UDK 599.323.4:577:591.16.

ACTIVITY OF MALATE-ASPARTATE SHUTTLE ENZYMES AND ANTIOXIDANT DEFENSE OF REPRODUCTIVE ORGANS AND EPIDYDYMAL SPERMATOZOA OF RATS

N. KUZMINA, D. OSTAPIV
Institute of animal biology NAAS

The activity of malate-aspartate shuttle and antioxidant defense enzymes of reproductive organs and epididymal spermatozoa of rats were studied. It was found that with changing of anatomic spot of rat reproductive organs: from testes to epidydymal spermatozoa, activity of antioxidant enzymes ($p < 0,05 – 0,001$), AST ($p < 0,001$) is getting lower, and in epidydymus MDH activity is maximal (2,10±0,17 nmol/min×mg protein).

Key words: malate dehydrogenase, aspartate aminotransferase, superoxide dismutase, glutathione peroxidase, catalase, spermatozoa, reproductive organs, rats.

Spermatozoa quantitative and qualitative characteristics in bull ejaculates are specified by biochemical processes that run in testicle and epididymal tissues, and sex cells in epididymis. Among biochemical processes important place take energy generating reactions, which are connected with malate-aspartate shuttle [1]. Due to shuttle mechanism, which is made by malate dehydrogenase (MDH) and aspartate aminotransferase (AST), occurs a regulation of relation between reduced and oxidited forms of nicotinamideadeninedinucleotide ($NADH/NAD^+$) in cytoplasm and mitochondria, substrate transamination and their incorporation in TCA cycle [2]. Furthermore, reproductive tissues and maturing spermatozoa characterize by oxidative processes that result in reactive Oxygen species (ROS) production, $O_2^{\bullet-}$ and H_2O_2 in particular. ROS generation in physiological normal values is necessary condition of spermatogenesis, capacitation and acrosomal reaction [3]. ROS exceeding generation and accumulation causes maturing spermatozoa metabolism violation and loss of fertilizing ability [4]. Optimal level content of ROS is maintained by multicomponent antioxidant system, key role in which play antioxidant enzymes (superoxide dismutase, SOD; glutathione peroxidase, GPO; catalase, CAT) [5]. However, defense mechanisms against ROS destructive action and their production regulation in tissues and maturing spermatozoa, sex cell energy providing in specific anatomical spots of reproductive system, their specifications, remain unknown.

In connection with this, antioxidant defense enzyme activity and malate-aspartate shuttle of rat reproductive organs and epidydimal spermatozoa.

Materials and methods

Researches were carried out on mature male rats (Rattus norvegicus var. Alba, line Wistar; n = 21; age – 5–6 months). Experimental procedures were carried out according to the European convention for the protection of vertebrate animals used for experimental and other scientific purposes (Stratsburg: Counsil of Europe, 18.03.1986) and Law of Ukraine "On protection of animals from cruelty" of 21.02.2006. Laboratory animals were decapitated under chloroformic narcosis. After decapitation epididymis and testicles were extirpated, and spermatozoa were washed from them with 0,9 % solution of sodium chloride. Epididymal and testicle tissues were homogenized in Potter homogenizer at 4ºC in 0,25 M sucrose solution (pH 7,4) and 6000 rev/min during 1 min. Homogenized tissue were centrifuged for 15 min at 8000 rev/min. In supernatant and epididymal spermatozoa activity of antioxidant defense enzymes (SOD [6], GPO [7] та CAT [8]) and malate-aspartate shuttle: AST [9] and MDH [10] were studied. Protein concentration in homogenized tissue and spermatozoa was determined with Folin phenol reagent [11]. Statistical analysis of gathered data was made by M. O. Plohinskiy [12].

Results and discussion

Rat epididymal and testicle tissue and epididymal spermatozoa characterize malate-aspartate shuttle enzyme and antioxidant defense activity. AST activity in rat epididymal and testicle tissues is 26,8 – 30,5 nmol/min×mg of protein, and in epididymal spermatozoa – in 2,6–3,0 times ($p < 0,001$) lower (10,3±1,12 nmol/min×mg of protein; tabl.).

Table 1

Activity of malate-aspartate shuttle enzymes and antioxidant defense of reproductive organs, n=21; M±m

Enzymes	Tissue		Epididymal spermatozoa
	Testicle	Epididymis	
AST, nmol/min×mg of protein	26,8±3,88	30,5±4,57	10,3±1,12***
MDH, nmol/min×mg of protein	0,45±0,088	2,10±0,17***	0,34±0,03
SOD, IU/ mg of protein	18,2±2,59	11,9±0,89*	9,7±0,83**
GPO, μmol/min×mg of protein	1,27±0,088	1,15±0,17	0,27±0,09***
CAT, μmol/min×mg of protein	2,10±0,27	2,12±0,40	0,36±0,05***

*Remark: difference is statistically valid comparing with testicle tissue * - $p < 0,05$; ** - $p < 0,01$ *** - $p < 0,001$

MDH activity is highest in epididymis (2,10±0,17 nmol/min×mg of protein), in testicle tissue lower on 78,6 % ($p < 0,001$) and in epididymal spermatozoa the lowest – 83,9% ($p < 0,001$).

