National Academy of Sciences of RA
Electronic Journal of
NATURAL SCIENCES

ՀՀ Գիտությունների Ազգային Ակադեմիա
Էլեկտրոնային ամսագիր
ԲՆԱԿԱՆ ԳԻՏՈՒԹՅՈՒՆՆԵՐ

Национальная Академия наук РА
Электронный журнал
ЕСТЕСТВЕННЫЕ НАУКИ

1(24), 2015

Biochemistry *Կենսաքիմիա* *Биохимия*

RELATIONSHIP BETWEEN LIVER POLYAMINES QUANTITY AND ARGINASE ISOENZYMES ACTIVITY AFTER ARGINASE INHIBITION BY N^G-HYDROXY-*L*-ARGININE

N. V. Avtandilyan, S. A. Karapetyan

Department of Biochemistry, Faculty of Biology, YSU, Armenia;
e-mail: nikolay.avtandilyan@gmail.com; tel: (+374) 93 789 267

ABSTRACT

The aim of the work is to perform the evaluation of the change of the polyamine rate after the inhibition of nonureotelic arginase activity through N^G-hydroxy-*L*-arginine. For the first time the change of the polyamine rate in the liver of the rats has been explained after the nonureotelic arginase activity inhibition. It has been discovered that in the cell, paralelly with the process of the inhibition of nonureotelic arginase activity, the polyamine rate is decreasing as well. The given research is indicating that the novel role of nonureotelic arginase is the participation in the regulation of biosynthesis of polyamines.

Key words: *polyamines, arginase, thin layer chromatography, N^G-hydroxy-L-arginine*

INTRODUCTION

Polyamines are essential components of the cell, and have wide range of influence [12]. The precursor of polyamines is L-ornithine, which is synthesized from L-arginine by arginase [14]. Ornithinedecarboxylase catalyzes the synthesis of putrescine from L-ornithine, after what this polyamine turns into spermidine, than into spermine by spermidine and spermine synthases respectively [10, 13]. It is not clear which isoform of arginases take part in the biosynthesis of polyamines.

There are two isoforms of arginases. One of them is ureotelic arginase, what is found in ureotelic organisms and is the last enzyme of urea cycle. Second is nonureotelic arginase (NUA), which have wide biological distribution, exists almost in all biological tissues of different organisms, do not participate in ammonia neutralization and has a unique role in cell metabolism [6, 8].

Currently is necessary to clarify the nature of arginase (ureotelic or nonureotelic) that takes part in the biosynthesis of polyamines [4, 5]. It has not been revealed the isoform of arginase which provide L-ornithine for the biosynthesis of polyamines [11, 15]. We assume that the necessary L-ornithine for the biosynthesis of polyamines is provided by nonureotelic isoform of arginase. N^G-hydroxy-*L*-arginine (NOHA) is an intermediate product of NOS reaction,which catalyze the generation of NO and citrulline from L-arginine [7]. It was shown that NOHA inhibits only nonureotelic arginase [1]. That is why it was used to evaluate the changes of polyamine quantity during the changes of nonureotelic arginase activity.

MATERIALS AND METHODS

Animals. Male adult white rats (30-36 week old, 200-220 *g*) were used for the experiment. The animals were killed under ether anesthesia followed by decapitation.

Chemicals. Spermine tetrahydrochloride, putrescine di-hydrochloride, spermidine trihydrochloride; 2,3-butanedione monoxime were obtained from Sigma Aldrich Co. Ltd. (Taufkirchen, Germany).

