Effects of Preconditioning on the Resistance to Acute Hypobaric Hypoxia and Their Correction with Selective Antagonists of Nicotinic Receptors

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Hypobaric hypoxic preconditioning increased the resistance of low resistant and highly resistant rats to acute hypobaric hypoxia at a critical height. Intergroup differences in the resistance of rats to acute hypobaric hypoxia were not observed after hypobaric hypoxia and one variational series with a wide range of resistance (4.5-24.5 min) appeared. Methyllycaconitine, an antagonist of subtype α_7 nicotinic cholinergic receptors, abolished the influence of hypobaric hypoxia on low resistant rats, but had no effect on highly resistant animals. Mecamylamine, a preferential antagonist of subtype $\alpha_4\beta_2$ and α_3-containing cholinergic receptors, did not modulate the effect of hypobaric hypoxia. By contrast, hypobaric hypoxia abolished the effect of mecamylamine on the resistance of rats that were not trained under conditions of hypobaric hypoxia (low resistant and highly resistant animals with low sensitivity to hypobaric hypoxia). We conclude that the same effect of hypobaric hypoxia is mediated by various mechanisms, which involve different nicotinic cholinergic receptors. They differ from the resistance mechanisms in non-trained rats.

Key Words: *acute hypoxia; preconditioning; nicotinic cholinergic receptors of the α_7 subtype and non-α_7 subtype; methyllycaconitine; mecamylamine*

Published data show that nicotinic cholinergic receptors (NCR) play an important role in the systemic response to hypoxia. NCR are expressed in all parts of the system for autonomic regulation of respiration and blood supply (from peripheral chemoreceptors and mechanoreceptors to sensory neurons and motoneurons in CNS) [3,5,6,10,11,15]. Homomeric α_7 receptors and heteromeric $\alpha_4\beta_2$ receptors are the most widely distributed subtypes of NCR in the brain. For example, they are expressed in neurons of the medulla oblongata that play a role in the regulation of breathing and cardiovascular function [3,4]. Subtype $\alpha_3\beta_4$ receptors are also identified in the medulla oblongata. These receptors are functionally active in relation to the vascular system [4,8]. A series of studies were devoted to evaluating the receptor specificity of cholinergic regulation in various organs. Vasodilation of major cerebral vessels and abdominal vessels is specifically realized via α_7 NCR [15] and $\alpha_3\beta_4$ receptors [3], respectively. The airway tone is regulated by α_7 and $\alpha_4\beta_2$ receptors, but not by α_3-containing subtypes of NCR [3]. The sinocarotid nerve receives information from the carotid arteries via α_3-containing NCR and, to a lesser extent, via α_7 receptors [9]. Impulses from the lungs and bronchi are transferred to the nucleus of the solitary tract in the medulla oblongata through α_3-containing NCR [6]. NCR belong to the ionotropic type of receptors. It can be suggested that these receptors play a role in the immediate mechanisms of adaptation to hypoxia. Acetylcholine simulates the effect of ischemic preconditioning in various organs. Recent studies showed that this effect of acetylcholine is realized via NCR [7,13,14].

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Here we studied the role of NCR in preconditioning after a single session of hypobaric hypoxia (HBH) in rats with low or high resistance to hypoxia.

MATERIALS AND METHODS

Experiments were performed on male outbred albino rats. The animals were tested in an altitude chamber. The lifespan to the second agonal inspiration (apnea) was evaluated at a critical height of 11,500 m (4% O_2, acute HBH). The animals were divided into groups of low resistant and highly resistant specimens with a lifespan <3 min and >7 min, respectively [1]. The rats were repeatedly subjected to 60-min HBH (5000 m, 11% O_2) after 3-4 weeks. They were tested at a critical height after 2-4 min. Control rats were not subjected to HBH before the second session of acute HBH.

The animals received a single intraperitoneal injection of selective NCR antagonists, methyllycaconitine (MLA, antagonist of α_7-containing NCR; Tocris) and mecamylamine (MEC, preferential antagonist of $\alpha_4\beta_2$ and α_3-containing NCR; Sigma). MLA (1.4 nmol/kg) or MEC (3.9 nmol/kg) was injected immediately or 30 min before HBH, respectively. The doses were selected in previous experiments [17]. The time of treatment was evaluated from the pharmacokinetics of these substances. Physiological saline was injected to animals of the reference group. All experimental groups of low resistant and highly resistant rats (control; HBH; HBH after treatment with MLA (MLA+HBH); and HBH after treatment with MEC (MEC+HBH) were homogeneous by the results of the first test.

