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Peculiarities of the Hypoxic State Formation in Rats under Nitrite Methemoglobinemia

KEYWORDS: hypoxic state, nitrite methemoglobinemia, lung, myocardium, mitochondria.

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ABSTRACT *In case of experimental methemoglobinemia induced in 80 adult male rats (Wistar) by 5-day injections of sodium nitrite in a dose of 5 mg / 100 g body weight, was studied the formation of the hypoxic state. The role of respiratory function, blood circulation, acid-base status of the blood and morphofunctional state of the heart and lungs tissue in the development of hypoxia in experimental conditions was revealed. It was shown, that the decreasing of hemoglobin concentration which is capable to oxygenation, was accompanied by a fall of blood oxygen, the oxygen tension and pH in arterial and mixed venous blood, changes in content and ratio of bicarbonate and buffer bases. Such changes can be regarded as a development of decompensated metabolic acidosis, however, without the formation of secondary tissue hypoxia due to the keeping of oxygen delivery by blood and its consumption ratio at the level > 3. The revealed changes at the tissue level, as well as in the respiratory and circulatory systems, have organ specific character. In the myocardium, there were signs of mitochondrial dysfunction in the absence of significant cytotoxic damage of the heart tissue, and in lung tissue a pronounced mitochondrial dysfunction is formed with the development of severe primary tissue hypoxia. The obtained results should be considered in the developing of advanced ways of hypoxic damage correction under formation of methemoglobinemia.*

INTRODUCTION

Currently, the mechanisms of studying problem of endogenous and exogenous sources nitrogen containing compounds influence on living organisms is extremely relevant due to intensive use of nitro compounds in human economic activity that become sources of active nitrogen species in the process of biotransformation (ANS) [1,2]. As clinical data and experimental results show, the organisms which are experiencing increased nitrite load have a number of metabolic disorders [3]. The main manifestation of the toxic action of these compounds is methemoglobinemia, which level is correlated with the degree of intoxication. At the same time, being a necessary element of the cells, the nitric oxide is capable to modulate vascular effects of reactive oxygen species (ROS) and amplify the pathogenic effect of ROS on the cell membranes and mitochondria of various tissues, including lung and myocardium [4]. Against this background, the complicated mechanisms of the so-called hemic hypoxia are formed - hypoxic state under methemoglobinemia, which is accompanied by the development of a pathological processes cascade. However, despite the intensive research, due to the multifactor effect on the organism of ANS, thus far there is no clear concept of the hypoxic state development under nitrite methemoglobinemia, in particular with regard to the formation of tissue hypoxia, which complicates the searching of effective correction ways of hypoxic damage.

The aim of this study was to determine the role of the respiratory and circulatory functions, acid-base status of the blood and morphofunctional state of the heart and lung tissue in the formation of tissue hypoxia under nitrite methemoglobinemia.

MATERIALS AND METHODS

Investigations were carried out on 80 adult male rats (Wistar) weighing 160-220 g. The methemoglobinemia was chosen as an experimental model, developing when injected subcutaneously methemoglobin-forming - aqueous solution of sodium nitrite ($NaNO_2$) - 5 mg of dry substance per 100 g of body weight.

All indicators were determined before injection of sodium nitrite (control) and on the 5th day after daily injection of methemoglobin-forming. The total content of hemoglobin (Hb) and methemoglobin (MetHb) in blood were determined by cyanide method [5]. The characterizing parameters of the lung function, gas exchange, oxygen parameters and acid-base state of blood were determined by mass spectrometer MX-6202 (Ukraine) and analyzers such as "Corning" (Hungary, UK), «Radelkis» (Hungary). Based on the obtained data there were calculated the respiratory minute volume ($\dot{V}_E$), the volume of alveolar ventilation ($\dot{V}_A$), the oxygen consumption ($\dot{V}_{O_2}$), the blood oxygen capacity (BOC), the oxygen content in arterial and mixed venous blood ($C_{a_{j_2}}$, $C_{\bar{v}}O_2$), the minute volume of blood circulation ($\dot{Q}$), the rate of oxygen transport by arterial blood ($q_{a_{j_2}}$) [6]. The lung diffusion capacity ($D_{L_{O_2}}$) was determined according to the equation $1/\theta V_c + 1/D_M = 1/D_{L_{O_2}}$, where - θV_c - the blood component of the lung diffusion capacity for oxygen, Dm – the membrane component of the lung diffusion capacity [7].

The preparation of tissue samples for electron microscopic studies was performed by the usual method [8], using reagents of Sigma (USA) and Fluka (Switzerland) firms. Viewing of ultrathin slices (40-60 nm in thickness) was performed using an electron microscope JEM 100CX (Japan). Morpho- and stereometric assessment of tissues was performed using a computer program for the morphometric counting Image Tool Version 3 (USA) on 130-150 fields in the control and experimental series of studies.

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Statistical processing was performed by applied program package «Microsoft Excel» using the Student's t test. The results were expressed as mean ± m, since the obtained data fit into the Maxwell distribution [9]. The differences between mean values were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

The injection of $NaNO_2$ was accompanied by an increase in the blood concentration of MetHb in 1.82 times compared with the initial value (Table 1).