Thin layer chromatography (TLC) of polyamines. The method of Seiler (1970) was used with some modifications. Tissues were extracted in 0.2 *M* cold $HC1O_4$ at a ratio of about 100 *mg/ml* $HC1O_4$. After extraction for 1 h in an ice bath, samples were pelleted at 15.000 *g* x 20 min in +4°*C*. Two hundred μl of $HClO_4$ extract were mixed with 400 μl of dansyl chloride (5 *mg/ml* in acetone) and 200 μl of saturated sodium carbonate were added. Excess dansyl reagent (5-(Dimethylamino) naphthalene-1-sulfonyl chloride) was removed by reaction with 100 μl (100 *mg/ml*) of added proline and incubation for 30 min. Up to 50 μl of dansylated extract were loaded on the pre-adsorbent zone of silica gel plates (PTLC-AF-V, silica gel CSCG, Russian), and the chromatogram was developed with chloroform:triethylamine (25:2, v/v) solvent system. The R_F values were calculated using the formula, R_F = distance traveled by solute spot/distance traveled by solvent front. After TLC, the dansylpolyamine bands were scraped, eluted in 2 *ml* ethyl acetate, and quantified in 505 *nm* (Genesys 10, USA) [5].

Separation and purification of arginase isoenzymes. The tissues homogenized in ice-cold 0,2 *M* Glycine buffer, pH 9,5; by using Potter-Elvehjem Glass Tissue Homogenizer. The homogenate was centrifuged at 1500 *g* for 30 min at 4°*C*. In the column (2,5×50 *cm*) containing Sephadex G-150 are added the crude extracts of tissues (10%). The column was balanced with K-phosphate buffer (pH 7,2) and was collected 40 fractions each one of for 4 ml [3, 4]. 4 *ml* of high-molecular-weight protein fraction after gel-filtration is passed through the column CM-cellulose (1.5x35 *cm*), balanced against 0.005 *M* Tris-HCl buffer, in pH 7.2, elution gradiented with the same buffer gradual increasing of molarity from 0 to 0.25 *M* KCl, elution speed is 24 *ml*/h, was collected 32 fractions each one of for 4 *ml*. After that in each fraction was determined arginase activity.

Colorimetric detrmination of arginase activity. In test-tube is added 1.5 *ml* glycine buffer, 0.5 *ml* supernatant, 0.2 *ml* $MnCl_2$ x $4H_2O$; 0.4 *ml* L-arginine. The control tube should contain the same, except of the 0.4 *ml* L-arginine. Enzyme catalysis interrupted with 1 *ml* 20% trichloroacetic acid. In sediment is determined the urea with Archibald's method [1]. N^G-hydroxy-L-arginine was added to arginase incubation mixture in 1 *ml* 0,2 *μM* quantity [7].

Statistical Analysis. Results expressed as means ± SD and means ± SE. Results examined by Student's *t*-test (single sample) using Statistica software (StatSoft 10.0).

RESULTS AND DISSCUSION

The aim of our work was to investigate the change of polyamines quantity in normal conditions and after the inhibition of NUA liver by NOHA.

After gel-filtration with Sephadex G-150 we have protein and arginase activity spectrums in two apexes in 13th and 25th fractions. 13th fraction contains ureotelic and 25th fraction nonureotelic isoform of arginase (nonureotelic arginase 1, NUA1). In previous studies were shown, that high-molecular fraction of liver during ion-exchange chromatography with CM-cellulose is divided into two apexes. First peak has low enzymatic activity, while second has high [1, 4, 5]. According to their physic-chemical properties (Km, effect of Mn^{2+}, molecular weight, intracellular localization, hormonal induction) the latter appears to be ureotelic and the first is nonureotelic isoform of arginase. Based on this, after gel-filtration, also was performed fractionation of high-molecular isoform of liver arginase with CM-cellulose.

After ion-exchange chromatography we have two apexes of arginase in 8th and 21th fractions. 8th fraction contains nonureotelic isoform (nonureotelic arginase 2) and 21th contains ureotelic isoform of arginase [2]. That is the reason that effect of NOHA on arginases activity and polyamines quantity were revealed using the mentioned fractions. After incubation (the influence of NOHA) one part of the mixture was used for arginase activity determination and the other part for the quantitative analysis of polyamines. 1 *ml* N^G-hydroxy-L-arginine was added to arginase incubation mixture in 0,2 *μM* quantity. Nonureotelic arginase 1 (NUA1) of liver is inhibited by 8.5%, NUA 2 by 27.48%, and UA by 1.3% when incubation mixture is added 0.2 *μM* of NOHA (*Fig. 1*). The latter shows that UA is not inhibited by NOHA, or the inhibition is insignificant, what can be the reason of partial purification of the enzyme. Further studies were directed to the monitoring of polyamines quantity change during the inhibition of nonureotelic arginase. In the fractions (normal conditions and after inhibition by NOHA) obtained after gel-filtration and ion-exchange chromatography was determined the quantity of polyamines with thin-layer chromatography (TLC).

