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Effect of Picrotoxin on Organism's Resistance to Acute Severe Hypoxia

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Organism's resistance to acute severe hypoxia (3% O_2) was studied after administration of $GABA_A$ receptor antagonist picrotoxin and adenosine receptor antagonist euphylline (aminophylline) and after neutralization of secondary hypocapnia by adding 7% CO_2 to the hypoxic mixture. Administration of picrotoxin to anesthetized rats increased animal resistance to hypoxia. The resistance to hypoxia decreased after treatment with euphylline. Neutralization of secondary hypocapnia by adding 7% CO_2 to the hypoxic mixture had no effect on animal lifespan.

Key Words: *picrotoxin; euphylline; resistance to severe acute hypoxia; hypocapnia; ultrasound*

The respiratory response to acute hypoxia consists of 2 phases: initial hyperventilation is followed after several minutes by a secondary decline of pulmonary ventilation below the initial rise, but above the basal prehypoxic level ("roll-off" phenomenon). The mechanisms of this biphasic response are poorly understood. The respiratory response to hypoxia is mediated by various neurotransmitter systems, including the glutamatergic, GABAergic, serotoninergic, and purinergic systems [6]. The initial stage of hyperventilation is associated with the involvement of NMDA glutamate receptors in the chemoreceptor regulation of respiration [11]. Published data show that $GABA_A$ receptor antagonist bicuculline abolishes phase 2 of the respiratory response (*i.e.*, decrease in pulmonary ventilation during hypoxia) [8,12]. Suppression of breathing during hypoxia is related to the development of secondary hypocapnia [7] and formation of physiologically active substances in the brain. One of these substances, adenosine, serves as an inhibitory neurotransmitter and suppresses respiration [10]. The resistance to hypoxia is evaluated under conditions of severe hypoxia (normobaric hypoxia with 3% O_2; and hypobaric hypoxia, "ascent" to an altitude of 11,000-12,000 m in a pressure chamber). Phase 2 of severe hypoxia is followed by a reversible respiratory arrest (apnea). The latency of apnea is considered as the lifespan [2,4,5]. The mechanisms of apnea during severe hypoxia remain unknown [6].

Here we studied the resistance of the respiratory and cardiovascular system to severe acute hypoxia after $GABA_A$ receptor blockade with picrotoxin. We also evaluated the effect of treatment with adenosine receptor antagonist euphylline and neutralization of secondary hypocapnia on the lifespan of rats during acute hypoxia.

MATERIALS AND METHODS

Acute experiments were performed on 36 male outbred albino rats weighing 400-450 g. The animals were intraperitoneally anesthetized with nembutal in a dose of 40-50 mg/kg. Blood pressure

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(BP) in the femoral artery was recorded using a micromanometer. Blood flow rate in the ascending aortic arch was measured with a miniature ultrasound transducer fixed at the end of a catheter (diameter 0.6 mm). The operating frequency was 2.7 MHz [3]. The transducer was introduced into the aorta through the right carotid artery. The data on blood flow rate in the ascending aorta and BP were processed on an analog-to-digital converter to estimate total peripheral vascular resistance and variations in stroke volume and minute volume of the heart (cardiac output). The heart rate was recorded with a cardiotachometer triggered by aortic pulse wave. Respiratory movements of the thorax were recorded with a tensiometric sensor.

Individual resistance to hypoxia was estimated during breathing gas mixture containing 3% O_2 in N_2. The latency between the start of breathing and development of apnea (lifespan) was evaluated. An apparatus for artificial ventilation was used when respiration did not spontaneously recover within 1.0-1.5 min after termination of gas mixture supply. Artificial lung ventilation was stopped with the appearance of spontaneous breathing. The animals with apnea latency of 1-4, not less than 9, and 5-8 min were classified as low (LR), high (HR), and medium resistant (MR) to hypoxia, respectively [2,4,5]. The groups of HR, LR, and MR rats included 13 (45%), 9 (31%), and 7 (24%) of 29 rats, respectively.

