

VETERINARSKI ARHIV 75 (1), 31-38, 2005

Arginase status in cattle reproductive system

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RAZMI, N., G. A. JELODAR, S. NAZIFI, A. DEHGhani: Arginase status in cattle reproductive system. Vet. arhiv 75, 31-38, 2005.

ABSTRACT

Seven healthy, sexually adult cows were slaughtered and the whole reproductive system of all animals was immediately collected. Different tissues, including ovaries with corpora lutea, muscular and mucosal layers of uterine horn, uterine body, cervix, vagina and vestibula, were carefully separated. Arginase specific activity (ASA) was determined and compared by the modified paranitrophenylglyoxal (PNPG) method. Results of this study indicate that the highest arginase-specific activity (79.01 ± 13.20 IU/mg of protein) is present in mucosal layer of vestibula, which did showed no significant difference with the mucosal layer of uterine horn (49.45 ± 8.73) and muscular layer of vestibula (49.04 ± 9.43) although there was a significant difference with the remaining parts of the reproductive system ($P < 0.05$). The finding of this study also indicates that this enzyme is present at different levels in all parts of cattle reproductive system, which may be related to different rate of cell proliferation, differentiation or some other unknown physiological and biochemical activities of the enzyme in this system.

Key words: arginase, reproductive system, cattle

Introduction

Arginase (L-arginine amidinohydrolase, EC 3.5.3.1) is a binuclear manganese metalloenzyme which 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 stage of the urea cycle (HERZFIELD and RAPER, 1976); arginase II is extrahepatic (HERZFIELD and RAPER, 1976; GLASS and

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KNOX, 1973; KAYSEN and STRECKER, 1973; KIM et al., 2001) and localized sub-cellularly in the mitochondrial matrix of kidney cells (SKRZYPEK-OSIECKA et al., 1983) and other tissues (POHJANPELTO and HOLTTA, 1983; SKOY et al., 1981; SCHNEIDER and DY, 1985), although 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 TOBOR, 1981) and differentiation (PEGY, 1986). Arginase activity at the site of wounds plays a role in the recovery of host tissues from inflammation and infection (GUOYAO and MORRIS, 1998). The distribution of arginase between the organs of normal human (REYERO and DONER, 1975; SPECTOR et al., 1982, 1983; ZAMECKA and POREMBSKA, 1988) and domestic animals (AMINLARI and VASEGHI, 1992) has been studied. Arginase activity has been identified in different parts of male and female reproductive systems such as the prostate and vagina (CAMA et al., 2003; WILSON, 2003), clitoral 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). To our knowledge there is no comprehensive publication focused on the presence and distribution of arginase activity in the female reproductive system, particularly in cattle. The purpose of this investigation was to evaluate the tissue arginase activity in different parts of cattle reproductive system which may be important in the productivity of this animal from the economic aspect.

Materials and methods

Seven apparently healthy, sexually adult cows were slaughtered at the slaughterhouse located at Fars province in the south of Iran.

Immediately after slaughter the whole reproductive system, including ovaries and uterus, were collected. All samples, kept on ice, were transferred to the laboratory within 45 minutes; tissues were separated, stripped of fat and extraneous materials, washed a

few times with physiological saline and then blotted. Tissue extracts were prepared by freezing 0.5 g. of the sample in liquid nitrogen, homogenizing with a hand-homogenizer, and suspending the homogenate in 4 millilitres of 0.025 M sodium phosphate buffer, pH 7.2. The suspensions were centrifuged for 15 minutes at 4000 g in an MSE high-speed refrigerated centrifuge. The supernatants were used as the source of enzyme. The activity of arginase was measured by modified the p-nitrophenyl glyoxal (PNPG) method (RAZMI, 1991). Arginine reacted with PNPG in 0.1 mol/lit sodium hydroxide to produce a coloured compound which absorbed maximally at 480 nm. Protein concentration in the crude extracts of different tissues was measured by the method of LOWRY et al. (1953). Data were analyzed statistically by analysis of variance (ANOVA). Differences between the means were statistically estimated by the Duncan test. All values were expressed in mean (\pm SEM) 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 the reproductive system are shown in Table 1.

Table 1. Mean (\pm SEM) of total soluble proteins and arginase activity of different parts of cattle reproductive system (n = 7)

Parts of reproductive system	Total soluble protein (mg/g of tissue)	Arginase activity (IU/g tissue)
Corpus luteum	31.75 (3.17)	0.92 (0.32)
Ovary	25.85 (1.22)	0.75 (0.22)
Uterine tube	22.21 (1.25)	0.93 (0.33)
Uterine horn (mucosa)	25.14 (1.88)	1.10 (0.22)
Uterine horn (muscle)	22.78 (1.09)	0.98 (0.23)
Uterine body (mucosa)	26.14 (2.13)	0.75 (0.29)
Uterine body (muscle)	22.07 (1.06)	0.76 (0.33)
Cervix (mucosa)	16.64 (1.84)	0.56 (0.34)
Cervix (muscle)	16.78 (1.37)	0.61 (0.36)
Vagina (mucosa)	19.85 (2.35)	0.77 (0.14)
Vagina (muscle)	19.00 (2.08)	0.62 (0.31)
Vestibula (mucosa)	15.85 (1.90)	0.13 (0.24)
Vestibula (muscle)	15.50 (1.70)	0.76 (0.37)

