УДК 577.112.3:547.495.9]:612.617
DOI: https://doi.org/10.17816/PAVLOVJ108589

Гипоксия-подобный эффект L-аргинина в семенных пузырьках и эпидидимисе крыс

Ю. А. Марсянова✉, В. И. Звягина

Рязанский государственный медицинский университет имени академика И. П. Павлова, Рязань, Российская Федерация

АННОТАЦИЯ

Введение. Влияние L-аргинина на обменные процессы опосредуется оксидом азота (II), пул которого регулируется несколькими ферментами. В литературе отмечается взаимное влияние дефицита кислорода и продукции NO. Кроме того, оба процесса можно регулировать с помощью экзогенного L-аргинина.

Цель. Оценить участие L-аргинина в развитии адаптационного ответа на хроническую нормобарическую гипоксию тканей мужской репродуктивной системы крыс и изучить его влияние на изменение метаболизма в условиях нормоксии.

Материалы и методы. Эксперимент проведен на крысах сток Wistar (самцы, n = 8), которые были разделены на следующие группы: (1) животные, получавшие в течение 10 дней инъекции L-аргинина 500 мг/кг массы тела; (2) животные контрольной группы, получавшие 0,9% раствор NaCl; (3) животные, подвергшиеся хронической нормобарической гипоксии, ежедневно наблюдались в гермокамере до снижения концентрации кислорода 10% в воздухе один раз в день в течение 14 дней; (4) животные контрольной группы, наблюдались в вентилируемой камере; (5) животные, подвергшиеся гипоксии и инъекциям L-аргинина. Материалом для анализа послужили митохондрии и безмитохондриальная фракция цитоплазмы семенных пузырьков, головки и хвоста эпидидимиса. Оценка показателей проводилась фотометрически с помощью диагностических наборов и наборов иммуноферментного анализа.

Результаты. При получении животными L-аргинина относительно группы контроля наблюдалось повышение в цитоплазме количества α-субъединицы гипоксией индуцируемого фактора в семенных пузырьках на 132% (p = 0,01), в хвосте эпидидимиса на 32% (p = 0,02) и снижение в митохондриях на 45% (p = 0,01) и 60% (p = 0,002) соответственно, снижение уровня сукцината на 40% (p = 0,005) и 51% (p = 0,0009), повышение концентрации молочной кислоты в цитоплазме на 194% (p = 0,03) и 253% (p = 0,018), снижение активности цитохромоксидазы с 0,96 [0,66; 1,69] у.е./мг белка до 0,27 [0,23; 0,32] (p = 0,0009) и с 1,04 [0,84; 1,33] до 0,26 [0,14; 0,37] (p = 0,003). Наблюдаемые изменения характерны для состояния гипоксии и объясняются переключением клетки на получение энергии гликолитическим путем, в отличие от митохондриального при нормоксии. Совместное влияние гипоксии и аргинина частично усиливали эффекты друг друга.

Заключение. L-аргинин вызывает в клетках гипоксия-подобное состояние посредством активации α-субъединицы гипоксией индуцируемого фактора, снижения активности цитохромоксидазы и увеличения функции гликолиза, а также частично усиливает эффекты хронической нормобарической гипоксии.

Ключевые слова: *L-аргинин; гипоксия; митохондрии; семенные пузырьки; эпидидимис; оксид азота*

Для цитирования:
Марсянова Ю.А., Звягина В.И. Гипоксия-подобный эффект L-аргинина в семенных пузырьках и эпидидимисе крыс // Российский медико-биологический вестник имени академика И. П. Павлова. 2023. Т. 31, № 3. С. 345–356. DOI: https://doi.org/10.17816/PAVLOVJ108589

Рукопись получена: 06.06.2022 **Рукопись одобрена:** 14.09.2022 **Опубликована:** 30.09.2023

DOI: https://doi.org/10.17816/PAVLOVJ108589

Hypoxia-Like Effect of L-Arginine in Seminal Vesicle and Epididymis of Rats

Yuliya A. Marsyanova✉, Valentina I. Zvyagina

Ryazan State Medical University, Ryazan, Russian Federation

ABSTRACT

INTRODUCTION: The effect of L-arginine on metabolic processes is mediated by nitric oxide (II), whose pool is regulated by several enzymes. In the literature, the mutual influence of oxygen deficit and NO production is described. Besides, both processes can be regulated by exogenous L-arginine.

AIM: To evaluate participation of L-arginine in the development of adaptive response to chronic normobaric hypoxia of tissues of reproductive system of male rats and to study its influence on metabolic changes in normoxia.

MATERIALS AND METHODS: The experiment was conducted on Wistar stock rats (males, n = 8) which were divided to the following groups: (1) animals receiving L-arginine injections of 500 mg/kg of body weight for 10 days; (2) animals of control group receiving 0.9% NaCl solution; (3) animals subjected to chronic normobaric hypoxia in a hermetic chamber, observed once a day for 14 days until the oxygen concentration in the air decreased by 10%; (4) animals of the control group observed in a ventilated chamber; (5) animals subjected to hypoxia and injections of L-arginine. The material for analysis was the mitochondria and mitochondria-free fraction of the cytoplasm of the seminal vesicles, of the head and tail of the epididymis. The parameters were evaluated photometrically using diagnostic and enzyme immunoassay kits.

