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FORMATION AND ROLE OF NITRIC OXIDE STORES IN ADAPTATION TO HYPOXIA

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1. INTRODUCTION

Periodic hypoxic episodes can exert either detrimental or protective effects on the organism, which are defined to a considerable extent by the regimen, that is, the ratio of duration and intensity of hypoxic exposures. It was shown that acute, chronic and intermittent hypoxia induce different responses of intracellular signaling pathways for transcription of different genes; physiological responses to hypoxic exposures differ correspondingly.[1] It is well known that chronic periodic hypoxia, which is observed, for example, in sleep apnea syndrome, can result in the development of pulmonary and systemic hypertension, myocardial infarction and cognitive disorders.[2] At the same time, dosed adaptation to intermittent hypobaric or normobaric hypoxia shows a range of direct and cross-protective effects, which have been widely used for the treatment and prevention of many diseases and to increase the efficiency of exercise training.[3] This is why studying the effect of hypoxia on key physiological processes is so important. One of such universal processes is the metabolism of nitric oxide (NO) tightly regulated by environmental oxygen.[4]

The effect of NO depends on the NO level. High concentrations of NO are very toxic while comparatively low concentrations of NO provide a regulatory function through activation of cyclic guanylate cyclase and cyclic GMP production in target cells.[5]

The direction of changes in NO synthesis is related to the extent and duration of hypoxia with different mechanisms of these changes. Hypoxic inhibition of endothelial NOS (eNOS) is evident as attenuation of endothelium-dependent vascular relaxation. After 48 hours of exposing rats to hypoxia, endothelium-dependent relaxation of the isolated aorta was reduced twofold.[6]

The decrease in NO production in acute hypoxia is reversible. For example, after 90-min hypoxia, plasma levels of the NO metabolites nitrite and nitrate in the blood from the aorta and renal artery were decreased to 48% and 73% of the initial level, respectively. After 1 hour of reoxygenation, these parameters returned to baseline.[7]

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Activity of NOS directly depends on the level of synthesized NO since NO regulates the NOS activity by the negative feedback mechanism. The synthesized NO binds to iron of NOS heme thereby inhibiting NOS; simultaneously apparent Km for O_2 increases.[8] Since O_2 and NO compete for NOS heme iron, the extent of NOS dependence on O_2 is related to the decomposition rate of the NO/heme iron complex, which depends, in turn, on O_2 concentration.[9] The level of O_2 as a substrate can limit NO production in hypoxia because the apparent sensitivity of NOS isoforms to O_2 is within the range of normal tissue O_2 concentrations.[10]

Another possible mechanism for hypoxic inhibition of NO production is the decrease in heat shock proteins HSP90 characteristic of hypoxia.[11] These proteins bind to eNOS and provide its activation in response to NO-stimulating agonists.[12]

Sensitivity of eNOS to O_2 shortage increases in the presence of inducible NOS (iNOS) induction. Indeed, a moderate decrease in O_2 tension in a cell culture, which generally does not induce any attenuation of NO synthesis, suppresses eNOS activity following the iNOS gene expression stimulated by bacterial lipopolysaccharide.[4]

The decreased NO production in acute hypoxia is due to inhibition of eNOS activity but not downregulation of the enzyme protein synthesis.[11] However chronic hypoxia can suppress both eNOS activity and eNOS gene expression in vessels of the systemic circulation simultaneously stimulating the latter in the pulmonary circulation. The amount of eNOS protein falls sharply in rat aorta while it increases in lungs as early as after 12 hours of hypoxia.[6] Percentage of pulmonary arterioles expressing eNOS begins to increase after 1 day of hypoxia. After that, this parameter increases for several days and then remains unchanged for 4 weeks. The NO overproduction in pulmonary arterioles is not always beneficial and may even contribute to the development of pulmonary hypertension depending on the relationship between NO and free oxygen species.[13]

Dosed intermittent hypoxia may be an efficient stimulator of NO synthesis and eNOS expression. However, intermittent hypoxia stimulates both activity and expression of all three NOS isoforms. Potentiation of constitutive NO production is generally beneficial for the organism because it protects the organism against hypertension, thromboses, vasospasm, oxidative stress, etc. At the same time, expression of iNOS results in NO overproduction and toxic effects of excessive NO. During a hypoxic exposure, expression of iNOS begins only after 6 hours of continuous hypoxia while the constitutive isoforms eNOS and nNOS become activated almost immediately.[14,15] Therefore the duration of an adapting session of hypoxia should not exceed 6 hours.

In our experiments, adaptation to intermittent hypobaric hypoxia was used for stimulation of endogenous NO production.[16] Adaptation was performed in the altitude chamber at the simulated altitude corresponding to 4000 m above sea level. The duration of hypoxic exposure was gradually increased from 10 minutes to 5 hours. The complete course of adaptation consisted of 40 daily sessions. Nitric oxide production was evaluated by total plasma level of nitrite and nitrate. Adaptation induced a gradual increase in plasma nitrite plus nitrate, and by the end of the adaptation course this parameter was twice that of the control level.

Protective effect of 8-day adaptation to hypoxia was evident as prevention of NO overproduction in brain and a significant increase in survival of rats exposed to acute hypoxia at a simulated altitude of 11,000 m.[17] A course of the NOS inhibitor N^{ω}-nitro-L-arginine with the last dose administered 24 hours prior to the acute hypoxia exposure