The respiratory and cardiovascular responses and latency of apnea during acute hypoxia were compared before and after systemic treatment with $GABA_A$ receptor antagonist picrotoxin (subconvulsive dose 1 mg/kg, 11 animals) and adenosine receptor antagonist euphylline (20 mg/kg aminophylline, 9 animals). The respiratory response in 7 animals was evaluated after central microinjection of 1 μl picrotoxin solution (4 mg/ml). Picrotoxin was administered through the occipital membrane into the fourth ventricle using a Hamilton microsyringe. To evaluate the role of secondary hypocapnia in apnea, the response of the test systems was compared after inhalation of gas mixture with 3% O_2 in N_2 and gas mixture with 3% O_2 and 7% CO_2 (9 animals).

RESULTS

Under control conditions, systemic BP decreased during breathing hypoxic mixture. The mean basal BP in HR animals was 110-120 mm Hg. BP in HR and LR rats decreased to 50 and 30 mm Hg (or more), respectively. Total peripheral vascular resistance in HR and LR rats decreased by 40-50 and 70%, respectively, compared to the basal level. Heart rate in HR and LR animals decreased by 10-20 and by more than 40-50 bpm, respectively. The decrease in heart rate in HR and LR rats was observed 50-60 and 10-20 sec after the start of hypoxic exposure, respectively. Cardiac output in HR animals increased by 20-30% or remained unchanged during severe hypoxia. Cardiac output in LR rats remained unchanged or slightly decreased under these conditions.

The classic biphasic respiratory response was clearly seen during severe hypoxia. Phase 1 (increase in the rate and amplitude of respiratory movements) in HR rats lasted for 1-2 min (Fig. 1, *a*). Phase 2 of the hypoxic response included a de-

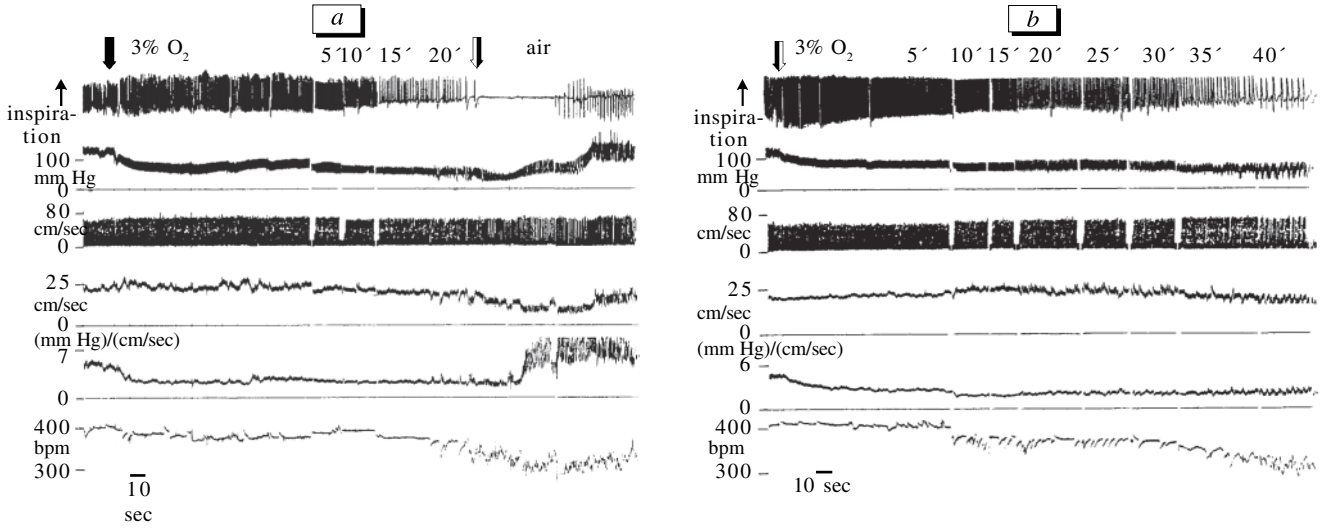

Fig. 1. Hemodynamic and respiratory parameters in a HR animal during inhalation of a gas mixture with 3% O_2 in N_2 before (*a*) and after picrotoxin administration (*b*). Here and in Fig. 2: respiratory movement (*1*), blood pressure (*2*), blood flow velocity in the ascending aortic arch (*3*), cardiac output (*4*), total peripheral vascular resistance (*5*), and heart rate (*6*). Arrows: start and cessation of breathing a gas mixture. Time scale 10 sec.

