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Activity of steroid sulphatase and estrogen sulphotransferase in the boar epididymis during the postpubertal period

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ABSTRACT

The activities of steroid sulphatase (StS) and estrogen sulphotransferase (EST) were determined in the epididymis of 18 boars. The animals were divided into three groups ($n = 6$) according to age (8, 12 and 16 months). The boars were anesthetized and castrated. The tissue samples of different epididymal parts (caput, corpus and cauda) were taken and homogenized. Activities of StS and EST were assessed using ^{3}H-estrone sulphate (^{3}H-E1S) and free ^{3}H-oestrone (^{3}H-E1) as substrates, respectively. The substrate conversion rates after 60 min of incubation were 51.25% for ^{3}H-E1S and 45.65% for ^{3}H-E1. The activities of both enzymes were significantly higher in the caput epididymis compared to the cauda epididymis ($p < 0.05$). A significant age-dependent increase of StS and EST activities ($p < 0.05$) was observed. These results suggest that the availability of estrogens in the boar epididymis may be locally controlled also by StS and EST. The age-dependent increase of StS and EST activities may be related to the process of "biochemical maturation" of the reproductive system during the postpubertal period.

1. Introduction

The spermatozoa become motile and gain the fertilizing ability during epididymal storage and transit. The boar testes produce large amounts of estrogens, which also may be found in semen. The main estrogen secreted by testis is estrone, predominantly in its sulphoconjugated form [1–4]. Estrogens play an important role in the regulation of development and function of the reproductive tract in the boar, for review see [5,6]. Estrogens in mice are involved in reabsorption of epididymal fluid, a process essential for the maintenance of normal male fertility [7,8]. In estrogen receptor alpha knockout (αERKO) mice and mice treated with estrogen receptor (ER) antagonist (ICI 182780), cauda epididymis sperm number, sperm motility and sperm fertilizing ability were reduced compared to intact/control animals [9]. Boars treated weekly with letrozole, an aromatase inhibitor, showed a reduced cauda sperm fertilizing ability and sperm number [10]. Morphological development of the caput and corpus epididymis was delayed in letrozole-treated boars [11].

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In the male reproductive tract, the main source of estrogens is testes. Estrogens are delivered from the testis to the epididymis through the tubules and via the blood. Epididymal cells [12–14] and spermatozoa [15,16] can also be a potential source of estrogens. Estrogens affect cells by binding to their specific receptors in target tissues. Both ERα and ERβ were found to be expressed in all three epididymal regions (caput, corpus and cauda) in boars from 2 to 8 months of age. However, in the older boars (1.5–2.5 years) ERβ was not demonstrated in the cauda [17]. Since only free estrogens are biologically active, whilst conjugated estrogens do not interact with the receptor [18], it was hypothesized that the availability of biologically active estrogens depends partially on locally expressed steroid sulphatase (StS) and estrogen sulphotransferase (EST [3,19]). StS catalyzes desulphation (i.e. activation of conjugated estrogens) and EST catalyzes sulphoconjugation (i.e. inactivation of free estrogens). The concentration of free estrogens in the epididymal fluid decreased from caput to cauda epididymis of boars [20]. The tissue concentration of free estradiol did not significantly differ between the epididymal regions, however the regional differences in the concentration of estrogen conjugates in epididymal tissue were observed [17].

Studies on the activity of StS and EST in the boar epididymis are limited. High conversion level of free estrogen to estrogen conjugates was demonstrated for the epididymis of two 2-year-old boars [21]. Activities of StS and EST were detected in the epididymal tissue (caput and corpus together) of three 200-day-old boars [3]. Boars become sexually mature between 5 and 9 months of age [22]. After reaching sexual maturity, the reproductive system undergoes so called ''biochemical maturation'' lasting to the age of 18 months. During this period the increase of protein content and the activity of the antioxidant system were observed in seminal plasma [23,24]. Epididymal protein secretion increased from 1-month-old to more than 8-month-old boars [25]. The biochemical changes seem to be essential for the production of semen with high biological properties.

There is no data concerning desulphation and sulphoconjugation of estrogens in different epididymal regions of the boar during the postpubertal period. Therefore, the aim of this study was to determine the activity of StS and EST in all three epididymal regions of 8-, 12- and 16-month-old boars.

