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EXPERIMENTAL STUDY OF ERYTHROCYTE ENERGY METABOLISM UNDER INHALATIONS OF NITRIC OXIDE

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Abstract

Biological effects of nitric oxide are multiply, including vasoactive activity, participation in neurotransmission and intercellular communication etc. These effects are associated with endogenous releasing of nitric oxide, but influence of exogenous administration of this substance does not study in details. In particular, systemic action of nitric oxide inhalations is not so clear.

The aim of this work was the investigation of nitric oxide inhalations action on some parameters of energy and oxidative metabolism of healthy rat blood.

Wistar rats were randomly divided into two groups: control group (without any manipulations; n=10) and main group (n=10) with inhalations by nitric oxide-containing gas flow (20 ppm). Lactate dehydrogenase and lactate level were estimated in rat blood samples. In addition, we calculated a number of integral coefficients of energy metabolism, such as substrate provision coefficient and coefficient of energy reactions balance.

Our experiments demonstrate that 10-days course of inhalations of low nitric oxide doses (20 ppm) increases the adaptive potential of healthy rats' organism. One of these positive effects is associated with activation of some components of energy metabolism. First of all, it realized through stimulation of catalytic activity of lactate dehydrogenase, including its erythrocyte pool. Observed metabolic effect provides the basis for pathogenic correction of diseases, associated with hypoxia, oxidative stress and energy deficiency.

Keywords: *nitric oxide, inhalations, energy metabolism, lactate dehydrogenase.*

Introduction

Data about multiply role of nitric oxide (NO) as a universal biological regulator is previously has for modulation of its endogenous synthesis and releasing [*Pisarenko O et al., 2009; Vanin A, 2009; Kalyanaraman B, 2013*]. For NO vasoactive activity, participation in neurotransmission and intercellular communication as well as other biological effects were demonstrated [*Dimmler S, Brune B, 1992; Zhang S, Shyder S, 1992; Almeida A et al., 2004; Manukhina E et al., 2006; Pisarenko O et al., 2009; Vanin A, 2009; Kalyanaraman B, 2013*]. In particular, nitric oxide was identified as a main endothelium relaxation factor [*Pisarenko O et al., 2009; Kalyanaraman B, 2013*].

At other side, results and molecular mechanisms of exogenous NO action on human and ani-

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mals organism is not studied in details. In addition, the sources of exogenous nitric oxide are not so numerous [*Lundberg J et al., 2008; Carlström M, 2021*]. They are different NO donors, including nitrovasodilators and synthetic substances [*Korobko V et al, 2014; Ritchie R et al, 2017; Bai Y et al, 2018*], and imitation of natural bound forms of nitric oxide, first of all – S-nitrosothiols and dinitrosyl iron complexes with some ligands [*Pisarenko O et al., 2009; Kalyanaraman B, 2013*].

Another way of exogenous NO production is using of different generating devices. In Russia single registered NO-generator is "Plazon", but it produced the gas flow with high NO concentration (500 ppm and more) [*Shekhter A et al, 2019; Igrunkova A et al, 2023; Zaborova V et al, 2023*]. That is why other device is necessary, and it was constructed by researchers from Russian federal nuclear center (Sarov). This apparatus can produce gas flow with low NO concentration (from 20 to 100 ppm) [*Martusevich A et al., 2014*].

Special way of extraorganismal stimulation of nitric oxide generation is using of short-wave ultrasound devices, producing oscillations on the frequency of NO (150±0,75 *GHz*). Until today we have only one similar apparatus, named "The orbit" (Saratov, Russia) [*Kirichuk V, Tsymbal A, 2012*]. There are some experimental papers on influence this device on functional and metabolic parameters of laboratory animals [*Vanin A, 2009; Martusevich A et al., 2013*], but it is difficult to establish the exact current concentration of nitric oxide in this case.

In early investigations we have shown that influence of gaseous nitric oxide on blood metabolic parameters in vitro is associated with NO concentration [*Martusevich A et al., 2013a; b*]. It was fixed that high NO concentration in gas phase (800 ppm) lead to wide spectrum of negative effects, including oxidative stress, energy deficiency, lactate accumulation, methemoglobin hypersynthesis etc. [*Martusevich A et al., 2013b*]. In opposite, use of low NO concentration (100 ppm) caused the stimulation of some components of energy metabolism and detoxication enzymes with moderate antioxidant effect [*Martusevich A et al., 2013b; Martusevich A et al., 2014*].

It is important that these data was registered for in vitro conditions, and they need for in vivo investigations. That is why the aim of this paper is scrutiny of effect of inhalation course with NO low concentration on erythrocytes energy metabolism in healthy rats.

