Journal of Steroid Biochemistry & Molecular Biology 143 (2014) 105–114

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry and Molecular Biology

journal homepage: www.elsevier.com/locate/jsbmb

Differential effects of estrogen exposure on arylsulfatase B, galactose-6-sulfatase, and steroid sulfatase in rat prostate development

Leo Feferman[a,b], Sumit Bhattacharyya[a,b], Lynn Birch[c], Gail S. Prins[c], Joanne K. Tobacman[a,b,*]

[a] Department of Medicine, University of Illinois at Chicago, Chicago, IL, United States
[b] Jesse Brown VA Medical Center, Chicago, IL, United States
[c] Department of Urology, University of Illinois at Chicago, Chicago, IL, United States

ARTICLE INFO

Article history:
Received 20 September 2013
Received in revised form
17 December 2013
Accepted 30 January 2014

Keywords:
Estradiol
Steroid sulfatase
Chondroitin sulfate
Versican
Glycosaminoglycans
Sulfatase

ABSTRACT

Sulfatase enzymes remove sulfate groups from sulfated steroid hormones, including estrone-sulfate and dehydroepiandrosterone-sulfate, and from sulfated glycosaminoglycans (GAGs), including chondroitin sulfates and heparan sulfate. The enzymes N-acetylgalactosamine-4-sulfatase (arylsulfatase B; ARSB) and N-acetylgalactosamine-6-sulfatase (GALNS), which remove sulfate groups from the sulfated GAGs chondroitin 4-sulfate (C4S) and chondroitin 6-sulfate, respectively, have not been studied in prostate development previously. In this report, the endogenous variation and the impact of exogenous estradiol benzoate on the immunohistochemistry and activity of ARSB and GALNS in post-natal (days 1–30) ventral rat prostate are presented, as well as measurements of steroid sulfatase activity (STS), C4S, total sulfated GAGs, and versican, an extracellular matrix proteoglycan with chondroitin sulfate attachments on days 5 and 30. Findings demonstrate distinct and reciprocal localization of ARSB and GALNS, with ARSB predominant in the stroma and GALNS predominant in the epithelium. Control ARSB activity increased significantly between days 5 and 30, but following estrogen exposure (estradiol benzoate 25 μg in 25 μl sesame oil subcutaneously on days 1, 3, and 5), activity was reduced and the observed increase on day 30 was inhibited. However, estrogen treatment did not inhibit the increase in GALNS activity between days 5 and 30, and reduced STS activity by 50% on both days 5 and 30 compared to vehicle control. Sulfated GAGs, C4S, and the extracellular matrix proteoglycan versican declined between days 5 and 30 in the control, but these declines were inhibited following estrogen. Study findings indicate distinct variation in expression and activity of sulfatases, sulfated GAGs, C4S, and versican in the process of normal prostate development, and disruption of these events by exogenous estrogen.

Published by Elsevier Ltd.

1. Introduction

Sulfatase enzymes comprise a group of cellular and extracellular enzymes that are key regulators of the degradation of sulfated glycosaminoglycans, including chondroitin sulfate, dermatan sulfate, keratan sulfate, heparin, and heparan sulfate, and of sulfated steroids, including estrone sulfate and dehydroepiandrosterone sulfate. Arylsulfatase B (ARSB; N-acetylgalactosamine-4-sulfatase) and N-acetylgalactosamine-6-sulfatase (GALNS) are enzymes that remove sulfate groups from the sulfated glycosaminoglycans (GAGs) chondroitin 4-sulfate (C4S) and chondroitin 6-sulfate (C6S), respectively. Deficiency of ARSB or GALNS leads to accumulation of sulfated glycosaminoglycans, resulting in the lysosomal storage diseases Mucopolysaccharidosis (MPS) VI from ARSB deficiency and MPS IVA from GALNS deficiency. Removal of the 4-sulfate group is required for the degradation of C4S, and removal of the sulfate group is required for activity of steroid hormones. Prior experiments in human mammary cell lines demonstrated that (1) estrone (100 pg/ml) and estradiol (200 pg/ml) exposure significantly reduced activity of steroid sulfatase (STS) and ARSB, but not of GALNS in MCF-7 and T47D cells; and (2) GALNS activity was significantly higher in primary mammary epithelial cells, whereas ARSB and STS activity were higher in primary myoepithelial cells [1]. In this report, we present in vivo data that expand on these prior in vitro observations of sulfatase activity and response to exogenous estrogen and report the endogenous sulfatase activity

* Corresponding author at: Department of Medicine, University of Illinois at Chicago, 840 S. Wood Street, CSN 440, M/C 718, Chicago, IL 60612, United States. Tel.: +1 312 569 7826; fax: +1 312 413 8283.
E-mail addresses: jkt@uic.edu, tobacmanj@aol.com (J.K. Tobacman).