It was shown that the quantity of polyamines is decreased during the inhibition of both isoforms of nonureotelic arginases (*Table 1*, *Fig. 2*). The latter let us conclude, that the first enzyme of the synthesis of polyamines (ornithinedecarboxylase) is deprived of ornithine, what in its turn brings to the quantity decrease of putrescine, spermine and spermidine.

The results confirmed that the required source of endogenous ornithine for the synthesis of polyamines in liver is the L-ornithine synthesized by nonureotelic arginase. A slight deviation is observed from liver NUA2. When

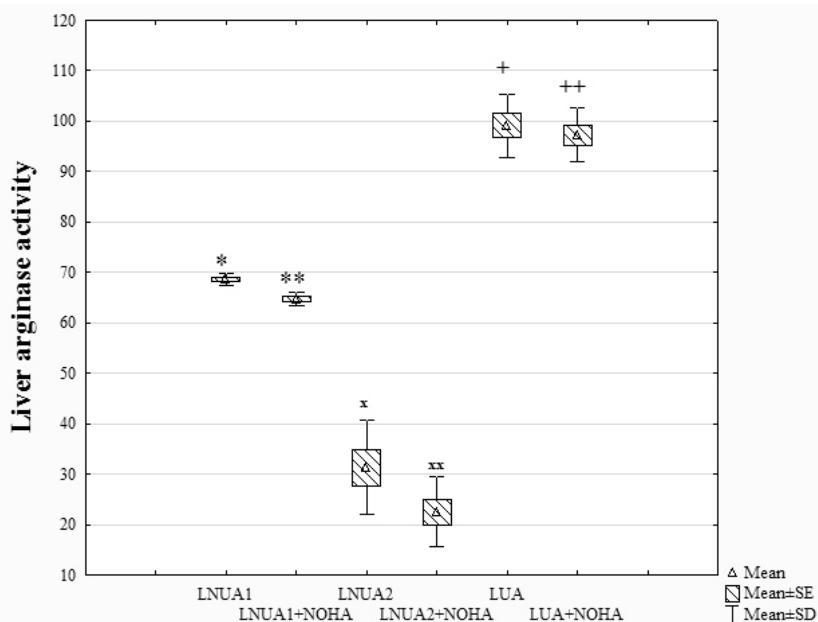

Fig. 1. Ureotelic and nonureotelic liver arginase activities in normal conditions and after the influence of NOHA, P<0.05; n=7. Liver arginases activity are represented in nM urea (product) in 1ml fraction in 1 minute incubation (reaction time) * - liver NUA1 activity in norm, ** - NUA1 activity after the influence of NOHA, x - liver NUA2 activity in normal, xx - NUA2 activity after the influence of NOHA, + - liver UA activity in normal, ++ - liver UA activity after the influence of NOHA.

liver NUA2 is inhibited the quantity of spermine does not take any changes (*Table 1*). Besides, the quantity of the other polyamines decreased, in case of liver NUA1. Studying of this exception is still continued, but considering the previous results of our laboratory, we can mention that the main quantity of L-ornithine for the synthesis of polyamines is provided with the liver NUA1. Such conclusions allow us to make the results of our recent studies [4, 5].

