Arginase status in bull reproductive system

Razmi, N.[1]*, Jelodar,Gh.A.[1], Nazifi,S.[2], Dehghan,A.[3]

[1]*Department of Basic Sciences, School of Veterinary Medicine, Shiraz University, Shiraz -Iran.*

[2]*Department of Clinical Studies, Faculty of Veterinary Medicine, Shiraz University, Shiraz -Iran.*

[3]*Graduated from the School of Veterinary Medicine, Shiraz University, Shiraz -Iran.*

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Abstract: **Seven healthy and sexually adult bulls were slaughtered and their reproductive system was isolated. Different tissues including: testes, epididymis, vas deferan, ampulla, accessory sex glands (seminal vesicle, prostate and bulbourethra), muscular and mucosal layer of pelvic and penile urethra were carefully dissected. Total soluble protein and arginase specific activity (ASA) were measured by Lowry and modified paranitrophenylglyoxal (PNPG) method, respectively. The results indicate that the highest arginase specific activity (51.28±8.79 $x10^{-3}$ IU/mg of protein) is present in muscle of pelvic part of urethra. Based on ASA, bulls reproductive system was categorized in three tissue groups: high, medium and low. Muscular layer of penile urethra with the highest ASA level (> 50 $X10^{-3}$ IU/mg tissue protein) is in the 1st group, Testes, bulbourethral gland and mucosal layer of penile urethra (30-40 $X10^{-3}$ IU/mg tissue protein) are in the2nd group and the rest parts (<25 $X10^{-3}$ IU/mg protein) are in 3rd group. Significant differences observed between classified tissues ($p<0.05$). The present study indicate that ASA is present at different levels in all parts of bull reproductive system. This condition may be related to different rate of cell proliferation and differentiation or some other unknown physiological and biochemical activities of the enzyme in this system.**

Key words: arginase, reproductive system, bull.

Introduction

Arginase (L-arginine amidinohydrolase, EC 3.5.3.1) is a binuclear manganese metalloenzyme that catalyzes the hydrolysis of L-arginine to form L-ornithine and urea through a metal-activated hydroxide mechanism (Christianson and Cox, 1999; Ash *et al.*, 2000). In mammals, two isoenzymes are identified: arginase I is found predominantly in hepatocytes where it catalyzes the final cytosolic step of urea cycle (Herzfield and Raper, 1976), and arginase II is extrahepatic (Herzfield and Raper, 1976; Glass and Knox, 1973; Kaysen and Strecker, 1973; Kim *et al.*, 2001) and localized subcellularly in the mitochondrial matrix of kidney cells (Skrzypek-Osiecka *et al.*, 1983) and other tissues (Pohjanpelto and Holtta, 1983; Skoy *et al.*, 1981; Schneider, 1985); however, little is known about the role of extrahepatic arginase found in erythrocytes, mammary gland, kidney, salivary gland, gastrointestinal tract and reproductive system (Marathe *et al.*, 1998). Arginase isozymes differ from each other in terms of their catalytic, molecular, and immunological properties. The major role of arginase, as the terminal enzyme of the urea cycle, was first detected in mammalian livers (Greenberg, 1960). Unlike arginase I, the primary function of arginase II appears to be in L-arginine homeostasis (Castillo *et al.*, 1993; Castillo *et al.*, 1994; Shi *et al.*, 2001), regulating L-arginine or L-ornithine pools for subsequent biosynthetic transformations (Morris, 2002). The importance of arginase may be in the production of ornithine for the synthesis of the polyamines putrescine, spermidine and spermine, which are required for normal cellular proliferation (Pegy and McCann, 1982; Tobor and

* **Corresponding author's email: razmi@shirazu.ac.ir, Tel: 0711-2286950, Fax: 0711-2286940**

Table 1: Mean (± SD) of total soluble proteins and arginase activity of different parts of bull reproductive system (n = 7 for each tissue). Standard deviation of mean.

Parts of reproductive system	Total soluble protein (mg/g of tissue)	Arginase activity (IU/g tissue)
Penile urethra(mucosa)	14.75 ± 4.37	0.53 ± 0.23
Testes	22.07 ± 3.20	0.72 ± 0.20
Epidydimis	23.28 ± 2.65	0.58 ± 0.19
Deferan duct	20.00 ± 3.92	0.40 ± 0.10
Ampulla	20.34 ± 3.86	0.45 ± 0.05
Pelvic urethra(mucosa)	28.57 ± 5.48	0.62 ± 0.21
Pelvic urethra(muscle)	21.14 ± 5.46	1.08 ± 0.13
Seminal vesicle	32.21 ± 7.78	0.78 ± 0.26
Bulbourethral gland	21.71 ± 5.19	0.68 ± 0.20
Prostate gland	18.85 ± 3.97	0.38 ± 0.12

