DOI 10.1007/s10517-016-3331-4

GENERAL PATHOLOGY AND PATHOPHYSIOLOGY

Modification of the Catalytic Properties of Erythrocyte Aldehyde Dehydrogenase in Rats after Nitric Oxide Inhalation

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 161, No. 1, pp. 9-12, January, 2016
Original article submitted March 27, 2015

We studied aldehyde dehydrogenase activity in erythrocytes from healthy rats and animals with thermal trauma after NO inhalation. NO had an activating effect on catalytic properties of aldehyde dehydrogenase in healthy rats and burned animals. The effect of NO was more pronounced during burn disease.

Key Words: *nitric oxide; blood; aldehyde dehydrogenase*

The biological role of NO as general low-molecular-weight biological regulator of physiological and metabolic processes has been demonstrated in numerous reports [1,2,6,7,9]. In light of this, it seems important to find the ways for controlling the endogenous level of this compound [10,14]. This goal is mainly achieved through modulation of NO synthase activity by administration of its substrate (L-arginine [2]) or modification of enzyme catalytic activity [2,8,11].

Published data show that other enzymatic and nonenzymatic systems are involved in the regulation of NO level [1,7,12,14]. Moreover, deposited forms of NO play an important role in the molecular, cellular, and body effects of this compound [1,2,4].

From practical point of view, analysis of the alternative pathways of NO formation is seems to be interesting. For example, biodegradation of organic nitrates (used as vasodilator drugs) into NO is catalyzed by the only enzyme, aldehyde dehydrogenase (ALDH; mainly fraction 2) [4,6-8,13,14). This process was studied in details. However, the effect of NO (product of this process) on enzyme catalytic activity is poorly known. This effect was described only in one report [6]. According to the laws of enzymatic hydrolysis [7,11], the reaction product with high probability will not be indifferent to the catalytic activity of the enzyme. This hypothesis is confirmed by the results of our previous studies. We revealed that various forms of NO have different *in vitro* effects on functional activity of ALDH. Moreover, stimulation with NO was followed by a dose-dependent response of the enzyme (biological threshold 0.15 mmol/liter) [4]. These data suggest that exogenous administration of NO *in vivo* modulates catalytic activity of ALDH. It is interesting to evaluate the dependence of this effect on the dose of study compound under conditions of severe systemic disorder.

This work was designed to study ALDH activity in erythrocytes from healthy rats and animals with thermal trauma after NO inhalations.

MATERIALS AND METHODS

Experiments were performed on 40 male Wistar rats. The animals were divided into 4 equal groups. Group 1 consisted of healthy rats that remained intact (single blood sampling). Group 2 rats daily inhaled a gas mixture containing 20 ppm NO (flow rate 2 liter/min, duration 5 min) for 10 days. The course duration and

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NO concentration correspond to those in pediatric and surgical practice [9,12]. The NO-containing mixture was generated with an experimental device for its synthesis [3,4] and delivered to a chamber with rats.

The animals of groups 3 and 4 were narcotized with Zoletil and Xyl (mixed anesthesia), exposed to thermal trauma by the original method, and received standard local treatment [15]. Group 4 rats received a course of inhalations (similarly to group 2 animals) starting from the next day after burn modeling.

Blood samples were obtained from the sublingual vein before the start and at the end of the experiment (day 11 for group 2; day 13 for groups 3 and 4). Erythrocyte ALDH activity was measured by the method of B. M. Kershengol'ts and E. V. Serkina with modifications [4].

The results were analyzed using Statisitca 6.0 software. Shapiro–Wilk test was used to check normality of data distribution. Taking into account the distribution of signs, the statistical significance of differences was evaluated by Kruskal–Wallis test.

RESULTS

The development of burn disease was accompanied by a 39.4% decrease in ALDH activity ($p<0.05$; Fig. 1), which is consistent with experimental data obtained on humans and rats [4]. A possible mechanism for enzyme inhibition is accumulation of utilized endogenous intoxication substrates.

After a course of NO inhalations, ALDH activity in healthy rats was by 2.69 times higher than in intact animals ($p<0.01$; Fig. 2). Taking into account that systemic treatment was performed with low doses of the agent (20 ppm), our results are consistent with published data. It was shown that *in vitro* exposure to low cNO oncentrations stimulates ALDH activity [4]. However, the level of enzyme catalytic activity after treatment of blood samples with NO depends on the direct effect of the test compound on ALDH function. By contrast, NO inhalation is accompanied by a strong systemic component of the response. It is partly related to metabolism optimization, reduction of endogenous intoxication, and improvement of blood flow in tissues (*e.g.*, due to the increase in microcirculation).