Under the influence of polyamines liver NUA1, kidney NUA2 and brain NUA are inhibited by mixed type of inhibition mechanism. Under the influence of polyamines liver NUA2 and kidney NUA1 are inhibited by uncompetitive type of inhibition mechanism. During mixed type of inhibition polyamines are connected as with NUA, as so with NUA-arginine complex, and during uncompetitive type of inhibition polyamines are connected only with enzyme-substrate complex. This fact gives some advantages to liver NUA1, what can bring us to the mentioned results.

Table 1. The quantity of polyamines in liver homogenate fractions after gel-filtration (LNUA1, in the fraction N25) and ion-exchange chromatography (LNUA2, in the fraction N8) in normal conditions and after the effect of NOHA on the activity of nonureotelic isoform of arginase, ($p<0,05$, $n=5$). PA-polyamines, the quantity of polyamines are represented in nM polyamine in 50 μl of the studied solution, SPM-spermine, SPD-spermidine, PUT-putrescine, LNUA-liver nonureotelic arginase, NOHA-N^G-hydroxy-L-arginine.

PA	PA quantity (LNUA1 in norm – before NOHA)	PA quantity (LNUA1+NOHA)	PA quantity (LNUA2 in norm)	PA quantity (LNUA2+NOHA)
PUT	3.4	2.2	1.4	1.1
SPD	4	2.6	2	1.6
SPM	10.6	9.4	9.4	9.4

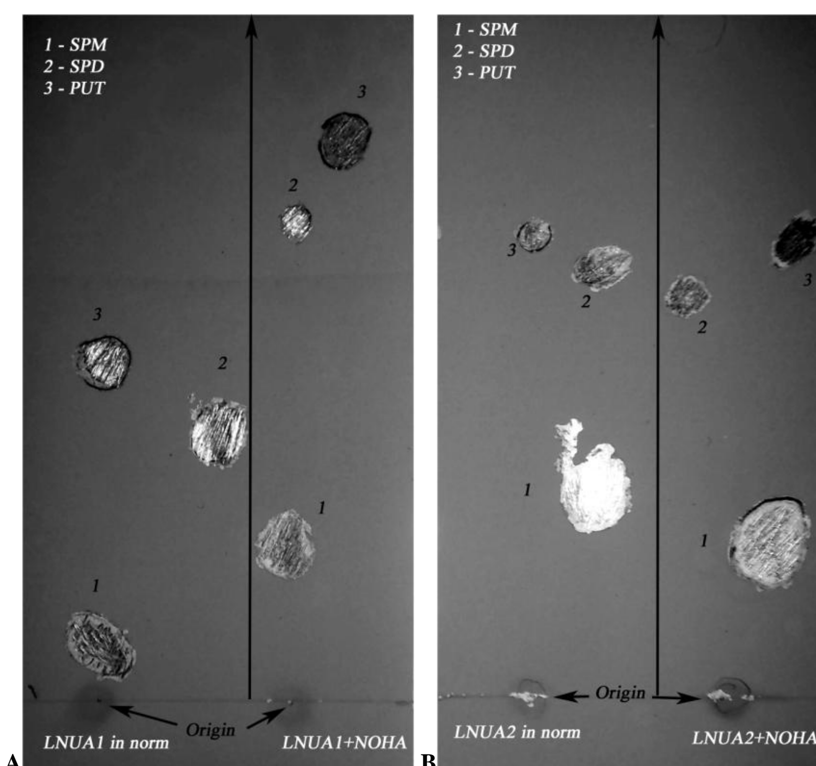

Fig. 2. TLC for liver homogenate densylpolyamines in normal conditions and after the effect of NOHA. Fraction N25 (A, after gel-filtration with Sephadex G-150, LNUA1) and fraction N8 (B, after ion-exchange chromatography with CM-cellulose, LNUA2), ($p<0,05$, $n=5$). SPM-spermine, SPD-spermidine, PUT-putrescine, LNUA-liver nonureotelic arginase, NOHA-N^G-hydroxy-L-arginine, RF-values for norm: $RF_{spm} = 0,35$; $RF_{spd} = 0,52$; $RF_{put}=0,63$ and after NOHA influence - $RF_{spm} = 0,41$; $RF_{spd} = 0,68$; $RF_{put} = 0,71$.