Tobor, 1981) and differentiation (Pegg 1986). Arginase activity at the site of wounds plays a role in the recovery of host tissues from inflammation and infection (Guoyao *et al.*, 1998). The distribution of arginase between the organs of normal human (Reyero and Doner, 1975; Spector *et al.*, 1982, 1983; Zamecka and Porembska, 1988), domestic animals (Aminlari and Vaseghi, 1992) and reproductive system of ram and cattle (Razmi *et al.*, 2004, Razmi *et al.*, 2005) have been studied. Arginase activity has been identified in different parts of male and female reproductive system such as: prostate, vagina (Cama *et al.*, 2003; Wilson, 2003), clitorial corpus and uterus, which may be important in synthesis of polyamines. Polyamines in turn may mediate the action of androgens (Mendez *et al.*, 2002). The existence of multiple forms of arginase in eukaryotes suggested a complex regulatory role of this enzyme in the metabolism, development and maintenance of these organisms. The mammalian arginase is well characterized (Beruter *et al.*, 1978; Viello-Breitburd and Orth, 1972). Arginase is present in abundance in mammary gland where the urea cycle is not present (Yip and Knox, 1972). The purpose of this investigation was to evaluate the tissue arginase activity in different parts of bull reproductive system which may be important in the productivity of this animal from economical point of view.

Materials and Methods

Seven apparently healthy and sexually adult bulls were slaughtered at the slaughterhouse located at Fars province in the south of Iran. Immediately after slaughter, whole reproductive system including ducts, testes, and accessory glands were collected. All samples, keeping on ice, were transferred to the laboratory within 45 minutes; tissues were separated, stripped from fat and extraneous materials, washed a few times with physiological saline and blotted.

Measurement of total soluble protein and arginase activity: Tissue extracts were prepared by freezing 0.5 gram of the sample in liquid nitrogen, homogenizing with a hand-homogenizer, and suspending the homogenate in 4 ml of 0.025 M sodium phosphate buffer (pH 7.2). The suspensions were centrifuged for 15 minutes at 4000g in an MSE high-speed refrigerated centrifuge. The supernatants were collected and arginase activity were measured by modified paranitrophenylglyoxal (PNPG) method (Razmi, 1991), the substrate, arginine reacted with PNPG in 0.1 mol/lit sodium hydroxide to produce a colored compound which absorbed maximally at 480 nm. The protein concentration in the crude extracts of different tissues was measured by Lowry method (1953). The arginase specific activity (ASA) of each sample was calculated by dividing the amount of arginase activity by the amount of total soluble protein (TSP).

Table 2: Mean (± SD[a]) arginase specific activity (ASA) in extracts of different parts of reproductive system in bull (n = 7 for each tissue studied). * Shows Significant difference observed between 3 groups (p<0.05).

Group of tissues	Parts of reproductive system	Specific activity x 10^{-3} of enzyme (IU/mg protein)
I*	Pelvic urethra(muscle)	51.28 ± 08.79
II*	Testes	32.60 ± 07.58
	Bulbourethral gland	31.47 ± 06.20
	Penile urethra(mucosa)	36.08 ± 07.25
III*	Epididymis	25.05 ± 05.38
	Deferan duct	19.91 ± 04.23
	Ampulla	22.05 ± 02.87
	Seminal vesicle	24.28 ± 04.52
	Prostate gland	20.25 ± 03.82
	Pelvic urethra(mucosa)	21.84 ± 03.08

The data were analyzed statistically by analysis of variance (ANOVA).The differences between the means were statistically estimated by the Duncan test. All values were expressed in mean (± SD) using a significant level of $p< 0.05$ (Norusis, 1993).

Results

Total soluble protein (TSP) concentration (mg/g of tissue) and arginase activity (IU/g of tissue) in different parts of reproductive system are presented in Table1. The highest TSP observed in vesicular gland and the same was lowest in mucosal layer of penile urethra. Arginase activity was highest in muscular layer of penile urethra and lowest in deferan duct.

The arginase specific activity (ASA) in different part of bull reproductive system is presented in Table 2. All tissues contained different amounts of arginase activity. Based on ASA, bull reproductive system was categorized in three groups: high, medium and low. Muscular layer of penile urethra with highest ASA level (>50 IU/mg tissue protein) was in 1st group. Testes, bulbourethral gland and mucosal layer of penile urethra (30-40 x10^{-3} IU/mg tissue protein) in 2nd group and the rest parts (<25 X10^{-3} IU/mg protein) were in 3rd group. Significant difference observed between ASA of three group tissues ($p<0.05$).