Table (1): The influence of methemoglobin-forming on indexes of the organism hypoxic state formation (M±m)

Parameters	Control	Methemo-globinemia
MetHb, %	2,10±0,25	4,45±0,48*
Hb, g%	13,8±0,38	11,9±0,29*
Hb «act», g%	13,5±0,38	11,57±0,33*
BOC, vol.%	18,1±0,38	15,2±0,52*
$P_{\grave{a}_{\hat{I}_2}}$, mmHg	96,8±2,13	82,0±3,02*
$P_{V_{\hat{I}_2}}$, mmHg	39,7±2,59	34,7±1,67
pH_a, r.u.	7,40±0,01	7,21±0,02*
pH_v, r.u.	7,36±0,01	7,19±0,02*
$\tilde{N}_{\grave{a}_{\hat{I}_2}}$, vol.%	16,94±0,32	14,07±0,23*
$C_{V_{O_2}}$, vol.%	11,43±0,27	9,60±0,29*
$\dot{Q}$, ml.min $^{-1}$/100 g	34,5±1,05	54,6±1,34**
$q_{\grave{a}_{\hat{I}_2}}$ ml.min $^{-1}$/100 g	5,84±0,14	7,79±0,19*
$\dot{V}_{O_2}$, ml.min $^{-1}$/100 g	1,90±0,19	2,53±0,23*
$\dot{V}_A / \dot{Q}$	1,05±0,06	0,62±0,06*
θV_c, ml.min $^{-1}\cdot$ mmHg$^{-1}\cdot$ml	1,10±0,08	1,74±0,012*
D_M, ml.min $^{-1}\cdot$ mmHg	0,52±0,02	0,56±0,02
D_L, ml.min $^{-1}\cdot$ mmHg	0,35±0,01	0,42±0,02*
Normal buffer base, mmol / l	47,3±0,25	46,6±0,20*
Deficit of the buffer bases, mmol / l	-2,9±0,45	-11,5±0,92**
Buffer base, mmol / l	44,4±0,22	35,1±0,32*
True bicarbonate, mmol / l	20,70±0,17	15,7±0,21*
Standard bicarbonate, mmol / l	20,0±0,19	15,5±0,29*
Total amount of carbon dioxide, mmol / l	21,7±0,20	16,9±0,15*

*** - the mean difference is significant at the 0.05 level;**
**** - the mean difference is significant at the 0.01 level**

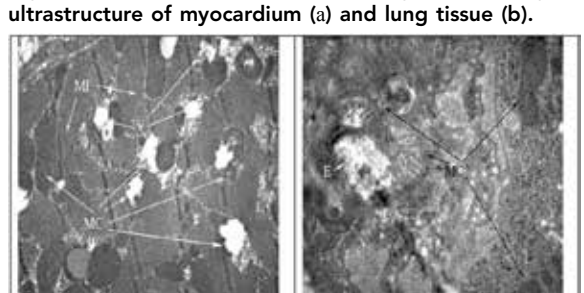
In connection with the transition of the actively functioning haemoglobin (Hb "act") in it's inactive, in respect of the respiratory gases transport, metform and with decreasing in concentration of total haemoglobin (Hb) by 14%, the amount of haemoglobin capable of oxygenation decreased by 16% (Table. 1). These changes led to a parallel drop in BOC by 16% and were accompanied by a decrease of oxygen tension in arterial ($P_{\grave{a}_{\hat{I}_2}}$) and mixed venous blood ($P_{V_{\hat{I}_2}}$) (by 15% and 13% respectively) (Table 1). Also it was revealed the decreasing of $c_{\grave{a}_{\hat{I}_2}}$ and $C_{V_{O_2}}$ by 15% and 16%, respectively (Table 1). The totality of detected changes is a prerequisite for the development in the organism of the hemic type of hypoxic state, accompanied with the arterial hypoxemia, and decreasing of arterial-venous difference in oxygen content. Furthermore, under the action of methemoglobin-forming there was a significant decrease of pH in the arterial (pHa) and mixed venous (pHv) blood in rats, with the pronounced increasing of base deficit and decreasing of the content of normal buffer base, the standard and true bicarbonates, total carbon dioxide content (Table 1). That is, one can state the development of decompensate metabolic acidosis at 5-day of $NaNO_2$ injections.

The formation of methemoglobinemia with concomitant hypoxic condition was accompanied by an increase of $\dot{Q}$ by 58% with no significant change in lung ventilation, in particular $\dot{V}_A$, resulting in ventilation-perfusion ratio ($\dot{V}_A / \dot{Q}$) decreases from baseline by 61%. The compensation of the oxygen delivery deterioration at the stage of the air - blood was carried out due to an increase of 1.2 times $D_{L_{O_2}}$ for the account θV_c, associated with the intensity of the blood flow in the pulmonary circulation, without significant changes in D_M (Table 1). Through the identification the set of changes $q_{\grave{a}_{\hat{I}_2}}$ increased by 33% compared to baseline levels, contributing to keeping the ratio of oxygen delivery by blood and the rate of it consumption ($\dot{V}_{O_2}$) (the last one on the 5th day of methemoglobin-forming injections has increased by 33%, Table 1) within 3.1÷3.8 [10]. However, one can assume that in these experimental conditions, the methemoglobin-forming directly affects on the tissues of body, which can lead to the development of primary (histotoxic) tissue hypoxia.