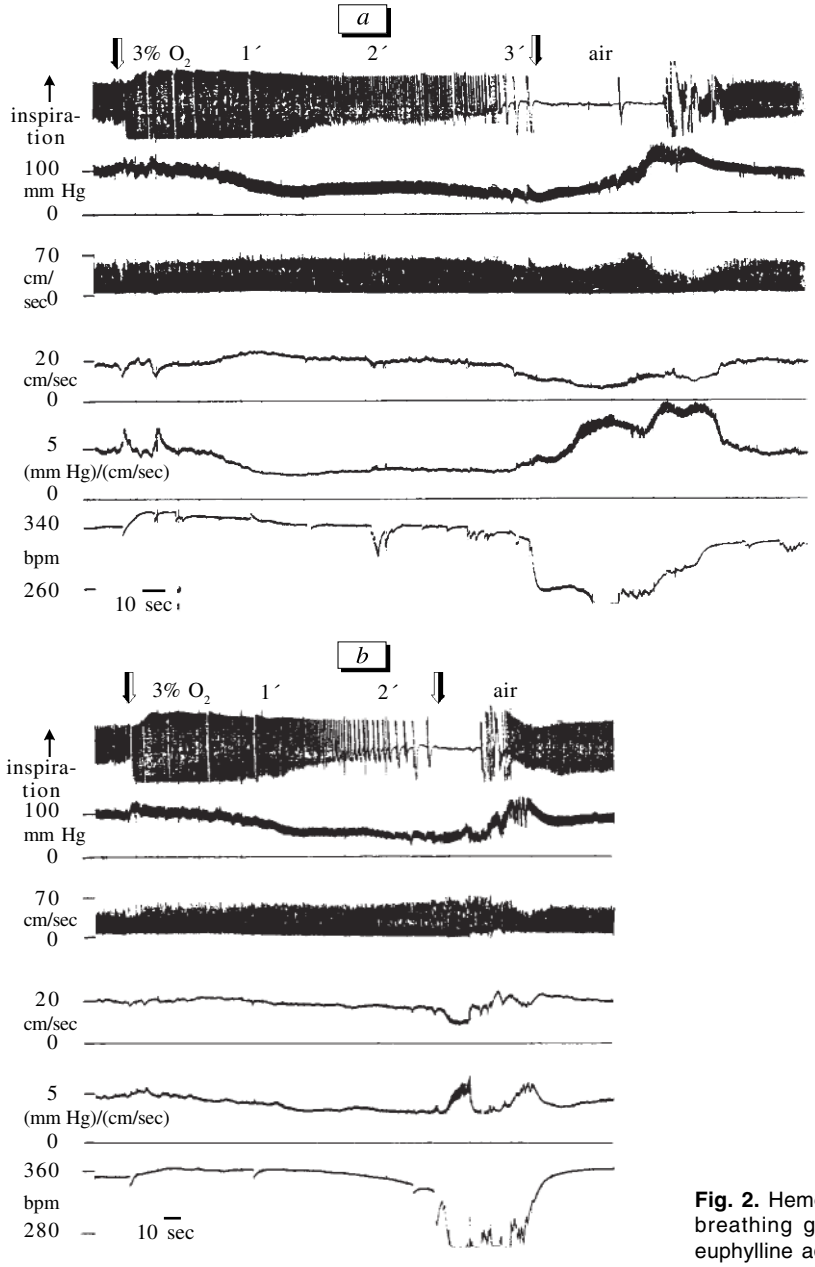

Fig. 2. Hemodynamic and respiratory parameters in LR rat during breathing gas mixture with 3% O_2 in N_2 before (*a*) and after euphylline administration (*b*).

crease in the amplitude of respiratory movements, which was not accompanied by variations in the respiratory rate (RR). Slow and high-amplitude respiratory movements were revealed after 5-6 min. This breathing pattern was observed for a long time (9-25 min) until the development of apnea.

Phase 1 in LR rats lasted for 30-50 sec. The shortened second phase (several slow inspirations) was followed by respiratory arrest. Variations in hemodynamic and breathing parameters in MR animals were similar to those in HR rats. However, the latency of apnea in MR rats was shorter (5-8 min).

Intravenous injection of picrotoxin in the subconvulsive dose increased the rate and amplitude of respiratory movements in some animals. These changes were not accompanied by variations in BP, cardiac output, and total peripheral vascular resistance. Other rats did not exhibit variations in respiratory and hemodynamic parameters. Picrotoxin increased BP and normalized RR in the animals, which were characterized by low basal level of BP and suppression of breathing.