Discussion

In mammals, the liver is the organ in which a full urea cycle is functional (Greenberg, 1960). The highest rates of arginine synthesis occur within the hepatic urea cycle, which is localized within periportal hepatocytes. Net arginine synthesis by the liver is only possible if the urea cycle is replenished by necessary intermediates such as ornithine. Arginase shares arginine as a common substrate with nitric oxide synthase (NOS), the enzyme that synthesizes NO, and NO is the principal mediator of penile erection and clitorial arousal (Bivalacqua *et al.*, 2001; Wilson, 2003).The presence of arginase in extra hepatic tissues might indicate that these tissues use arginase for purposes other than urea synthesis. Our data show that arginase is present in almost all parts of bull reproductive system with different ranges of activity .The highest arginase specific activity was observed in muscular layer of pelvic urethra. Highest ASA was also reported in mucosal layer of penile urethra of ram (Razmi *et al.*, 2004). Low arginase specific activity in other parts of reproductive system might be due to either low cell division and differentiation rate of these area (Pegg, 1986) or lower soluble proteins in tissues (ASA was obtained in IU/mg of soluble proteins of each tissue). Sign of arginase activity have been detected in genitalia of female rabbit (Wilson, 2003). The arginase activity in reproductive system is important from this point of view that, arginase will compete with nitric oxide synthase (NOS) for a common

substrate, arginine, to produce ornithine or NO. Arginase is found in abundance in tissues with high proliferation and differentiation rate which NOS is mainly present in tissues which require vasodilation and have arousal function (Pegg, 1986). High ASA in pelvic urethra which obtained in this study may be due to high proliferation and differentiation rate of cells in this part or less participation in sexual arousal (Cama *et al.*, 2003; Pegg, 1986). Administration of small amount of arginase inhibitor, increased blood flow to genitalia of both male and female rabbit (Wilson, 2003). NO is also synthesized in rat uterus and its production is regulated by progesterone (Yallampalli and Dong, 2000). NO synthase is identified in human clitorial corpus cavernosum (Burnett, 1997) and vagina (Hoyle *et al.*, 1996). Arginase II may co localize in these tissues and inhibition of arginase in female may enhance smooth muscle relaxation and sexual arousal (Cama, 2003). The results of this study showed that arginase is present, but probably does not play a significant role in ammonia detoxification, in different parts of bull

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بررسی وضعیت آرژیناز در دستگاه تولید مثلی گاونر

نعمت‌اله رزمی [۱] * غلامعلی جلودار [۱] سعید نظیفی [۲] عبدالرسول دهقانی [۳]

۱) گروه علوم پایه، دانشکده دامپزشکی دانشگاه شیراز، شیراز - ایران.

۲) گروه علوم درمانگاهی، دانشکده دامپزشکی دانشگاه شیراز، شیراز - ایران.

۳) دانش آموخته دانشکده دامپزشکی دانشگاه شیراز، شیراز - ایران.

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تعداد هفت رأس گاو نر بالغ و سالم ذبح گردید، بلافاصله تمام سیستم تولید مثلی جدا و سپس از تفکیک قسمت‌های مختلف شامل: بیضه‌ها، اپیدیدیم، وازدفران، آمپولا و غدد ضمیمه جنسی (شامل عدد وزیکولار، پروستات و بالبویورترا)، لایه‌های ماهیچه‌ای و مخاطی بخش لنگی پیشابراهی یوریترا با دقت جدا گردید. میزان پروتئن تام و فعالیت ویژه آرژیناز (ASA) به ترتیب با روش لوری و روش اصلاح شده پارانیتروفنیل گلی اکسال (PNPG) تعیین گردید. نتایج نشان داده حداکثر فعالیت ویژه آنزیم (پروتئین بافت $IU/mg \times 10^{3}$ ۵۱/۲۵۸/۷۹) در لایه عضلانی بخش لنگی یورترا می‌باشد. براساس میزان فعالیت ویژه آنزیم (ASA) بخش‌های مختلف سیستم تولید مثلی گاو به سه دسته (زیاد، متوسط و کم) تقسیم گردید. لایه ماهیچه‌ای بخش آلتی یورترا با بیشترین میزان ASA (پروتئین بافتی $IU/mg \times 10^{3}$ ۳۰-۴۰) در گروه دوم و بقیه بافت‌ها ($IU/mg \times 10^{3}$ ۲۵) در گروه سوم قرار گرفتند. از نظر آماری بین گروه‌های بافتی تعریف شده، تفاوت معنی داری در میزان ASA مشاهده گردید (۰/۰۵). نتیجه این مطالعه نشان داد که آنزیم آرژیناز با مقادیر متفاوت در بخش‌های مختلف سیستم تولید مثلی گاو نر وجود دارد که ممکن است با میزان متفاوت تکثیر و تمایز سلولی و یا فعالیت‌های مختلف فیزیولوژیکی و بیوشیمیایی این آنزیم در این بافت‌ها مرتبط باشد.

واژه‌های کلیدی: آرژیناز، دستگاه تولید مثلی، گاونر.

*) نویسنده مسؤول: تلفن: ۰۷۱۱-۲۲۸۶۹۵۰ نمابر: ۰۷۱۱-۲۲۸۶۹۴۰ Email:razmi@shirazu.ac.ir