Similar results were obtained in experiments on animals with thermal trauma receiving a course of NO inhalations (Fig. 3). ALDH activation in these animals was more pronounced than in healthy specimens. ALDH activity under these conditions was higher than in untreated rats (by 6.67 times, $p<0.01$) and intact animals (by 4.05 times, $p<0.01$). Moreover, the observed increase in enzyme catalytic activity was more pronounced than in healthy rats after the course of NO inhalations (by 50.4%, $p<0.05$).

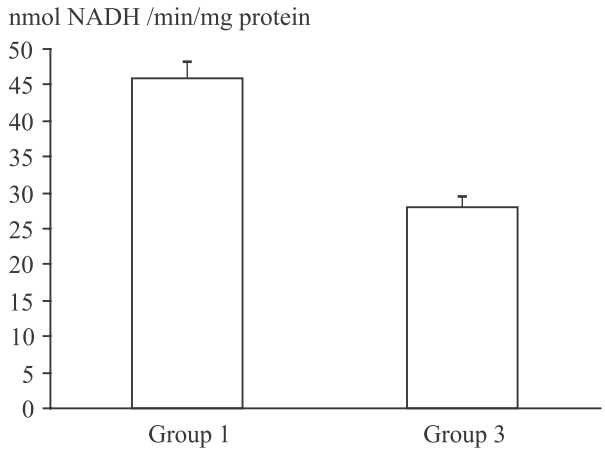

Fig. 1. ALDH activity in erythrocytes from healthy rats (group 1) and animals with thermal trauma (group 3).

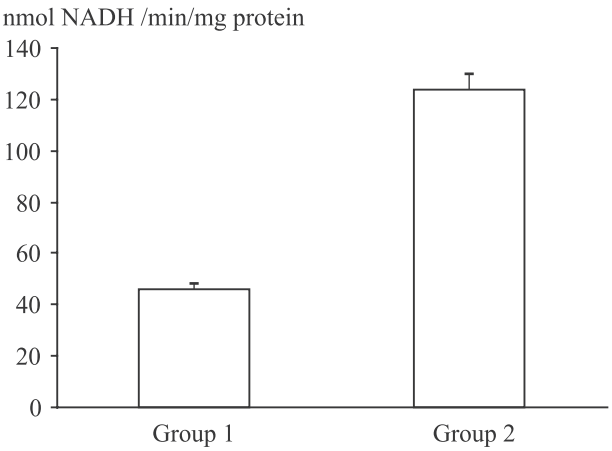

Fig. 2. ALDH activity in erythrocytes from healthy rats (group 1) and after a course of NO inhalations (group 2).

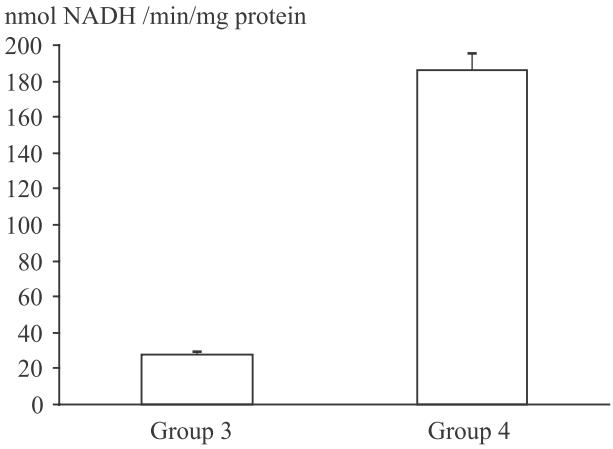

Fig. 3. ALDH activity in erythrocytes from rats with thermal trauma not receiving (group 3) and receiving a course of NO inhalations (group 4).

This metabolic reaction can be mediated by several mechanisms. Combined effect of these processes contributes to the observed changes in enzyme function. For example, gaseous NO in low concentrations

(20 ppm) that affect only Cys-302 thiol groups of the studied enzyme [4,6,11] are rapidly utilized by GSNO reductase (thioredoxin system), catalase, deoxyhemoglobin, cytochrome C, and other molecules [7,10,14]. Apart from with direct stimulatory effect of NO on ALDH, the influence of this compound is realized via optimization of energy metabolism, detoxification function of the liver [5], improvement of microcirculation with the primary involvement of a NO-dependent endothelial component for blood flow regulation [13], and antioxidant action of NO in low doses [1,5].

Our study of the effect of systemic treatment with NO (inhalation) revealed *in vivo* activating effect of this compound on catalytic activity of ALDH. These results are consistent with previous data on an *in vitro* effect of study compound in human blood samples. This effect is observed in healthy rats and animals with thermal trauma. It should be emphasized that the effect is more pronounced during burn disease.

This work was supported by the grant of the President of the Russian Federation for Young Doctors of Sciences (grant No. MD-7256.2015.7).

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