Central microinjection of 1 μl picrotoxin solution (4 mg/ml) into the fourth ventricle impaired the respiratory rhythm, which manifested in cyclic variations in RR, irregularity of respiratory movements, and periodic breathing (in some animals).

The respiratory and cardiovascular responses to hypoxia in picrotoxin-treated rats differed from the control. BP in HR animals decreased to 60-70

mm Hg over the first 5-7 min. BP decreased to 60 mm Hg in the follow-up period and remained unchanged for at least 30 min. Heart rate increased by 10-20 bpm over the first 8-10 min, decreased to the basal level after 8-10 min, and remained unchanged during 30-min breathing the hypoxic mixture. Cardiac output increased by 30-40% and remained unchanged until apnea.

Hypoxia-induced changes in the respiratory parameters after injection of picrotoxin were more pronounced compared to the control. The amplitude and rate of respiratory movements increased more significantly. The respiratory response was biphasic, but the amplitude and rate of breathing decreased later. A 2-fold decrease in RR was observed after 25-30 min. This breathing pattern persisted for a long time until the development of apnea (40 min or more; Fig. 1, *b*).

The response of picrotoxin-treated LR and MR rats to hypoxia was accompanied by less significant changes in hemodynamic parameters. BP decreased to a lesser extent. Heart rate remained unchanged until apnea. No changes were found in cardiac output. The respiratory response was more significant after picrotoxin injection.

The lifespan of HR and LR rats in severe hypoxia increased by 2-2.5 times after intravenous injection of picrotoxin in the subconvulsive dose. These data suggest that GABA accumulation in brain tissue plays an important role in the development of apnea during severe hypoxia. Our findings are consistent with the results of studies performed by Japanese investigators. They showed that extracellular GABA concentration in the solitary nucleus increases during phase 2 of the hypoxic response. Moreover, microinjection of bicuculline into this structure reduced suppression of breathing (increase in the respiratory volume) [12]. Bicuculline in subconvulsive doses prevented the decrease in activity of the diaphragmatic nerve during hypoxia [8]. Systemic administration of picrotoxin in subconvulsive doses maintained the biphasic response and increased activity of the sublingual nerve during hypoxia [6]. Picrotoxin increased organism's resistance during posthemorrhagic hypoxia [1].

Intravenous injection of euphylline was followed by a decrease in BP (by 40-50 mm Hg) and total peripheral vascular resistance, but had no effect on other hemodynamic parameters. Heart rate and amplitude of respiratory movements remained unchanged under these conditions. BP retuned to the basal level after 10-15 min. euphylline did not modulate the response to hypoxia. However, the lifespan of animals decreased by 1.5-2 times during severe hypoxia.

We hypothesized that adenosine receptor blockade with euphylline is followed by an increase in the lifespan of animals, since this treatment can abolish the inhibitory effect of adenosine on breathing [10]. However, this effect was not revealed. Adenosine causes dilation of cerebral vessels during hypoxia and, therefore, serves as a regulator of blood circulation in the brain. Published data show that theophylline prevents dilation of cerebral vessels during hypoxia [9]. Euphylline-induced decrease in blood supply to the brain under hypoxic conditions probably prevails over the improvement of breathing due to adenosine receptor blockade. These changes contribute to a decrease in the lifespan of animals during severe hypoxia (Fig. 2, *a*, *b*).

Previous studies showed that secondary hypocapnia due to hyperventilation plays a role in the suppression of breathing during prolonged hypoxia [7]. Comparison of the latency of apnea during "pure" hypoxia (3% O_2) and hypoxia with 7% CO_2 (neutralization of secondary hypocapnia) showed that the lifespan of some animals does not increase, but even decreases. Hence, hypercapnia does not play an important role in the development of apnea (at least, during severe hypoxia).

We conclude that $GABA_A$ receptor blockade with picrotoxin in the subconvulsive dose increases organism's resistance to severe acute hypoxia. The resistance to severe acute hypoxia decreases after adenosine receptor blockade with euphylline, which is probably related to inhibition of cerebral vasodilation under hypoxic conditions. Secondary hypercapnia does not play a role in the development of apnea during severe hypoxia.

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