RESULTS: The animals receiving L-arginine showed increase in the amount of α-subunit of hypoxia-induced factor in the cytoplasm of seminal vesicles by 132% (p = 0.01), in the tail of epididymis by 32% (p = 0.02) and reduction in mitochondria by 45% (p = 0.01) and 60% (p = 0,002), respectively, a decrease in succinate levels by 40% (p = 0.005) and 51% (p = 0.0009), an increase in the concentration of lactic acid in the cytoplasm by 194% (p = 0.03) and 253% (p = 0.018), a decrease in cytochrome oxidase activity from 0.96 [0.66; 1.69] RU/mg of protein to 0.27 [0.23; 0.32] (p = 0.0009) and from 1.04 [0.84; 1.33] to 0.26 [0.14; 0.37] (p = 0.003), relative to the control group. The observed changes are characteristic of the state of hypoxia and are explained by the cell switching over to glycolytic pathway of energy production, in contrast to mitochondrial pathway in normoxia. The combined effect of hypoxia and arginine partially enhanced each other's effects.

CONCLUSION: L-arginine causes hypoxia-like state in cells through activating α-subunit by hypoxia-induced factor, reducing cytochrome oxidase activity, increasing glycolysis, and also partially enhances the effects of chronic normobaric hypoxia.

Keywords: *L-arginine; hypoxia; mitochondria; seminal vesicles; epididymis; nitric oxide*

For citation:
Marsyanova YuA, Zvyagina VI. Hypoxia-Like Effect of L-Arginine in Seminal Vesicle and Epididymis of Rats. *I. P. Pavlov Russian Medical Biological Herald.* 2023;31(3):345–356. DOI: https://doi.org/10.17816/PAVLOVJ108589

Received: 06.06.2022 **Accepted:** 14.09.2022 **Published:** 30.09.2023

ECO•VECTOR

LIST OF ABBREVIATIONS

ASS — argininosuccinate synthetase
ATP — adenosine triphosphate
CF — cytoplasmic fraction
CNH — chronic normobaric hypoxia
COX — cytochrome-c-oxidase (цитохромоксидаза)
eNOS — endothelial NO-synthase
ETC — electron transport chain
HIF — hypoxia-inducible factor
iNOS — inducible NO-synthase
nNOS — neuronal NO-synthase
L-arg — L-arginine
LDH — lactate dehydrogenase
MF — mitochondrial fraction
NAD — nicotineamide-adenine-dinucleotide
NADH — nicotineamide-adenine-dinucleotide, reduced form
NOS — nitric oxide synthase
PHD — prolyl hydroxylase domain
ROS — reactive oxygen species
SDH — succinate dehydrogenase
VEGF — vascular endothelial growth factors

INTRODUCTION

The proteinogenic amino acid L-arginine (L-arg) is of great importance for an organism not only as a structural component of proteins, but also as a donor of nitrogen atoms in synthesis of polyamines, urea, and in production of ammonium salts, and also for synthesis of NO. The signaling role of nitric oxide (II) is no longer in doubt and is not limited to the regulation of vasodilation, which makes L-arginine an attractive object of study.

The key enzyme for the synthesis of L-Arg is argininosuccinate synthetase (ASS) [1], while the amino acid itself can be used as a substrate in the synthesis of NO by nitric oxide synthases (NOS), and be also degraded to urea with participation of arginase, here; both enzymes compete with each other [2]. NOS is represented by three isoforms: two Ca-dependent constitutive forms named according to the tissues where they were first found — endothelial NO-synthase (eNOS) and neuronal NO-synthase (nNOS), and also Ca-independent inducible NO-synthase (iNOS).

In the literature, the mutual influence of arginase and all the three NOS isoforms on each other was noted. A great number of studies in this field are devoted to participation of these enzymes in the development of endothelial dysfunction [3]. An increase in the synthesis of NO contributes to S-nitrosylation of arginase leading to its activation, and decreased level of the substrate — L-Arg [4]. Entry of exogenous L-Arg causes dose-dependent increase in the amount of nitric oxide (II) in blood and increases expression of eNOS [5] and iNOS [6]. A disorder in binding of calcium due to decrease in phosphorylation of eNOS by serine residue in position 1177 and increase in phosphorylation of threonine in 495, leads to reduction of the activity of this enzyme, which is observed in hypoxia. Here, enhanced expression of arginase is noted [7]. Thus, participation of L-Arg in saturation of tissues with oxygen may be a decisive factor due to the direct effect on synthesis of nitric oxide (II) in hypoxia. This effect has found use in the treatment of renal insufficiency, among other things, due to activation of signaling pathways of nitric oxide (II) [8], also of disorders of bone tissue [9], chronic obstructive pulmonary disease [10], chronic uteroplacental ischemia [11], and sexual dysfunction [12]. Evaluation of NO level in blood is an important diagnostic criterion for development of pathological processes, and also has a prognostic significance [13]. Increased production of NO in hypoxia can be a successful mechanism of adaptation not only from the point of view of enhancement of vasodilation, but also from positions of signaling, since, by activating guanylate cyclase, nitric oxide (II) induces synthesis of fetal hemoglobin which has a higher affinity to oxygen than adult hemoglobin.

The expected effect of increase in NO synthesis in response to introduction of L-Arg is refuted in the studies on the NB9 human neuroblastoma cells expressing nNOS [14]. It was shown in the experiment that in the presence of high amount of arginine, NO synthase switches over from production of NO to generation of reactive oxygen species (ROS), which can probably be explained by the acceleration of transition of nitric oxide (II) to peroxynitrite in these conditions, which is a potent oxidizer belonging to reactive nitrogen species. This phenomenon is considered as one of mechanisms of indirect toxic effect of the amino acid. There is evidence of neurotoxicity of nitric oxide (II) synthesized by nNOS and iNOS, in hypoxia [15–16].