MATERIAL AND METHODS

Animals: Experiments were executed on 20 healthy male Wistar rats. Two groups of animals were collected: control group (n=10; without any manipulations) and main group (n=10). Rats of main group got daily inhalations of NO-containing gas mixture (NO concentration – 20 ppm; flow speed – 2 l/*min*; inhalation time – 10 *min*) during 10 days. NO-containing gas mixture was generated with experimental device, constructing on Russian federal nuclear center (Sarov, Russia).

Method characteristics: For energy metabolism estimation we used lactate dehydrogenase activity in direct (LDGdir) and reverse (LDGrev) reactions. Enzyme catalytic activity was studied in erythrocytes hemolysate with G.A. Kochetkov's method [*Martusevich A et al., 2013b; Martusevich A et al., 2014*]. Lactate concentration in erythrocytes was estimated with automatic analyzer SuperGL Ambulance. In addition, we calculated number of integral coefficients of energy metabolism, such as substrate provision coefficient and coefficient of energy reactions balance (ERB). They are calculated with special formulas:

$$ERB = \frac{(LDGdir)^2}{(LDGrev)^2} \times 100$$

where LDGdir – activity of lactate dehydrogenase in direct reaction; LDGrev - activity of lactate dehydrogenase in reverse reaction)

$$SP = C(Lactate) \times \frac{LDGdir}{LDGrev}$$

where (C(Lactate) – erythrocytes lactate level; LDGdir – activity of lactate dehydrogenase in direct reaction; LDGrev - activity of lactate dehydrogenase in reverse reaction)

Statistics: Statistical analysis of the data was performed with Statistica 6.0 program. Data were expressed as means ± SE, the Student's t-test was used for detection of statistical difference.

Results and Discussion

It was stated that NO inhalations stimulates the direct and reverse reactions of erythrocytic lactate dehydrogenase (Fig. 1), but this effect is strongly marked for direct activity of enzyme (+105% vs +78% for reverse one to animals of control group; $p<0.05$). These findings characterize effect of NO exposure as positive for investigated parameter of energy metabolism of red blood cells.

Estimation of lactate level in blood plasma demonstrated (Fig. 2) that NO inhalations cause the minimal decreasing of this metabolite concentration (at 7%; $p<0.1$ to control group). At that time, in erythrocytes we fixed clear elevation of lactate level (at 18%; $p<0.05$). On our opinion, these dynamics may be associated with indicated activation of lactate dehydrogenase and exchange of lactate between blood plasma and intraerythrocytic cytoplasm.

Complex analysis of investigated part of energy metabolism was produced with calculation of substrate provision coefficient and coefficient of energy reactions balance. It was observed that both indicated indexes were significantly increased after course of NO inhalations as compared with rats of control group (Fig. 3).

In particular, coefficient of energy reactions balance is charactering only catalytic properties of lactate dehydrogenase was elevated at 32% to control level ($p<0.05$). Substrate provision coefficient is additionally using lactate concentration demonstrated more clear increasing (at 36%; $p<0.05$ to animals of control group). This tendency indicates on optimization of energy metabolism under course of NO inhalations for initiation of investigated intermediate link of erythrocytes energy production.

In 1989 B. Brune and E.G. Lapetina shown that nitric monoxide initiated a special cytozolic protein with molecular weight 37 kD, which will be identified as key enzyme of glycolysis – glyceraldehydes-3-phosphatedehydrogenase [*Dimmler S, Brune B, 1992*]. This effect was supported by J. Zhang and S.H. Snyder (1992) on the neuronal cells culture and after – for other cells and enzymes (for example, phosphofructokinase of pancreatic onset cells and neurons) [*Mohr S et al., 1999; Almeida A et al., 2004*]. Some articles dedicate to the role of nitric oxide and NO-synthase

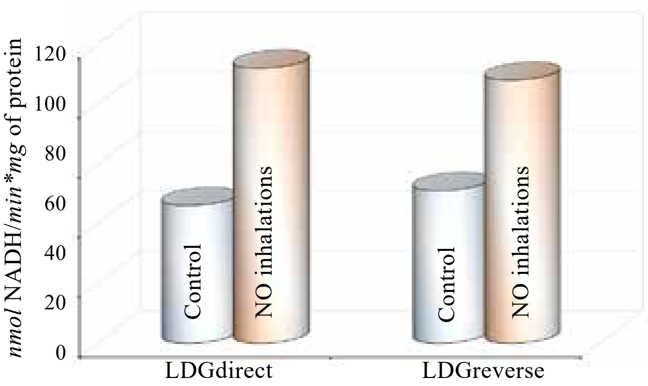

Figure 1. *Influence of NO inhalations of activity of lactate dehydrogenase in direct and reverse reactions (LDGdirect - activity of lactate dehydrogenase in direct reaction; LDGreverse - activity of lactate dehydrogenase in reverse reaction; "*" – level of statistical difference is p<0.05 to control value)*

