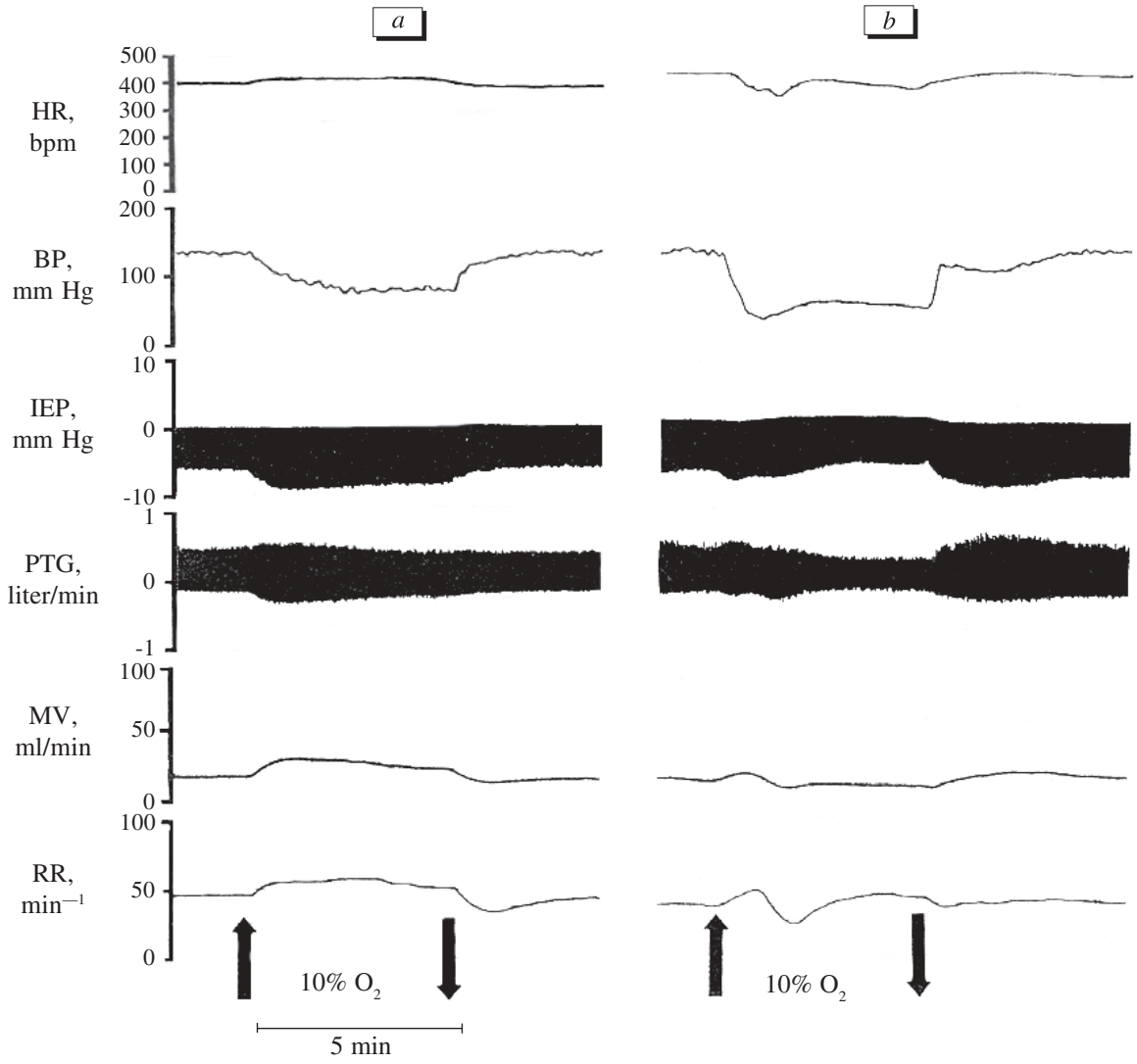

Fig. 1. External respiration and hemodynamics in rats breathing 10% hypoxic gas mixture before (*a*) and after administration of MK-801 (*b*). BP, mean systemic blood pressure; IEP, intraesophageal pressure; PTG, pneumotachogram; MV, minute ventilation; RR, respiratory rate. Bottom: time point. Arrows: start and termination of breathing a gas mixture.

TABLE 1. Parameters of Respiration and Mean Systemic BP during Inhalation of 10% Hypoxic Gas Mixture before and after NMDA Receptor Blockade with MK-801 (*M*±*m*)

Parameter	Control			MK-801		
	baseline level	1st minute	4th minute	baseline level	1st minute	4th minute
MV, ml/min	23.6±1.5	41.3±3.6**	29.3±2.7*	21.0±2.7	30.4±4.5	21.0±3.2
Respiratory rate, min^{-1}	57.8±3.9	59.2±3.5	54.9±2.9	49.3±4.6	59.7±4.6	54.0±3.0
RV, ml	0.41±0.03	0.70±0.05**	0.54±0.05*	0.42±0.04	0.49±0.05+	0.39±0.06
$\overline{BP}$, mm Hg	117±4	77±6**	55±7**	97±9	48±6**+	33±3**+

Note. *p<0.05 and **p<0.001 compared to baseline level; +p<0.01 compared to the control.

minute) and did not differ from the baseline. MV significantly increased during phase I and tended to increase during phase II of the respiratory reaction to hypoxic mixture under control conditions. These changes were related to an increase in the depth of breathing. The respiratory rate remained practically unchanged.