Highest SOD activity is determined in testicle tissue (18,2±2,59 IU/mg of protein), lower on 34,6 % (11,9±0,89 IU/mg of protein) in epididymis and the lowest (9,7±0,83 IU/mg of protein) in epididymal spermatozoa. Analogical, GPO activity lowers with production and maturation of spermatozoa: testicle → epididymis → epididymal spermatozoa, correspondingly, from 1,27±0,088

μmol/min×mg of protein in testicle tissue, lower on 9,5 % in epedidymis and on 78,2 % ($p < 0,001$) in epididymal spermatozoa. Herewith, CAT activity of testicle and epididymal tissues is equal (2,1 μmol/min×mg of protein) and lowers on 82,9 % in epididymal spermatozoa. Thus, changing in rat reproductive organs: testicle → epididymis → epididymal spermatozoa characterizes by gradual lowering of AST and antioxidant enzymes activity. Herewith, maximal MDH activity is in epididymis.

Thus, discovered higher AST activity, comparing to MDH, in testicle and epididymis, points out intensive disposal of aspartate in processes of tissue anabolism and catabolism, and maximal MDH activity in epididymis indicates energetic substrates high necessity in this rat reproductive system anatomical spot. Simultaneously, high SOD activity in testicle tissue, where spermatogenesis is carried out, indicates that enzyme performs a valuable role in cell protection in the time of differentiation, when chromatin is in noncondensated state and particularly sensitive to superoxide anion radical impact. High $O_2^{\bullet-}$ utilization intensity leads to formation of H_2O_2 high quantity, which activates CAT. GPO activity in testicle tissue, comparing to CAT, is lower, which is specified by high H_2O_2 accumulation and activation of higher efficiency utilization system. In epididymis takes place final spermatozoa maturation stage, during which essential reconstruction of cytoplasmic membrane are implemented, involving ROS. That is why antioxidant defense system enzymes carry out regulation role, controlling ROS levels. Lowered level of epididymis antioxidant defense activity may indicate spermatozoa maturation completion and ability to fertilize oocyte.

Conclusions

1. Activities of AST in testicle and epididymis tissues are equal (26,8 – 30,5 nmol/min×mg of protein), and lower in 2,6 – 3,0 times ($p < 0,001$) in epididymal spermatozoa.
2. MDH activity is highest in epididymis (2,10±0,17 nmol/min×mg of protein), in testicle tissue lower on 78,6 % ($p < 0,001$) and in epididymal spermatozoa the lowest – 83,9% ($p < 0,001$).
3. SOD highest activity is in testicle tissue (18,2±2,59 IU/mg of protein), lower on 34,6 % (11,9±0,89 IU/mg of protein) in epididymis and the lowest (9,7±0,83 IU/mg of protein) in epididymal spermatozoa.
4. CAT activity in testicle and epididymal tissues is high (2,1 μmol/min×mg of protein) and on 82,9 % lower in epididymal spermatozoa
5. GPO activity lowers with production and maturation of spermatozoa: testicle → epididymis → epididymal spermatozoa, correspondingly, from 1,27±0,088 μmol/min×mg of protein in testicle tissue, lower on 9,5 % in epedidymis and on 78,2 % ($p < 0,001$) in epididymal spermatozoa.

Research perspectives. It is reasonable to continue research work with studying enzymes of malate-aspartate shuttle and antioxidant defence in male organs of agricultural animals – boar and bull.

References

1.Burgos C. Studies in vitro on shuttle systems of mouse spermatozoa. Biochem / Burgos C., Coronel C.E. et al. // J. — 1982. — V. 208, No. 2. — P. 413-417.

2.Mitchell M. Disruption of mitochondrial malate-aspartate shuttle activity in mouse blastocysts impairs viability and fetal growth. / Mitchell M., Cashman K.S., Gardner D. et al. // Biology of Reproduction. — 2009. — V. 80, No. 2.— P. 295-301.

3.Ford W.C. Regulation of sperm function by reactive oxygen species // Human eproduction. — 2004. — V. 10, No. 5. — P. 387–399.

4.Gomez E. Development of an image analysis system to monitor the retention of residual cytoplasm by human spermatozoa: correlation with biochemical markers of the cytoplasmic space, oxidative stress, and sperm function / Gomez E., Buckingham D.W., Brindle J., et al. // J. Androl. — 1996. —V. 17. — P. 276–287.