RESULTS

Repeated testing of control rats under conditions of acute HBH had various effects on the resistance of low resistant and highly resistant specimens (Fig. 1). The lifespan remained practically unchanged in low resistant animals, but tended to decrease in highly resistant specimens. HBH was followed by a significant increase in the resistance of low resistant rats and had a normalizing effect on highly resistant specimens (Fig. 1). Therefore, hypoxic preconditioning increases the resistance of animals. This effect was particularly pronounced in low resistant rats, which is consistent with published data [1].

Intergroup differences in the resistance of low resistant and highly resistant rats to acute HBH were not observed after HBH. The lifespan of animals was arranged in one variational series with a wide range of resistance to HBH (4.5-24.5 min; Fig. 2, *1*, *3*). Hence, the resistance to HBH under conditions of preconditioning is mediated by the mechanisms that differ from

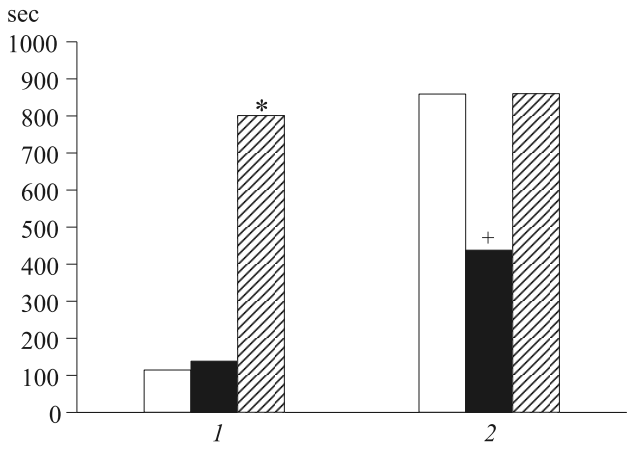

Fig. 1. Median lifespan (LS) of low resistant and highly resistant rats during acute HBH after various experimental exposures. Low resistant animals (*1*); highly resistant animals (*2*). Light bars, LS_1 after primary testing (random division of rats to low resistant and highly resistant specimens, n=11/10); dark bars, LS_2 after repeated testing in control groups (n=6/4); shaded bars, LS_2 in groups of HBH (n=5/6). *p<0.025: between LS_1 and LS_2 (nonparametric exact Fisher test). +Tendency.

the mechanisms for resistance of non-trained animals at a critical height. No correlation was found between the resistance of low resistant and highly resistant rats to acute HBH before and after HBH (ρ=0.297 and 0.330 respectively; n=5 and 6, respectively; p>0.05, Pierson test).

MLA injection to low resistant rats was followed by a 2-fold decrease the effect of HBH. MLA had a modulatory effect on the upper limit, but not on the low limit of resistance (Fig. 2, *3*, *4*). MEC did not modify the influence of preconditioning (Fig. 2, *3*, *5*). Our previous studies showed that both antagonists in the specified doses increase the resistance of low resistant rats to acute HBH [15]. Comparative study showed that the effect of MLA+HBH on the resistance of rats to acute HBH is similar to that of MLA. The action of MEC+HBH was similar to the effect of preconditioning (Fig. 3, *1*). These results indicate that MLA abolishes the influence of preconditioning. However, MLA has a direct effect on the resistance of non-trained animals. MEC did not modify the influence of preconditioning. We did not observe the additive effect of two factors under these conditions. Hence, preconditioning abolishes the effect of MEC on the resistance of non-trained rats.

Our results indicate that in low resistant rats, the mechanisms for immediate adaptation differ from the inherited or acquired mechanism (due to the primary exposure to acute HBH) for acute hypoxia tolerance. NCR of the α_7 subtype are an essential component in the mechanism of adaptation in low resistant rats. An *in vitro* (neocortex) and *in vivo* study (myocardium) showed that activation of α_7 NCR is analogues