2. Materials and methods

The investigation was carried out on 18 Polish Landrace boars in a pig insemination station in the north-east of Poland. The animals were kept in individual pens and fed on standard diet. The animals were divided into three groups ($n = 6$) according to age (8, 12 and 16 months). The boars were anesthetized with azaperone (20 mg/kg i.m.) and ketamine (20 mg/kg i.v.) and castrated. The protocol was approved by the Local Ethics Commission for Animal Experiments. After castration, the epididymides were separated from the testes, placed in ice-cold phosphate buffer (PBS) and transported to the laboratory.

The epididymides were divided into three regions (caput, corpus and cauda [25]). The tissue samples of each epididymal region were collected and washed with saline to remove spermatozoa. The samples were cut into small pieces and washed with Ringer solution (Polfa, Warsaw, Poland) until a clear supernatant was obtained following centrifugation. After the final wash, 5 g of the tissue pulp was homogenized in 15 ml Ringer–Hepes buffer, consisting with Ringer solution and Hepes buffer (39:1; Sigma–Aldrich Co., St. Louis, MO, USA). The homogenate was filtered to remove connective tissue. All steps were performed on ice.

Activities of steroid sulphatase (StS) and estrogen sulphotransferase (EST) were assessed as was described for bovine placenta [19]. For determination of the StS activity, 1 ml of incubation medium (Ringer–Hepes buffer with 0.1% BSA; Sigma–Aldrich Co., St. Louis, MO, USA), the substrate (0.6 pmol ^{3}H-estrone sulphate [^{3}H-E_1S; NEN Science Products Inc., Brussels, Belgium] dissolved in 0.1 ml Ringer solution with 0.1% BSA) and 0.2 ml homogenate was added to 15 ml glass tubes (Wheaton Science Products, Millville, NJ, USA). The samples were incubated at 37 °C in a water bath with shaking for 1, 5, 15, 30 and 60 min. Control samples contained 1 ml incubation medium, the substrate and 0.2 ml incubation buffer instead of homogenate (medium blank) or heat inactivated homogenate (tissue blank). The controls were incubated for 60 min and the incubates were stored at −20 °C until assay. Each reaction was run in duplicates and the experiments were repeated four times.

The formed free ^{3}H-E_1 was extracted from the whole samples (incubates) with 2 ml × 3 ml toluene (Sigma–Aldrich Co., St. Louis, MO, USA). The samples were mixed on a overhead shaker for 20 min. The aqueous phase was frozen and the toluene phase was decanted. The pooled extracts containing ^{3}H-E_1 were dried and dissolved in scintillator (Perkin Elmer Inc., Waltham, MA, USA). The ^{3}H-radioactivity was measured with a liquid scintillation counter (LSA Tri-Carb 2800TR, Perkin Elmer Inc., Waltham, MA, USA). The ^{3}H-radioactivity in the remaining aqueous phase (^{3}H-E_1S) was also counted. The extraction efficiency was 87.9%. The activity of StS was expressed as the percentage of substrate conversion. The total ^{3}H-radioactivity in toluene extracts and aqueous phases was set as 100% value for calculation of conversion rate.

For determination of EST activity, ^{3}H-E_1 (0.6 pmol; NEN Science Products Inc., Brussels, Belgium) was used as substrate. Additionally, 0.5 μmol of 4-nitrophenylsulphate-potassium salt (Sigma–Aldrich Co., St. Louis, MO, USA) for inhibition of StS and 50 mmol of 3′-phosphoadenosine-5′-phosphosulphate (Sigma–Aldrich Co., St. Louis, MO, USA) as a co-factor were used for the reaction. The formed ^{3}H-E_1S was measured after removal of free ^{3}H-E_1 by extraction with toluene (Sigma–Aldrich Co., St. Louis, MO, USA). The samples were then hydrolyzed with 0.3 standard units of arylsulphatase (Merck KGaA, Darmstadt, Germany) and again extracted as described above.

Statistical analysis was performed on raw data using GraphPad PRISM program (GraphPad Software Inc., San Diego, CA, USA). The data were shown as a percentage of added radioactivity (mean ± SEM). The statistical significance of differences among various incubation times, epididymal regions or age groups was assessed using one-way ANOVA followed by Bonferroni's multiple comparison test. The level of significance was set at $p < 0.05$ for all analyses.