5.Fernandez-Santos M.R. Sperm characteristics and DNA integrity of iberian red deer (cervus elaphus hispanicus) epididymal spermatozoa frozen in the presence of enzymatic and nonenzymatic antioxidants / Fernandez-Santos M.R.,. Martinez-Pastor F., Garcia-Macias V. et al. // J. Androl. — 2007. — V. 28, No. 2. — P. 294–305.

6.[Chevari S.N. Detecting of blood antioxidant parameters and their diagnostic meaning in old age / Chevari S.N., Andyan T.A. Shtrenger Ya.I. // Lab. Work. — 1991. — 10. — P. 9 – 13.] – Russain.

7.[Moim V. M. Simple and specific method of glutathione peroxidase detection in erythrocytes // Lab. Work. — 1986. — 12. — P.16 – 19.] – Russain.

8.Korolyuk M.A. Catalase activity detecting method / Korolyuk M.A., Ivanova L.I., Mayorova I.G., Tokarev V.E. // Lab. Work. — 1991. — 12. — P. 9 – 10.

9.[Reitmann S. A colorimetric mechod for the determination of serum glutamic oxaloacetic and glutamic puruvic transaminases. / Reitmann S. Frankel S. // Amer. J. Clin. Path. — 1957. — V. 28, No. 1. — P. 56 – 63.] – Russian.

10.[Kochetov G.A. Practical guidance on enzymology. Moscow: High School, 1980. — 380 p.] – Russian.

11.Lowry O.H. Protein measurement with Folin phenol reagent /Lowry O.H., Rosebrough N. J., Fair A. L., Randall R. J. // J. Biol. Chem. — 1951. — Vol. 193, No. 1. — P.264–275.

12. [Plokhinskiy N.A. Biometry. Moscow: MSU. — 1970. — P. 53–60.] – Russian.

УДК 599.323.4:577:591.16.

ЗВ’ЯЗКИ МІЖ АКТИВНІСТЮ ЕНЗИМІВ МАЛАТ-АСПАРТАТНОГО ШУНТА І АНТИОКСИДАНТНОГО ЗАХИСТУ СПЕРМИ

Н. Кузьміна, Д. Остапів
Інститут біології тварин НААН

Вивчали зв'язки між ензимами малат-аспартатного шунта і антиоксидантного захисту в свіжоотриманих еякулятах бугаїв та кнурів. Встановлено, що між активністю малатдегідрогенази (МДГ) і супероксиддисмутази (СОД) існує позитивна залежність (η = 0,367 – у бугая та 0,205 – у кнура). У бугая максимум активності глутатіонпероксидази (ГПО - 1,01±0,09 мкмоль/хв×мг протеїну) проявляється за активності МДГ 0,20 – 0,30 нмоль/хв×мг протеїну (η = 0,407). У спермі кнура, утворений H_2O_2 за посередництва МДГ,

утилізується каталазою (КАТ; η = 0,326). На противагу, у спермі бугая МДГ негативно корелює з активністю КАТ (η = 0,566). Активність аспартатамінотрансферази (АСТ) проявляє негативний зв'язок з СОД сперми кнура (η = 0,446), а у бугая - слабкий (η = 0,281). Активність АСТ у спермі кнура та бугая підвищує активність ГПО (η = 0,356 і 0,425) і знижує КАТ (η = 0,440 і 0,458).

Ключові слова: малат-аспартатний шунт, антиоксидантна система, активні форми Оксигену, спермії.

СВЯЗИ МЕЖДУ АКТИВНОСТЬЮ ЭНЗИМОВ МАЛАТ-АСПАРТАТНОГО ШУНТА И АНТИОКСИДАНТНОЙ ЗАЩИТЫ В СПЕРМЕ БЫКОВ И ХРЯКОВ

Н. Кузьмина, Д. Остапив
Институт биологии животных НААН

Изучали связи между энзимами малат-аспартатного шунта и антиоксидантной защиты в свежеполученных эякулятах быков и хряков. Установлено, что между активностью малатдегидрогеназы (МДГ) и супероксидисмутазы (СОД) существует положительная зависимость (η = 0,367 – у быка и η = 0,205 – у хряка). У быка максимум активности глутатионпероксидазы (ГПО; 1,01±0,09 мкмоль/мин×мг протеина) проявляется при активности 0,20 – 0,30 нмоль/хв×мг протеина МДГ (η = 0,407). В сперме хряка, образованный H_2O_2 при участии МДГ, утилизируется каталазой (КАТ; η = 0,326). В противовес, в сперме быка МДГ негативно коррелирует с активностью КАТ (η = 0,566). Активность аспартатаминотрансферазы (АСТ) проявляет отрицательную связь с СОД спермы хряка (η = 0,446), а у быка - слабую (η = 0,281). Активность АСТ в сперме хряка и быка повышает активность ГПО (η = 0,356 и 0,425) и снижает КАТ (η = 0,440 и 0,458).

Ключевые слова: малат-аспартатный шунт, антиоксидантная система, активные формы Оксигена, спермии.