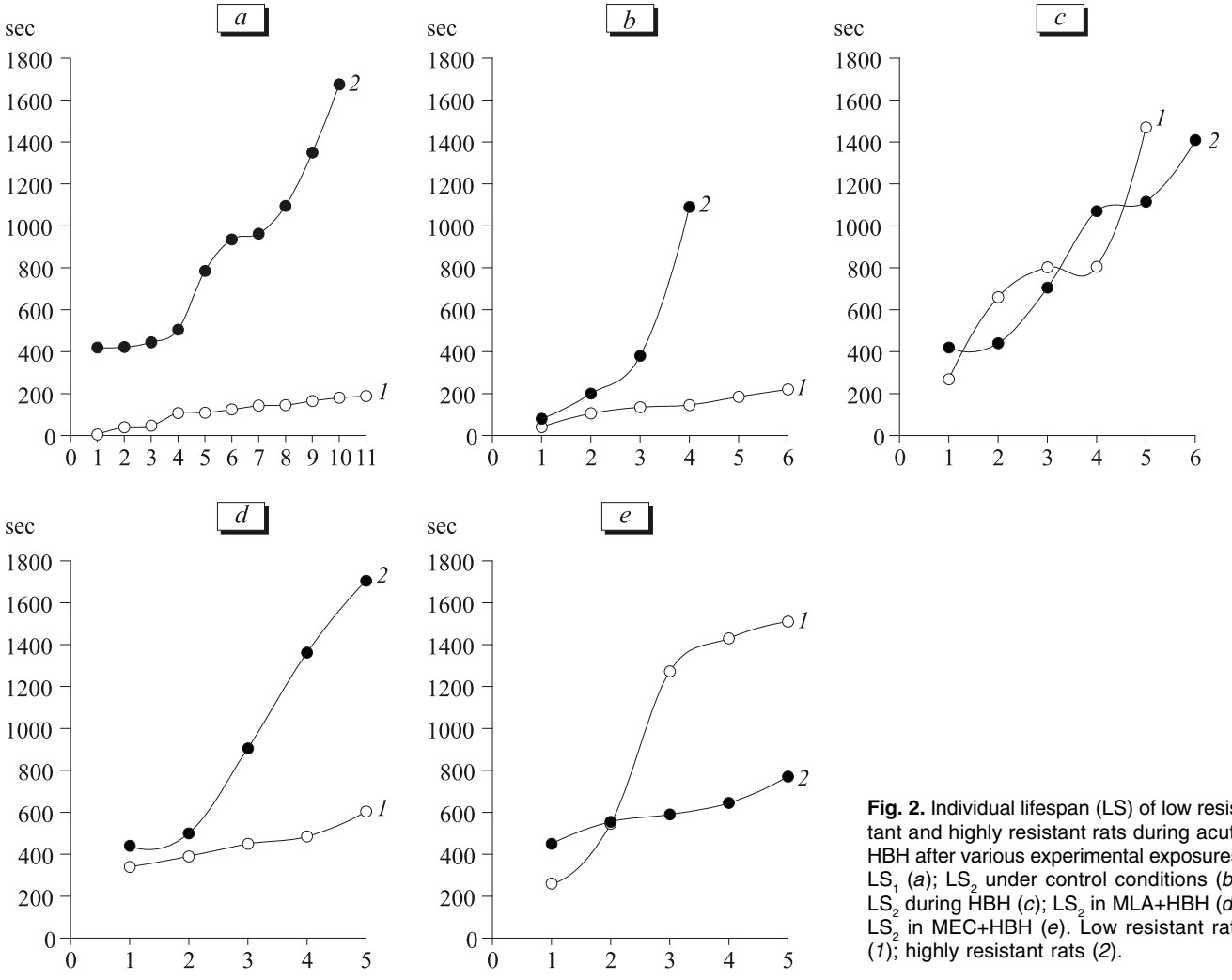

Fig. 2. Individual lifespan (LS) of low resistant and highly resistant rats during acute HBH after various experimental exposures. LS_1 (*a*); LS_2 under control conditions (*b*); LS_2 during HBH (*c*); LS_2 in MLA+HBH (*d*); LS_2 in MEC+HBH (*e*). Low resistant rats (*1*); highly resistant rats (*2*).

to ischemic preconditioning under various conditions [13,14]. In low resistant rats, non-α_7 NCR are not involved in the mechanisms of adaptation. Moreover, they become ineffective during this process. Previous studies revealed the existence of alternative mechanisms of hypoxia tolerance. Chronic hypoxia blocks the cholinergic pathway for sensitivity of the sinocarotid nerve to acute hypoxia or hypercapnia, which is realized via α_3-containing NCR. It should be emphasized that this pathway is ineffective before adaptation [9]. Other authors described the relationship between hypoxia/hypercapnia, NCR, and cardiac vagal neurons [10]. A key factor of variations in cholinoreception under conditions of hypoxia is increased dependence of NCR on oxygen (due to coupling of receptor function with activity of purinergic receptors) [9,10]. Coupling with these receptors is specific for NCR and observed in various parts of the nervous system (from the oxygen-sensitive peripheral component [9] and medulla oblongata [10] to the overlying structures of the brain, including the neocortex [12]). This is typical of α_3-containing MEC-sensitive subtype of NCR [9] and α_7 subtype of receptors [10,12]. Changes in the efficiency of NCR contribute to ATP-independent processes of signal transduction in various functional stages [9,10].