In addition to the listed pathways of L-Arg metabolism, an important role in maintaining the pool of this amino acid is played by one of enzymes of ornithine ASS cycle whose expression is regulated by hypoxia-inducible factor (HIF): slowdown of transcription is associated with binding of HIF with the enhancer in promoter of ASS1 enzyme gene [1]. In mammals, HIF is represented by three forms: HIF1, HIF2, HIF3. All the three variants are active in the form of heterodimer consisting of α and β subunits. HIF1α is oxygen-sensitive HIF monomer which in conditions of normoxia undergoes hydroxylation and polyubiquitination by

prolyl hydroxylase domain (PHD) and von Hippel-Lindau protein, respectively, which leads to its proteasomal degradation. When this protein manages to avoid proteolysis, HIF1α associates with HIF1β, penetrates the nucleus and performs the function of the transcription factor. Among non-canonical, that is, non-hypoxic ways of preserving high HIF1α concentration, is increase in its nitrosylation [17] with simultaneous inhibition of PHD by nitric oxide (II) [18]. These mechanisms permit to suggest that introduction of exogenous L-Arg to animals in conditions of normoxia may contribute to switching of cell metabolism to anaerobic way of energy production causing pseudohypoxia. Another non-canonical way of HIF activation is associated with the deficit of succinate dehydrogenase (SDH) which leads to increase in the concentration of succinate that allosterically inhibits PHD [19].

In general, mitochondrial dysfunction can provoke an abnormal response to hypoxia. Thus, accumulation of pyruvate facilitates activation of HIF, however, buildup of reduced nicotine amide adenine dinucleotide (NAD) [20], the same as deficit of lactate, activates PHD, which leads to HIF degradation and development of pseudonormoxia — the state when in conditions of oxygen deficit, the adaptive response typical of hypoxia, does not develop. The stabilized transcription factor activates expression of genes encoding glycolysis enzymes including lactate dehydrogenase (LDH). It can be suggested that the activity of this enzyme in this case will play a decisive role in formation of adaptation, and lactate is assigned the signaling role of triggering these changes.

Besides, HIF participates in regulation of mitochondrial metabolism by activating the expression of the second type of IV subunit of cytochrome-c-oxidase (COX) which replaces Cox411 expressed in normoxia. Such replacement is accompanied by reduction of ROS production. In addition, reduction of ROS is promoted by complex II reversal of electron-transport chain (ETC) — succinate dehydrogenase which is also controlled by NO [21].

HIF-mediated transcriptional response to hypoxia occurs only after several hours. However, it is important that cells quickly respond to reduction of the level of oxygen and restructure metabolism in the first minutes of exposure to hypoxia. One of early response mechanisms is binding of HIF1α with proteins in the outer membrane of mitochondria, which provides control of membrane potential, synthesis of ATP, release of cytochrome out of the intermembrane space, finally reducing the risk of apoptosis [22].

The second strategy of adaptation to hypoxia is directed not to intracellular restructure, but to improving the supply of oxygen to tissues through enhanced vasodilation and angiogenesis, which is promoted by HIF through activation of iNOS expression and vascular endothelial growth factors (VEGF).

Impairment of the supply of energy to the cell in hypoxia and of reoxygenation underlie the pathogenesis of various conditions, in particular, male infertility. L-Arg is a part of drugs used to solve the problem of sexual dysfunction [23]. The effects of this amino acid are mediated both through participation in protein synthesis and by the action of the produced NO: increased blood flow to the male genitals and activation of spermatogenesis, normalization of erection, antioxidant protection. It should be taken into account that not all tissues need increased oxygen supply for proper functioning. Thus, the epididymis where accumulation, maturation and storage of spermatozoa occur, is referred to tissues with low sensitivity to hypoxia [24], which prevents early activation of germ cells.

Thus, L-Arg can influence changes in cell metabolism, facilitating the development of a hypoxia-like response. Among the totality of factors about the influence of L-Arg, it seems interesting to investigate its effects on cellular metabolism and mitochondrial function in hypoxic conditions *in vivo*.

The **aim** of this study to evaluate the participation of L-arginine in the development of an adaptive response to chronic normobaric hypoxia in the tissues of the male reproductive system of rats and to study its effect on changes in metabolism in normoxia.

MATERIALS AND METHODS

Working with animals. The study with participation of animals was approved at a meeting of the Commission for the Control of the Management and Use of Laboratory Animals (Protocol No. 16, 2018), the work was performed in accordance with the 'European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes' (Strasbourg, 1986), the Order of the Ministry of Health of the Russian Federation of April 1, 2016 No. 199n 'On Approval of the Rules of Good laboratory Practice' and the Order of the Ministry of Health of the USSR of August 12, 1977 No. 755 'On Measures for Further Improvement of Organizational Forms of Work Using Experimental Animals'. The animals were kept in standard conditions.

The experiment was performed on 40 sexually mature Wistar male rats of 200g–280 g weight, which were divided to 5 groups (n = 8):

- ***group 1*** (normoxia) — control for group 2;
- ***group 2*** (chronic normobaric hypoxia, CNH) — modeling of CNH;
- ***group 3*** (NaCl) — control for group 4;
- ***group 4*** (L-Apr) — introduction of exogenous L-arginine;
- ***group 5*** (CNH + L-Arg) — introduction of exogenous L-arginine in conditions of CNH; comparison

groups for this series of experiment were groups 2 and 4.