The highest TSP concentration was observed in corpus luteum and the same was lowest in the muscular layer of vestibula. Arginase activity was higher in the mucosal layer uterine horn and lower in the mucosal layer of vestibula. The arginase specific activity in different parts of cattle reproductive system is presented in Table 2. All tissues contained different amounts of arginase-specific activity. Highest arginase-specific activity (79.01 ± 13.20 IU/mg of protein) was observed in the mucosal layer of vestibula, which did not show significant difference with the mucosal layer of the uterine horn (49.45 ± 8.73 IU/mg of protein) and the muscular layer of the vestibula (49.04 ± 9.43 IU/mg of protein) but showed a significant difference with the remaining parts of the reproductive system.

Table 2. Mean (\pm SEM) ASA in extracts of different parts of the reproductive system in cattle (n = 7)

Parts of reproductive system	Specific activity $\times 10^{-3}$ of enzyme (IU/mg protein)
Uterine body (mucosa) ^b	28.81 (04.33)
Corpus luteum ^b	28.94 (06.02)
Ovary ^b	29.04 (06.38)
Vagina (muscle) ^b	32.65 (07.44)
Uterine tube ^b	33.57 (13.60)
Cervix (mucosa) ^b	41.78 (07.94)
Uterine horn (muscle) ^b	43.20 (07.58)
Uterine body (muscle) ^b	34.27 (07.37)
Cervix (muscle) ^b	36.12 (07.85)
Vagina (mucosa) ^b	39.01 (08.09)
Vestibula (mucosa) ^a	79.01 (13.20)

Values in same column with different superscripts have a statistically significant difference ($P < 0.05$)

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 is an enzyme which shares a common substrate with nitric oxide synthase (NOS), the

enzyme which synthesizes NO, the principal mediator of penile erection and clitoral 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 cattle reproductive system, with differing ranges of activity. The highest arginase specific activity was observed in the mucosal layer of vestibula. Low arginase specific activity in other parts of the reproductive system might be due to either low cell division and differentiation rate of these areas (PEGY, 1986) or to lower soluble proteins in tissues (ASA was obtained in IU/mg. of soluble proteins of each tissue). Signs of arginase activity have been detected in the genitalia of the female rabbit (WILSON, 2003). Arginase activity in the reproductive system is important from the 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 a high proliferation and differentiation rate in which NOS is mainly present in tissues which require vasodilation and have an arousal function (PEGY, 1986). High ASA in the vestibula obtained in this study may be due to the high proliferation and differentiation rate of cells in this part, or less participation in sexual arousal (CAMA, 2003; PEGY, 1986). NO is the principal mediator for penile erection and arginase may down-regulate its production in penile corpus cavernosum, causing alteration of normal penile homeostasis and erectile dysfunction in diabetic patients (BIVALACQUA et al., 2001). Administration of a small amount of arginase inhibitor increased blood flow to the genitalia of both male and female rabbits (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 clitoral corpus cavernosum (BURNETT, 1997) and vagina (HOYLE et al., 1996). Arginase II may co-localize in these tissues and inhibition of arginase in the female may enhance smooth muscle relaxation and sexual arousal (CAMA, 2003). The results of this study show that arginase is present in different parts of the cattle reproductive system, that it probably plays no significant role in ammonia detoxification, and that it might be important in polyamine biosynthesis, which is necessary for S phase of cell cycle (PEGY, 1986) or blood flow regulation in the reproductive system in balance with NOS.

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Received: 27 September 2003

Accepted: 14 January 2005

RAZMI, N., G. A. JELODAR, S. NAZIFI, A. DEGHANI: Aktivnost arginaze u organima za razmnožavanje goveda. *Vet. arhiv* 75, 31-38, 2005.

SAŽETAK

U istraživanje je bilo uključeno 7 zdravih krava. Odmah nakon klanja, životinjama su bili odstranjeni svi organi za razmnožavanje iz kojih su prikupljeni uzorci različitih tkiva: jajnici sa žutim tijelom, slojevi mišićnog i sluzničnog dijela iz roga maternice, tijela maternice, cerviksa, te vagine i vestibuluma. Za utvrđivanje specifične aktivnosti arginaze (ASA) te usporedbu te aktivnosti u različitim tkivima, primijenjena je preinačena paranitrophenylglyoxal (PNPG) metoda. Rezultati su pokazali da je najviša razina specifične aktivnosti arginaze ($79,01 \pm 13,20$ IU/mg bjelančevina) utvrđena u sluzničnom sloju vestibuluma. Navedena vrijednost nije bila statistički značajno različita u odnosu na sluznični sloj roga maternice ($49,45 \pm 8,73$) i mišićni sloj vestibuluma ($49,04 \pm 9,43$) već samo u odnosu na ostale dijelova organa za razmnožavanje ($P < 0,05$). Rezultati istraživanja pokazuju da je u organima za razmnožavanje goveda prisutna različita aktivnost arginaze što se može povezati s različitim stupnjem proliferacije stanica, diferencijacijom ili s drugim nepoznatim fiziološkim odnosno biokemijskim aktivnostima ovog enzima.

Cljučne riječi: arginaza, organi za razmnožavanje, govedo