MLA did not modulate the preconditioning effect in highly resistant rats. MEC tended to narrow the range of resistance (due to the upper limit; Fig. 2, *4*, *5*). MLA produced no direct effect on the resistance of rats to acute HBH [15]. In animals of this group, the α_7 subtype of NCR is not involved in the preconditioning and inherited (or acquired) mechanism of hypoxia tolerance. MEC similarly decreases the resistance of rats that were trained or non-trained to HBH (Fig. 3, *2*) [15]. The decrease in the resistance of these animals was insignificant. The modulatory effect of MEC on the resistance of non-trained rats probably did not change under conditions of HBH. If this assumption is true, MEC-sensitive NCR do not play a role in the mechanisms of immediate adaptation. However, previous experiments with the hippocampus showed that

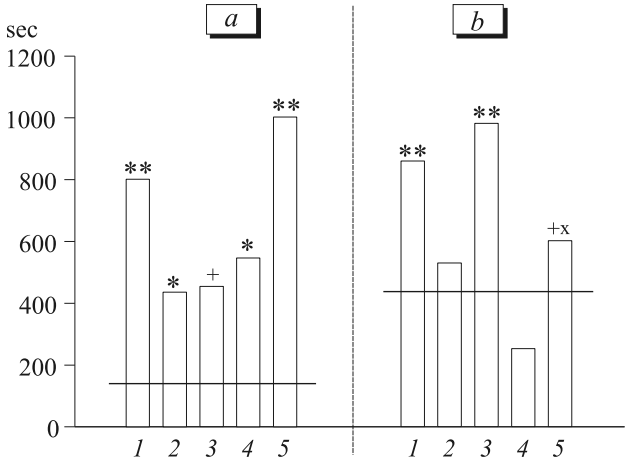

Fig. 3. Direct and indirect (mediated by HBH) effects of antagonists for NCR of the α_7 (MLA) and non-α_7 subtype (MEC) on the lifespan (LS) of low resistant and highly resistant rats during acute HBH after repeated testing. Low resistant rats (*a*); highly resistant rats (*b*). HBH (*n*=5/6 for *a* and *b*, respectively; *1*); MLA (*n*=6/8, *2*); MLA+HBH (*n*=5/5; *3*); MEC (*n*=18/6; *4*); MEC+HBH (*n*=5/5; *5*). Horizontal line, LS_2 in control groups. Significant differences, nonparametric EFT (exact Fisher test). **p*<0.05 and ***p*<0.025 compared to LS_2 in the control group; ^{+}p<0.05 compared to LS_2 in the HBH group (for *3* and *5*). ^{x}Tendency.

MEC abolishes the effect of ischemic preconditioning that is induced by anticholinesterase agents [8]. Low efficiency of MEC can result from the opposite effects, which are realized via various NCR of the non-α_7 subtype.

MEC had no effect on the lower limit of the adaptive effect of HBH in highly resistant rats (Fig. 2, *5*). Hence, the effect of MEC is manifested only in specimens with high sensitivity to HBH. By contrast, MEC is ineffective in rats with low sensitivity to HBH. NCR of the non-α_7 subtype probably become ineffective during adaptation of these rats (similarly to low resistant animals). Therefore, highly resistant rats are heterogeneous by the mechanisms of preconditioning.

These data illustrate the existence of 3 mechanisms of hypoxic preconditioning. One of these mechanisms involves NCR of the α_7 subtype. MEC-sensitive non-α_7 NCR (low resistant rats) lose activity during this process. NCR do not play a role in the other two mechanisms (subgroup of highly resistant rats with high sensitivity to HBH). Moreover, MEC-sensitive NCR of the non-α_7 subtype become ineffective under these conditions (subgroup of highly resistant rats with low sensitivity to HBH). The mechanisms for adaptation and resistance to acute HBH differ and can be alternative in non-trained rats. Low resistant and highly resistant rats probably differ in the composition of NCR, which determines the pathway of adaptation. It remains unclear whether the differences in the composition of receptors in low resistant and highly resistant rats are inherited or acquired (after primary testing). The observed differences can also result from combined effect of these factors.

The mechanisms of resistance to hypoxia of different severity can be attributed to various structures of the brain. Adaptation to hypoxia of medium severity (*e.g.*, HBH) results from autonomic regulation of the cardiovascular and respiratory systems, whose nerve centers are located in the medulla oblongata [10]. The brain structures that are most sensitive to oxygen deficiency play a key role under fatal conditions (acute HBH). However, the resistance of low resistant rats to acute HBH can be reduced at the level of pulmonary afferents. Electrical stimulation or activation of sensory C-fibers via NCR can cause apnea (test parameter in our experiments) during the first tens of seconds of severe hypoxia. It is also typical of decerebrate animals [2].

Our results illustrate the existence of various pathways for an immediate increase in the *in vivo* tolerance of low resistant and highly resistant rats. These mechanisms involve specific NCR of the α_7 and non-α_7 subtypes.

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