Modeling of CNH. To model CNH, a hermetic chamber of 1.2 L volume was used (length × width × height: 10.5 × 10.5 × 11.0 cm) connected to МАГ-6-П-К multi-channel gas analyzer (O2: measurement interval 0%–100%; CO2: measurement interval 0%–12%), so that the chamber and gas analyzer formed a closed space. Duration of staying of animals in the hermetic chamber was determined not by time periods, but individually for each animal on the basis of the reading on the gas analyzer, the experiment lasted until the level of oxygen in the chamber decreased to 10%. The manipulations were repeated daily once a day in the morning hours for 14 days. During the experiment, the control group of animals was placed in the ventilated chamber [25].

Introduction of L-arginine. The animals received exogenous L-Arg (98.5%; Диаэм, Russia) on the basis of 500 mg of L-Arg per kg of body mass as 20% solution in 0.9% NaCl during 10 days; injections were made intraperitoneally using a disposable insulin syringe once daily in the morning. The control group of animals was injected with 0.9% NaCl.

The animals exposed to CNH who were administered injections of exogenous L-Arg, received the drug from day 5 to day 14 of the experiment for modeling hypoxia, 30 minutes before the session.

Obtaining the biomaterial. On the 14th day of the experiment, immediately after the end of the procedures, the rats were subjected to anesthesia (a mixture of Zoletil® 100 and Xylanit® at a dose of 6 mg/kg of body mass with a disposable insulin syringe intramuscularly). Then, seminal vesicles, the head and tail of the epididymis were taken, from which homogenates were obtained using Potter S homogenizer (Sartorius AG, Germany) in a Tris-HCl buffer pH = 7.4 containing 0.25 M sucrose solution, in the ratio of 1 part of tissue (mg) to 9 parts of buffer (ml). All procedures were performed at the temperature no higher than 4°C. The obtained homogenates were centrifuged twice: for 15 minutes at 3,000 g — for precipitation of nuclei and undamaged cells and at 14,000 g — for precipitation of mitochondria. The study material was mitochondria-free cytoplasmic fraction (CF) and mitochondrial precipitate resuspended in the isolation medium — the mitochondrial fraction (MF) — with addition of Triton-X100 detergent.

Methods of laboratory analysis. The concentration of NO metabolites was determined by the intensity of pink coloring in the reaction of azocoupling of naphthylethylenediamine with diazotized sulfanilamide (Griess reagent; NevaReactiv, Russia) which is formed in the presence of nitrites, the coloring intensity depends on the amount of nitrites and nitrates reduced to nitrites using VCl_3 (Acros Organics, USA); the results were expressed in nmol/mg of protein.

The concentration of succinate was determined using Succinate Colorimetric Assay Kit (Sigma-Aldrich Co, США) by the intensity of coloring formed in the coupled reaction succinatoxidase/peroxidase, with the results expressed in μmol/mg of protein.

The amount of HIF1α was determined using Hypoxia Inducible Factor 1 Alpha kit for immune enzyme assay (Cloud-Clone Corp., USA), the results were expressed in ng/mg of protein.

The above parameters were measured in a 96-well microplate on Stat Fax 3200 plate photometer (Awareness Technology Inc., USA).

The concentration of lactic acid was determined using Lactic Acid-Olveks kit (Olveks Diagnosticum, Russia) by intensity of coloring in the coupled reaction lactate oxidase/peroxidase, with the results expressed in μmol/mg of protein.

The activity of lactate dehydrogenase was determined using LDH kit (Olveks Diagnosticum, Russia) by kinetic method by reaction of reduction of pyruvate to lactate and oxidation of NADH to NAD+. The speed of oxidation of NADH to NAD+ was proportional to LDH activity. The activity of enzyme was expressed in RU/mg of protein

The activity of cytochrome-c-oxidase (COX) was determined using kinetic method by the decrement of the optical density of the reduced cytochrome C solution in the reaction of the cytochrome oxidation with oxygen in the presence of the enzyme, the reaction rate proportional to the activity of the enzyme, was expressed in RU/mg of protein.

The above parameters were determined by photometric method using Stat Fax 1904+ biochemical analyzer (Awareness Technology Inc., USA).

The activity of succinate dehydrogenase (SDH) was determined by the decrement of the optical density of potassium ferricyanide solution in the reaction of reduction in the presence of the enzyme, the of reaction rate proportional to the activity of the enzyme, was determined photometrically using SF-2000 spectrophotometer (OKB Spectr, Russia), and expressed in nmol of succinate/mg of protein.

The content of the total protein was determined photometrically (CFK-3-01-ZOMZ photocolorimeter, Russia) by Lowry method using CliniTest-BL kit (ECO Service, Russia).

Statistical data processing was performed using Excel 2013 (Microsoft, USA) and Statistica 12.0 (Stat Soft Inc., USA). The type of the distribution was determined using Shapiro–Wilk test. The nonparametric Mann–Whitney U-test was used to assess the significance level in the groups. The results are presented as Median [Quartile 1; Quartile 3] — Me [Q1; Q3]. The level of differences in comparison of two independent samples was considered statistically significant with error

probability $p < 0.05$, in comparison of three independent samples, Bonferroni correction was applied and the level of differences was considered statistically significant with error probability $p < 0.0167$. At $0.05 \le p < 0.10$, a tendency to change in parameters was considered probable.