The confirmation of this hypothesis obtained in electron microscopic study of lung tissue and myocardium. It should be emphasized that the changes at the tissue level, as well as in the respiratory and circulatory systems, have expressed organ specific character.

The increase in blood flow velocity under methemoglobinemia was largely ensured, firstly, due to good preservation of myocardial ultrastructure with the localization of tissue edema in vacuole-like structures, which are observed between the intact myofibrils (Fig. 1a).

Figure (1): The influence of methemoglobin-forming on ultrastructure of myocardium (a) and lung tissue (b).



a – The ultrastructure of myocardium after 5 days injection of $NaNO_2$: vacuole (V) among unchanged myofibrils (MF); partially or fully vocalized and destructured, as well as native mitochondria (MC).x 9600

b – The ultrastructure of lung tissue after 5 days injection of $NaNO_2$: development of edema (E), partially or fully vocalized and destructured mitochondria (MC); small amount of native organelles. x 9600

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Secondly, by the end of 5-day of $NaNO_2$ injections in the mitochondrial apparatus (MCA) of heart tissue were formed the changes required to maintain of optimal energy metabolism during the straining at the function of the circulatory system.

The typical for tissue hypoxia violations of mitochondria (MC) ultrastructure: the partial or complete vacuolation, destruction of crists and mitochondrial membranes, etc. (Fig. 1a), were compensated by the activation of MC morphogenesis, accompanied by a moderate (30-35%) swelling and increasing in the amount of MC surfaces per unit volume of tissue (Table 2) that it is important to increase the capacity of the MC respiratory chain, which provides a membrane-bound enzymes activities [11].

Table (2): The influence of methemoglobin-forming on morpho- and stereometric parameters of mitochondria in the myocardium and lung tissue (M±m)

Experimental conditions	Total number of mitochondria, unit/μm²		Number of structurally altered mitochondria, %		Mean diameter of mitochondria, μm		Total surface of mitochondria in unit volume of tissue, μm²	
	Myocardium							
	SS MC	IMF MC	SS MC	IMF MC	SS MC	IMF MC	SS MC	IMF MC
Control (a=150)	12,5±1,6	8,4±1,1	4,1±0,6	2,7±0,4	0,50±0,04	0,76±0,07	8,3±0,8	6,8±0,9
Methemoglobinemia (a=140)	23,8±3,7**	17,1±2,2**	12,8±5,7**	11,6±4,3**	0,67±0,08*	1,03±0,10*	13,7±1,5*	8,9 ±0,7*
Lung tissue								
Control (a=150)	9,6±0,2		4,6±0,5		0,39±0,01		5,7±0,5	
Methemoglobinemia (a=140)	10,1±0,7		9,7±0,7**		0,63±0,03**		6,8±0,7	

* - the mean difference is significant at the 0.05 level; ** - the mean difference is significant at the 0.01 level; a - the number of regions randomly chosen for calculations; SS MC - subsarcolemmal mitochondria; IMF MC – intramyofibrillar mitochondria

Therefore, after 5 days of methemoglobin-forming injection in the myocardium the signs mitochondrial dysfunction without expressed histotoxic damaged of heart tissue are showing.

The absence of expressed respiratory response to the development of methemoglobinemia may be, to some extent, explained by the changes in morpho-function state of lung tissue. There was the development of edema, the inhibition of the surfactant system, the formation of hemorrhagic syndrome in some alveoli. It should be emphasized that from MCA there was not revealed the significant compensatory response, as it is observed in myocardium. The activation of MC morphogenesis did not happen; while significantly increasing the amount of structural damaged organelles with its vacuolization and elements of destruction (Fig. 1b). There was a sharp increase of the MC swelling with the increasing of its average diameter of more than of 50% from the initial level. It is usually regarded as an indicator of reducing the MC energy capacity and the first stage of organelles death by necrotic type (Fig. 1b) [12]. Thus, under the influence of $NaNO_2$ the expressed mitochondrial dysfunction in the lung tissue was formed, which may also indicate the development of pronounced primary tissue hypoxia.

CONCLUSION

It was shown that the 5-day injection of methemoglobin-forming - $NaNO_2$ - leads to the development in the organism of the hemic type of hypoxic condition, which is accompanied by arterial hypoxemia and decompensates metabolic acidosis, but without the formation of secondary tissue hypoxia. Moreover, it was revealed the significant changes at tissue level, which, as in the systems of respiration and circulation, have the expressed organ specific character: the signs of mitochondrial dysfunction without substantial histotoxic damage in heart tissue were observed in myocardium, but in lung tissues the expressed mitochondrial disfunction was formed, that indicates the development of pronounced primary tissue hypoxia. The obtained results should be considered in development of advanced ways for correction of hypoxic damages, evolving during methemoglobinemia.

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