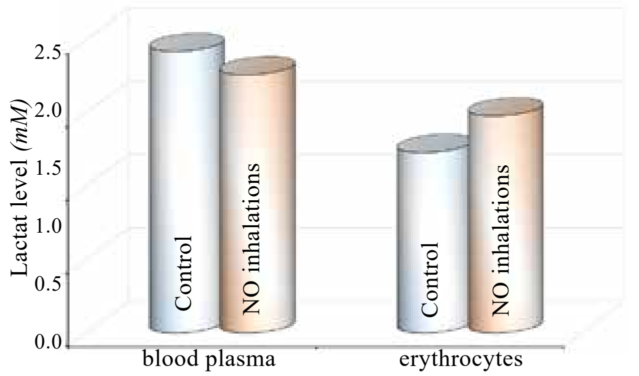

Figure 2. *Lactate level in erythrocytes and blood plasma of rats with and without NO inhalations ("*" – level of statistical difference is p<0.05 to control value) el of statistical difference is p<0.05 to control value)*

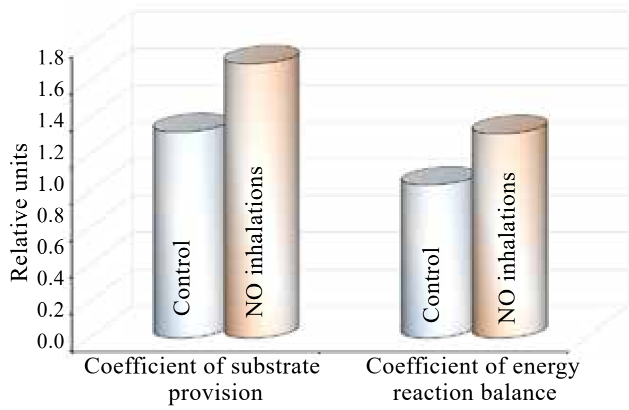

Figure 3. *Calculated coeffificent of erythrocyted energy mebabolism in rats with and without NO inhalations ("*" – level of statistical difference is p<0.05 to control value)*

in adaptation to hypoxia and tissue hypoperfusion by modification of cell respiration processes [*Manukhina E, 2006*]. Mechanisms of this effect is not studied in details, but indicated and other data postulates the crucial role of NO in energy metabolism regulation [*Brune B, Lapetina E, 1989; Dimmler S, Brune B, 1992; Mohr S et al., 1999; Almeida A et al., 2004; Martusevich A, 2014*]. In addition, there are some data about action of substance on intacellular glucose transport in muscles [*Zhang S, Shyder S, 1992; Almeida A et al., 2004*]. So, results of previous investigations allow concluding the influence of NO on energy metabolism, but specialties and mechanisms of this effect are discussed still now.

On the base of our results the influence of NO low doses on erythrocytes energy metabolism consists of number of components. In particular, NO inhalations lead to total stimulation of lactate dehydrogenase, initiating the activation of its direct and reverse reactions. It is important that systemic administration of gaseous nitric oxide has a prevalent action on direct reaction of this enzyme, leading to generation of pyruvate, a main energy substrate in Krebs cycle.

At other side, dynamics of lactate level is disproportional. We observed the decreasing of lactate concentration in blood plasma, but it was elevated in erythrocytes. On our opinion, these changes are associated with utilization of metabolite by lactate dehydrogenase and its transport into erythrocytes due to increased permeability of membranes. This phenomenon may have an adaptive value, because lactate transport can recharge its erythrocyte pool for substrate provision of lactate dehydrogenase during its activation.

Indicated tendency is confirmed by changes of integrative coefficients of energy metabolism, such as coefficients of energy reactions balance and substrate provision. Our results demonstrated that its level was significantly increased after the exposure (at 32 and 36% to initial value, respectively). Given the nature of these parameters, we can conclude not only on the stimulating effect in respect of lactate dehydrogenase, but also about the coherence of this process to changes in the concentration of lactate in plasma and erythrocytes. This allows concluding that NO-dependent activation of investigated part of erythrocyte energy metabolism under inhalations of NO low concentration (20 ppm).

Conclusion

In whole, our experiments demonstrate that 10-days course of inhalations of low NO doses (20 ppm) increases the adaptive potential of healthy rats' organism. One of these positive effects is associated with activation of some components of energy metabolism. First of all, it realized through stimulation of catalytic activity of lactate dehydrogenase, including its erythrocyte pool. Observed metabolic effect provides the basis for pathogenic correction of diseases, associated with hypoxia, oxidative stress and energy deficiency.

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