RESULTS

In modeling of CNH, there was a decrease in the activity of COX, LDH MF in tissues of seminal vesicles, head and tail of the epididymis, and in the content of lactate MF in seminal vesicles and head of the epididymis, in the concentration of NO metabolites and in the activity of LDH CF in the tail of the epididymis, and decrease in the content of NO metabolites and increase in the lactic acid CF in seminal vesicles (Table 1).

In the experiment with introduction of exogenous L-Arg to the animals, there was a decrease in the amount of HIF1α, content of nitric acid (II) metabolites and succinate and COX activity in mitochondria of

Table 1. Changes in Biochemical Parameters of Seminal Vesicles, Head and Tail of Epididymis of Rats in CNH (n = 8; Me [Q1; Q3])

Parameter	Group 1 (Normoxia)	Group 2 (CNH)	p
SEMINAL VESSELS			
Nitric oxide (II) metabolites, nmol/mg of protein	974 [906; 1064]	717 [628; 784]	***0.003***
HIF1α MF, ng/mg of protein	25.2 [20.6; 35.2]	14.8 [11.6; 20]	***0.024***
HIF1α CF, ng/mg of protein	4.4 [3.9; 5]	3 [2.9; 4.3]	0.16
Succinate, μmol/mg of protein	1123 [989; 1453]	828 [617; 957]	***0.007***
SDH, nmol of succinate/mg of protein	0.32 [0.02; 0.41]	0.32 [0.24; 0.46]	0.71
Lactic acid MF, μmol/mg of protein	5.87 [4.62; 6.92]	1.76 [1.41; 1.95]	***0.01***
Lactic acid CF, μmol/mg of protein	0.75 [0.62; 0.85]	1.66 [1.2; 1.99]	***0.008***
LDH MF, RU/mg of protein	798 [602; 1146]	1125 [1003; 1379]	0.21
LDH CF, RU/mg of protein	3061 [2211; 3907]	1272 [1224; 2550]	0.13
COX, RU/mg of protein	1.34 [1.14; 1.72]	0.31 [0.25; 0.41]	***0.0005***
HEAD OF EPIDIDYMIS			
Nitric oxide (II) metabolites, nmol/mg of protein	1023 [900; 1544]	897 [667; 1046]	0.13
HIF1α MF, ng/mg of protein	14 [12; 20.5]	12.2 [10; 16.8]	0.27
HIF1α CF, ng/mg of protein	4 [3.9; 4.4]	3.9 [2.8; 4.1]	0.43
Succinate, μmol/mg of protein	1202 [1092; 1868]	838 [660; 928]	***0.0039***
SDH, nmol of succinate/mg of protein	0.35 [0.1; 0.52]	0.4 [0.28; 0.67]	0.49
Lactic acid MF, μmol/mg of protein	5.65 [4.38; 7.47]	2.65 [2.23; 3.03]	***0.0009***
Lactic acid CF, μmol/mg of protein	1.71 [1.19; 1.93]	1.94 [1.66; 2.2]	0.24
LDH MF, RU/mg of protein	3383 [1659; 4088]	804 [709; 977]	***0.001***
LDH CF, RU/mg of protein	5799 [5431; 6590]	3088 [2247; 3724]	***0.00009***
COX, RU/mg of protein	0.89 [0.64; 1.05]	0.35 [0.26; 0.49]	***0.007***
TAIL OF EPIDIDYMIS			
Nitric oxide (II) metabolites, nmol/mg of protein	1055 [902; 1207]	825 [766; 868]	***0.01***
HIF1α MF, ng/mg of protein	20.5 [19; 23]	12.8 [11.1; 13.7]	***0.01***
HIF1α CF, ng/mg of protein	2.5 [2.3; 3]	4.3 [3.9; 4.6]	***0.014***
Succinate, μmol/mg of protein	1355 [1240; 1608]	958 [760; 1078]	***0.03***
SDH, nmol of succinate/mg of protein	0.22 [0.18; 0.67]	0.26 [0.19; 0.63]	0.96
Lactic acid MF, μmol/mg of protein	6.25 [4.84; 8.12]	4.38 [2.75; 6.55]	0.48
Lactic acid CF, μmol/mg of protein	1.18 [0.82; 1.6]	1.6 [1.58; 1.65]	0.16
LDH MF, RU/mg of protein	2732 [1592; 3140]	2882 [2394; 3529]	0.37
LDH CF, RU/mg of protein	7158 [5684; 7359]	3532 [2810; 4327]	***0.018***
COX, RU/mg of protein	1.23 [0.91; 2.21]	0.54 [0.36; 0.64]	***0.03***

Notes: L-Arg — L-arginine; CNH — chronic normobaric hypoxia; CF — cytoplasmic fraction; MF — mitochondrial fraction; SDH — succinate dehydrogenase; LDH — lactate dehydrogenase; COX — cytochrome-c-oxidase, HIF — hypoxia-inducible factor

all the examined tissues; reduction of the level of lactic acid and LDH activity in seminal vesicles, reduction of lactate with simultaneous increase in LDH activity in the mitochondria of the epididymis tail (Table 2). In the cytoplasm of the studied tissue, there was increase in the following parameters: lactic acid in the seminal vessels, head and tail of the epididymis, HIF1α in the seminal vessels and tail of the epididymis, LDH activity only in the tail of the epididymis.