In animals breathing hypoxic mixture for 4 min and not receiving MK-801, MV and respiratory volume (RV) were below the level observed by the 1st minute of treatment (Table 1). However, MV slightly exceeded the baseline level due to 32% increase in RV (Table 1).

The cardiovascular system exhibited a single-phase reaction to transition from normoxia to hypoxia (Fig. 1). Mean BP decreased by 1.5 and 2 times in phases I and II of the respiratory reaction, respectively (Table 1). We did not perform special analysis of changes in HR. Inhalation of hypoxic mixture was usually followed by a slight decrease in HR under control conditions.

The test parameters returned to normal after termination of breathing hypoxic mixture.

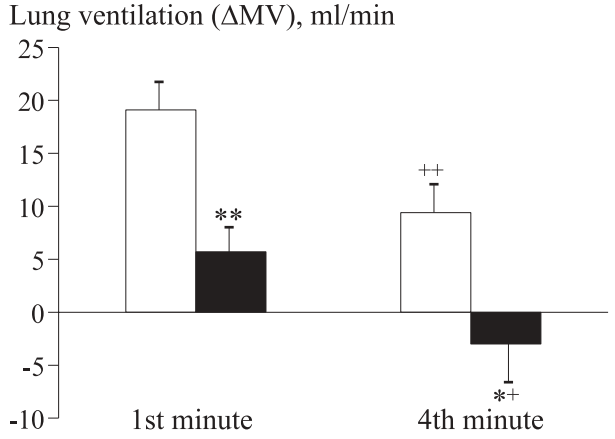

Fig. 2. Minute ventilation in rats on the 1st and 4th minutes of breathing hypoxic mixture before and after administration of MK-801 (compared to the baseline level). Light bars: control. Dark bars: MK-801 administration. *p<0.02 and **p<0.01 compared to the baseline level; +p<0.002 and ++p<0.001 compared to the control.

Blockade of NMDA receptors was produced by intravenous injection of MK-801. Breathing and circulation were monitored over 30 min after MK-801 administration. NMDA receptor blockade produced little effect on external respiration and circulation. Lung ventilation tended to decrease (by 11%) 30 min after treatment, which was related to deceleration of respiratory movements (by 15%). The mean systemic BP decreased by 17% (p>0.05, Table 1).

The biphasic respiratory response to hypoxia persisted after injection of MK-801 (Fig. 1). Under conditions of breathing hypoxic mixture MV tended to increase by 1.5 times in phase I, but decreased to the baseline level in phase II (Table 1).

We evaluated differences (Δ) between phases I and II of the respiratory reaction to hypoxia before and after MK-801 injection. After NMDA receptor blockade the increase in MV during phase I of the respiratory reaction to hypoxia was related not only to deepening of breathing, but also to an increase in the respiratory rate.

The absolute respiratory rate tended to increase in animals receiving MK-801 and exposed to hypoxic hypoxia. The study of hypoxic changes in the respiratory rate (Δ) before and after NMDA receptor blockade revealed a more significant effect of an increase in the rate of respiratory movements. After administration of MK-801 the respiratory rate increased by 20% in phase I, but decreased to the baseline level in phase II of hypoxia (Table 1). The increase in the respiratory rate in rats is consistent with the results of previous experiments on dogs exposed to hypoxia after NMDA receptor blockade [3].

In animals receiving MK-801 hypoxia produced less significant changes in RV: only by 16% in animals treated with the preparation during phase I of the respiratory reaction (less significantly than in control rats, Table 1).

Cessation of breathing and sharp decrease in BP during hypoxia were revealed in 4 rats. These animals were excluded from further observations.

In order to diminish the influence of baseline lung ventilation and BP parameters we estimated the difference between parameters of external respiration in phases I and II of the respiratory reaction to hypoxia and the corresponding baseline levels recorded before and after administration of the preparation. Pairwise differences between groups were calculated.

Phase I of the respiratory reaction to hypoxic hypoxia characterizes sensitivity of the respiratory system to oxygen deficiency. Sensitivity of the respiratory system to hypoxia significantly decreased after MK-801 administration and NMDA receptor blockade. In phase I of hypoxia MV increased more significantly than after treatment with MK-801 (by 2 times, Fig. 2). In phase I of the respiratory reaction to hypoxia ΔRV in control rats increased more significantly than in animals receiving MK-801 (by 4.5 times). As differentiated from ΔRV, the respiratory rate increased by 7 times after blockade of NMDA receptors. These changes reflect internal reconstruction of the central regulation of respiration and pronounced decrease in the sensitivity of the respiratory system to hypoxic hypoxia.

The resistance to hypoxia decreased. After NMDA receptor blockade parameters of external respiration (MV and RV) did not increase and even decreased compared to the control and phase I of the respiratory reaction. Variations in the rate of respiratory movements had similar effect. These changes, as well as more significant decrease in BP and cessation of breathing in 40% animals, indicate that organism's resistance to hypoxic hypoxia decreases under conditions of NMDA receptor blockade. Our results suggest that MK-801 and other antagonists of NMDA receptors should not be used as neuroprotectors for patients with ischemic injury of the brain.

We conclude that the blockade of NMDA receptors decreases sensitivity of the respiratory system and organism's resistance to hypoxia.

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