SUMMARY

Summing up we can mention, that during inhibition of nonureotelic isoforms of liver arginase, the quantity of polyamines are decreased. Taking into consideration this results we can conclude, that in mammalian cells the required source of endogenous L-ornithine for the synthesis of polyamines is provided by the nonureotelic isoform of arginase. Apart from the fundamental value, our study can also have a practical importance. In pathological conditions (cancer, neurodegenerative, autoimmune diseases) the change of NUA activity, can lead to the change of the quantity of polyamines, what can have influence to the course of the diseases.

Nonureotelic isoenzymes of liver arginase take part in the biosynthesis of polyamines, because they influence on polyamines quantity. We should also mention that one or two of liver nonureotelic isoenzymes (liver NUA1 after gel-filtration and liver NUA2 after ion-exchange chromatography) can participate in biosynthesis of proline, NO, guanidine compounds (creatin) and histones. It is logical that this different isoenzymes involved in mentioned processes have different binding sites for polyamines and various types of inhibition mechanisms [4, 5]. Further investigation should be done to lighten questions mentioned above.

REFERENCES

[1] *Давтян М.А*. Эволюционные аспекты оброзования и нейтрализаций аммиака / III Сисакяновские чтения, 2005, с. 106-154.

[2] *Давтян М.А., Алчуджян Н.Х.* Выделение и очистка изоферментов аргиназы печени крыс / Биология. Межвуз. сб. науч. тр. ЕГУ, Ереван, 1979, вып. 1, с. 94-98.

[3] *Archibald R.M.*, 1945. The colorimetric determination of urea / J. Biol. Chem., 167: 507.

[4] *Avtandilyan N.V., Karapetyan S.A., Davtyan M.A*., Polyamines are noncompetitive inhibitors for nonureotelic arginases in the different tissues of rats. National Academy of Sciences of RA / Electronic J. of Nat. Sci., 1 (20), 2013: 7-10.

[5] *Avtandilyan N.V.*, The research of the role of nonureotelic arginases in polyamines biosynthesis in brain and kidney in vitro / Biolog. Journal of Armenia, 1 (65), 2013: 26-32.

[6] *Buniatian H.CH., M.A. Davtian*, Urea synthesis in brain / J. Neurochem., 1966, 13: 743–753.

[7] *Daghigh F., Fukuto J.M., Ash D.E.*, Inhibition of rat liver arginase by an intermediate in NO biosynthesis, N^G-hydroxy-L-arginine: implications for the regulation of nitric oxide biosynthesis by arginase / Biochem. Biophys. Res. Commun., 1994, 202: 174-180.

[8] *David E. Ash*, Structure and function of arginases / J. Nutr. 2004, 134: 2760S–2764S.

[9] *D.Agostino L, Di Luccia A.*, Polyamines interact with DNA as molecular aggregates / Eur J Biochem 269: 4317-25, 2002.

[10] *Hui Li et al.*, Regulatory role of arginase I and II in nitric oxide, polyamine, and proline syntheses in endothelial cells / Am J Physiol Endocrinol Metab., 2001, 280: E75–E82.

[11] *Joshua L.* Deignan et al. Polyamine homeostasis in arginase knockout mice / Am J Physiol Cell Physiol, 2007, 293: 1296-1301.

[12] *Moinard C., Cynober L., deBrandt J.P.*, 2005. Polyamines: metabolism and implications in human diseases / Clin Nutr 24: 184–197.

[13] *Morris Jr., S.M.*, Recent advances in arginine metabolism: Roles and regulation of the arginases / Br. J. Pharmacol., 2009, 157: 922-930.

[14] *Pegg A.E., McCann P.P.*, Polyamine metabolism and function / Am J Physiol, 1982: 243:C212-C221.

[15] *Wallace H.M., Fraser A.V., Hughes A.*, 2003. A perspective of polyamine metabolism / Biochem J, 376 (Pt 1): 1–14.