Table 2. Effect of Arginine on Changes of Biochemical Parameters of Seminal Vesicles, Head and Tail of Epididymis of Rats in Chronic Normobaric Hypoxia (n = 8; Me [Q1; Q3])

Parameter	Group 3 (NaCl)	Group 4 (L-Arg)	Group 5 (CNH + L-Arg)
SEMINAL VESICLES			
NO (II) metabolites, nmol/mg of proteins	792 [703; 911]	650 [578; 713] ***p_{3-4} = 0.03***	734 [601; 1007] p_{2-5} = 0.71 p_{4-5} = 0.43
HIF1α MF, ng/mg of protein	23.6 [20.4; 30.4]	13 [9.6; 17.7] ***p_{3-4} = 0.01***	10.5 [8.9; 13] p_{2-5} = 0.16 p_{4-5} = 0.16
HIF1α CF, ng/mg of protein	3.1 [2.9; 4.5]	7.2 [5; 8.2] ***p_{3-4} = 0.01***	5.3 [4.7; 6.2] ***p_{2-5} = 0.007*** p_{4-5} = 0.43
Succinate, μmol/mg of protein	985 [847; 1376]	594 [533; 655] ***p_{3-4} = 0.005***	644 [624; 690] p_{2-5} = 0.49 p_{4-5} = 0.23
SDH, nmol of succinate/mg of protein	0.25 [0.12; 0.36]	0.26 [0.15; 0.36] p_{3-4} = 0.96	0.52 [0.33; 0.64] p_{2-5} = 0.13 p_{4-5} = 0.04
Lactic acid MF, μmol/mg of protein	4.76 [3.96; 7.76]	2.59 [2.27; 2.99] ***p_{3-4} = 0.007***	4.8 [4.38; 5.69] ***p_{2-5} = 0.007*** ***p_{4-5} = 0.005***
Lactic acid CF, μmol/mg of protein	0.94 [0.61; 1.86]	2.76 [1.6; 3.66] ***p_{3-4} = 0.03***	2.49 [1.99; 3.07] p_{2-5} = 0.052 p_{4-5} = 0.56
LDH MF, RU/mg of protein	1440 [741; 2373]	565 [384; 783] ***p_{3-4} = 0.04***	625 [426; 1040] p_{2-5} = 0.19 p_{4-5} = 1.0
LDH CF, RU/mg of protein	4071 [2823; 5028]	3542 [1909; 5909] p_{3-4} = 0.75	3448 [2935; 3970] ***p_{2-5} = 0.014*** p_{4-5} = 0.96
COX, RU/mg of protein	0.96 [0.66; 1.69]	0.27 [0.23; 0.32] ***p_{3-4} = 0.0009***	0.46 [0.35; 0.66] p_{2-5} = 0.23 p_{4-5} = 0.07
HEAD OF EPIDIDYMIS			
NO (II) metabolites, nmol/mg of proteins	999 [876; 1225]	651 [609; 701] ***p_{3-4} = 0.001***	1043 [910; 1576] p_{2-5} = 0.13 ***p_{4-5} = 0.0009***
HIF1α MF, ng/mg of protein	12.4 [10.6; 15.5]	5.9 [5.6; 7.4] ***p_{3-4} = 0.002***	16.2 [15.5; 17.1] p_{2-5} = 0.16 ***p_{4-5} = 0.0009***
HIF1α CF, ng/ mg of protein	3.2 [2.4; 3.3]	2.7 [2.3; 3.1] p_{3-4} = 0.32	3.8 [3.5; 4.8] p_{2-5} = 0.71 ***p_{4-5} = 0.007***
Succinate, μmol/mg of protein	1140 [928; 1456]	539 [448; 599] ***p_{3-4} = 0.0009***	1295 [1276; 1459] ***p_{2-5} = 0.0009*** ***p_{4-5} = 0.0009***
SDH, nmol succinate/mg protein	0.43 [0.23; 0.55]	0.48 [0.38; 0.58] p_{3-4} = 0.43	0.36 [0.33; 0.40] p_{2-5} = 0.1 P_{4-5} = 0.56
Lactic acid MF, μmol/mg of protein	4.47 [3.84; 5.92]	2.72 [1.71; 5.36] p_{3-4} = 0.16	12.1 [10.86; 13.4] ***p_{2-5} = 0.0009*** ***p_{4-5} = 0.003***
Lactic acid CF, μmol/mg of protein	1.4 [0.96; 1.83]	2.61 [2.41; 2.79] ***p_{3-4} = 0.001***	2.75 [2.42; 3.86] p_{2-5} = 0.43 p_{4-5} = 0.03

Continuation of the table 2

LDH MF, RU/mg of protein	1175 [720; 1839]	1741 [1438; 2627] $p_{3-4} = 0.27$	2466 [1328; 3284] **$p_{2-5} = 0.004$** $p_{4-5} = 0.71$
LDH CF, RU/mg of protein	3765 [3294; 4511]	4607 [4010; 5515] $p_{3-4} = 0.23$	4088 [3247; 4775] $p_{2-5} = 0.16$ $p_{4-5} = 0.27$
COX, RU/mg of potein	0.9 [0.81; 1.05]	0.29 [0.21; 0.34] **$p_{3-4} = 0.00002$**	0.70 [0.47; 1.19] $p_{2-5} = 0.04$ **$p_{4-5} = 0.005$**
TAIL OF EPIDIDYMIS			
NO (II) metabolites, nmol/mg of proteins	1239 [881; 1568]	520 [476; 568] **$p_{3-4} = 0.002$**	712 [665; 846] $p_{2-5} = 0.23$ $p_{4-5} = 0.01$
HIF1α MF, ng/mg of protein	22 [17; 24.3]	8.8 [7.8; 10.5] **$p_{3-4} = 0.002$**	12.3 [9.8; 14.3] $p_{2-5} = 0.31$ $p_{4-5} = 0.08$
HIF1α CF, ng/mg of protein	3.1 [2.7; 3.9]	4.1 [3.9; 4.7] **$p_{3-4} = 0.02$**	6.2 [4.7; 6.4] $p_{2-5} = 0.13$ $p_{4-5} = 0.1$
Succinate, μmol/mg of protein	1606 [1211; 2006]	794 [682; 867] **$p_{3-4} = 0.0009$**	1161 [895; 1491] $p_{2-5} = 0.23$ **$p_{4-5} = 0.007$**
SDH, nmol of succinate/mg of protein	0.24 [0.15; 0.5]	0.19 [0.11; 0.22] $p_{3-4} = 0.31$	0.3 [0.26; 0.35] $p_{2-5} = 0.71$ **$p_{4-5} = 0.013$**
Lactic acid MF, μmol/mg of protein	6.76 [4.96; 8.77]	3.07 [2.69; 5.1] **$p_{3-4} = 0.03$**	7.63 [5.42; 10.09] $p_{2-5} = 0.16$ $p_{4-5} = 0.03$
Lactic acid CF, μmol/mg of protein	1.12 [0.77; 1.49]	3.95 [1.95; 4.63] **$p_{3-4} = 0.018$**	2.35 [1.85; 2.99] **$p_{2-5} = 0.014$** $p_{4-5} = 0.43$
LDH MF, RU/mg of protein	1685 [902; 2556]	3456 [2993; 3895] **$p_{3-4} = 0.046$**	3475 [2331; 5748] $p_{2-5} = 0.79$ $p_{4-5} = 0.79$
LDH CF, RU/mg of protein	5289 [4088; 5845]	8375 [7407; 10510] **$p_{3-4} = 0.005$**	6507 [4636; 8196] **$p_{2-5} = 0.08$** $p_{4-5} = 0.16$
COX, RU/mg of protein	1.04 [0.84; 1.33]	0.26 [0.14; 0.37] **$p_{3-4} = 0.003$**	0.16 [0.1; 0.22] **$p_{2-5} = 0.001$** $p_{4-5} = 0.27$

Notes: L-Arg — L-arginine; LDH — lactate dehydrogenase; MF — mitochondrial fraction; SDH — succinate dehydrogenase; CNH — chronic normobaric hypoxia; COX — cytochrome oxidase; CF — cytoplasmic fraction

In correction of hypoxia with exogenous L-Arg, a buildup in the concentration of lactate in mitochondria of seminal vesicles was noted in comparison with CNH, and in the amount of HIF1α and activity of LDH in the cytoplasm; increase in the level of succinate, lactic acid and activity of LDH in mitochondria of the head of epididymis; reduction of COX activity in the mitochondria of the tail of epididymis, and increase in the content of lactate and activity of LDH in the cytoplasm. The combined effect of factors (CNH + L-Arg) compared with the action of L-Arg alone led to increase in the content of lactate in mitochondria of seminal vesicles; in NO metabolites and succinate — in both parts of the epididymis; in the amount of HIF1α (MF and CF), lactic acid and COX activity — in the head of the epididymis; in the activity of SDH In mitochondria of the tail of epididymis.

DISCUSSION

Some changes observed in the conditions of introduction of exogenous L-Arg, have a tendency similar to the model of CNH. In particular, this can be traced in

the accumulation of lactic acid [26], HIF1α [27] and in the activity of COX [25]. Increase in the amount of HIF1α on exposure to different substances even at normal oxygen tension, is characterized as a state of pseudohypoxia. The transcription activity of HIF1α contributes to further transformations of metabolism, which explains the character of changes in the concentration of lactate and activity of COX. In other words, it can be said that L-Arg exhibits hypoxia-like effect.

The effect of L-Arg on the stabilization of HIF1α explains the similarity of the metabolic response in this experimental model with the response in CNH. However, here, tissue specificity of reaction is observed. Thus, in seminal vesicles, L-Arg provided an almost complete similarity to the changes characteristic of hypoxia (an increase in the concentration of lactate, in the amount of HIF1α and LDH activity in the cytoplasm, decrease in COX activity), while in the experiment with exogenous introduction of L-Arg to animals with the underlying CNH, HIF1α accumulated in the cytoplasm more effectively than in hypoxia alone. The combined influence of these factors probably produced some cumulative effect, which was a cause for increase in LDH activity in the cytoplasm of seminal vesicle cells relative to condition of hypoxia, and for accumulation of lactate in mitochondria relative to both CNH and L-Arg. Presumably, these changes are based on HIF-mediated activation of LDH expression. The observed decrease in the content of HIF1α in MF in hypoxia or when receiving L-Arg, can be associated with an increase in its transcriptional activity caused by the systematic exposure to these factors. We assume that during the experiment it was possible to achieve a stable transcriptional response, which reduces the significance of the need to develop acute response by direct binding of HIF1α with proteins in the outer membrane of mitochondria.

The absence of statistically significant changes in the activity of SDH, with simultaneous depletion of succinate pool and accumulation of lactate, indicates switching of the function of electron transport chain (ETC) as the main supplier of protons in conditions of normoxia from complex I to complex II. In this way a cell tries to compensate for ineffective in the discussed conditions oxidation of coenzyme NADH by reducing pyruvate to lactate to support glycolysis and citrate cycle, and the work of ETC is supported through oxidation mainly of succinate. With this, production of L-Arg did not influence the activity of ATP-synthase.

The effect of administered L-Arg in the epididymis was similar to that in seminal vesicles. A distinctive feature was accumulation of lactate in the cytoplasm of tissues and increase in the activity of LDH in the tail of the epididymis. In our earlier studies, we described the differences between the functional areas of the epididymis in accumulation of lactate and HIF1α [25–27] in conditions of CNH. Administration of exogenous L-Arg demonstrates the opposite effects: increase in the activity in the tail in the absence of changes in the head of the epididymis. Interestingly, in animals of CNH + L-Arg group, increased COX activity was noted in the head of epididymis, with the lowest activity of the enzyme in the tail. Increased activity of SDH with accumulation of succinate in this case can evidence restructure of the work of ETC: in the absence of the effective electron transport through cytochrome-c-oxidase, the final acceptor may be fumarate, which leads to increase in the concentration of succinate, as well as to enhancement of its signaling function and reduction of ROS production. It is known that even in conditions of normoxia, tissues are differently saturated with oxygen, and the tail of the epididymis is characterized by low oxygen content and by a more active expression of HIF1α [24], which permits to make an assumption about the influence of exogenous arginine on cells of the tail of epididymis in hypoxia, as a potent stimulus for signaling and switching of metabolism to production of energy by glycolysis.

Decline in the concentration of nitric oxide (II) metabolites in hypoxia can be explained by a probable HIF1-induced slowdown of ASS expression which is responsible for biosynthesis of arginine in the majority of body tissues [2], which leads to reduction of the bioavailability of this amino acid as a substrate for synthesis of NO. Course administration of exogenous L-Arg at a dose of 500 mg/kg of body mass did not lead to the expected increase in NO in tissues at the time of completion of the experiment. This phenomenon can be explained, on the one hand, by hypoxia-like effect of L-Arg, and on the other hand, by the activation of arginase through nitrosylation [4], which in combination led to reduction of the level of NO metabolites. This mechanism probably underlies adaptation of tissues and their protection from excess nitric oxide (II).

CONCLUSION

Parenteral administration of L-arginine to animals induces development of pseudohypoxia in the cells of seminal vesicles and tail of the epididymis, which partially enhances the effects observed in chronic normobaric hypoxia. In the head of the epididymis, this regularity is less pronounced. Being a precursor to nitric oxide (II), L-arginine promotes reduction of the activity of cytochrome-c-oxidase in all the studied tissues and increases the role of glycolysis in the epididymis, as indicated by increase in the activity of lactate dehydrogenase and accumulation of lactate.

The changes are especially evident in the caudal part of the epididymis, where reversal of the work of succinate dehydrogenase is as well observed, which improves protection of the cell in chronic normobaric hypoxia. Thus, L-arginine exhibits a hypoxia-like effect

through regulating the activity of hypoxia-induced factor and enzymes involved in energy metabolism, which is the reason for exogenous administration of this amino acid permits to control the processes of adaptation to oxygen deficit.

ADDITIONALLY

Funding. This study was not supported by any external sources of funding.
Conflict of interests. The authors declare no conflicts of interests.
Contribution of the authors: *Yu. A. Marsyanova* — carrying out the main stages of the study, collecting and processing material, statistical processing, analysis and interpretation of data, writing the text of the article; *V. I. Zvyagina* — development of the concept and design of the study, evaluation of data interpretation, review and editing of the article. The authors confirm the correspondence of their authorship to the ICMJE International Criteria. All authors made a substantial contribution to the conception of the work, acquisition, analysis, interpretation of data for the work, drafting and revising the work, final approval of the version to be published and agree to be accountable for all aspects of the work.

Финансирование. Авторы заявляют об отсутствии внешнего финансирования при проведении исследования.
Конфликт интересов. Авторы заявляют об отсутствии конфликта интересов.
Вклад авторов: *Марсянова Ю. А.* — проведение основных этапов исследования, сбор и обработка материала, статистическая обработка, анализ и интерпретация данных, написание текста статьи; *Звягина В. И.* — разработка концепции и дизайна исследования, оценка интерпретации данных, проверка и редактирование статьи. Авторы подтверждают соответствие своего авторства международным критериям ICMJE (все авторы внесли существенный вклад в разработку концепции и подготовку статьи, прочли и одобрили финальную версию перед публикацией).

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ОБ АВТОРАХ

***Марсянова Юлия Александровна**;
ORCID: https://orcid.org/0000-0003-4948-4504;
eLibrary SPIN: 4075-3169; e-mail: yuliyamarsyanova@yahoo.com

Звягина Валентина Ивановна, к.б.н., доцент;
ORCID: https://orcid.org/0000-0003-2800-5789;
eLibrary SPIN: 7553-8641; e-mail: vizvyagina@yandex.ru

AUTHOR'S INFO

***Yuliya A. Marsyanova**;
ORCID: https://orcid.org/0000-0003-4948-4504;
eLibrary SPIN: 4075-3169; e-mail: yuliyamarsyanova@yahoo.com

Valentina I. Zvyagina, Cand. Sci. (Biol.), Associate Professor;
ORCID: https://orcid.org/0000-0003-2800-5789;
eLibrary SPIN: 7553-8641; e-mail: vizvyagina@yandex.ru

* Автор, ответственный за переписку / Corresponding author