0960-0760/$ – see front matter. Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.jsbmb.2014.01.017

and expression and the impact of estrogen exposure on sulfatase activity in a rodent model of prostate development.

This study, by elucidating differences between activity and distribution of sulfatases in both the native prostate and following estrogen treatment, presents a novel approach to prostate morphogenesis, focusing on activity and expression of sulfatases that modify chondroitin sulfate and on the associated changes in total sulfated GAGs, C4S, and the ECM proteoglycan versican. Decline in ARSB activity has been shown in malignant prostate tissue, malignant colon tissue, malignant mammary cells, and metastatic colonic cell lines [1–6]. In human prostate cells, decline in ARSB produced increases in total sulfated GAGs, C4S, and versican [2,3]. Previously, both versican and chondroitin sulfate have been identified as biomarkers of more aggressive prostate cancer [7,8]. Versican is a large, aggregating extracellular matrix proteoglycan with chondroitin sulfate attachments that interacts with multiple cell surface receptors and recruits signaling molecules to the cell surface, thus modulating signaling pathways and stromal–epithelial interactions [3,9–11].

The profiles of ARSB, GALNS, and STS enzyme activity and localization and of total sulfated GAGs, C4S and versican in the developing rat prostate have not previously been addressed. In the rodent, prostate development occurs predominantly in early postnatal life, and high doses of exogenous natural estrogens caused developmental and differentiation defects in the adult prostate [12]. In this report, measurements of ARSB, GALNS, and steroid sulfatase (STS) activity, total sulfated GAGs, C4S, and the proteoglycan versican on days 5 and 30 of post-natal development in rat prostate tissue are presented. The impact of an intermediate dose of exogenous estradiol benzoate (25 μg) exposure on days 1,3, and 5 of post-natal life on these parameters and on ARSB and GALNS immunohistochemistry on post-natal days 1, 3, 6, 10, 15, and 30 is presented. Since decline in ARSB activity has been associated with prostate neoplasia [2,3], insight into the interactions among estrogen, ARSB, GALNS, and STS in prostate development may lead to better understanding of the effects of steroid hormones on stromal–epithelial interactions and on mechanisms of prostate carcinogenesis.

2. Methods

2.1. Animal care and treatment

All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Illinois at Chicago. 96 male Sprague-Dawley rats (Zivic-Miller, Pittsburgh, PA) were treated with subcutaneous injections of either 25 μl sesame oil alone (controls) or high-dose estrogen (25 μg estradiol benzoate in 25 μl sesame oil) on post-natal days 1, 3, and 5 as previously described [12,13]. Twelve animals (6 control and 6 estrogen-treated) were euthanized on days 1, 3, 6, 10, 15, and 30, and the prostates were removed, formalin-fixed, paraffin-embedded and sectioned, as previously detailed [14–17]. In addition, prostate tissue from similarly estrogen-treated and vehicle control animals was obtained from rats sacrificed on day 5 (total $n = 12$) and on day 30 (total $n = 12$).

2.2. ARSB and GALNS immunohistochemistry

Sections of the estrogen-treated and control ventral prostate tissues were mounted on the same slide and immunostained. Antigen retrieval was done with pH 6.1 citrate buffer at 90 °C for 40 min. For ARSB, the slides were incubated overnight with rabbit polyclonal antibody (1:50; Open Biosystems, Huntsville, AL), then for 1 h with anti-rabbit IgG-HRP at 1:1000 dilution. Color was developed with 3,3′-diaminobenzidine (DAB) for 5 min. For GALNS, slides were incubated overnight with rabbit polyclonal antibody (1:200; Open Biosystems, Huntsville, AL), followed by anti-rabbit IgG-HRP at 1:1000 dilution for 1 h and DAB for 5 min and counterstained with hematoxylin. The sections were blocked with a serum-free Universal Blocking Solution from Biocare Medical (Concord, CA). Negative control used normal rabbit IgG diluted in buffer at the same dilution as used for the primary antibody (1:50 for ARSB; 1:200 for GALNS), and all other staining procedures were similar. Positive controls were sections of adult human prostate. Digitized images were obtained with QCapture software (QImaging, Surrey, BC, Canada) at 20× magnification. Background and brightness were modified with GIMP Portable software (Portable Apps, New York, NY) or with Adobe Photoshop (CS2). Qualitative assessments of intensity and distribution of ARSB and GALNS immunostaining were made by three study investigators (LF, GSP, JKT), who agreed on the descriptive findings that are reported.

2.3. Determination of ARSB, GALNS, and STS activity in prostate tissue homogenates

Homogenates were prepared from ventral prostate tissue of estrogen-treated and control rats on days 5 and day 30 of post-natal development (total $n = 24$). Arylsulfatase B (ARSB; N-acetylgalactosamine-4-sulfatase) activity measurements were performed using a fluorometric assay, as previously, with 20 μl of tissue homogenate, 80 μl of assay buffer (0.05 M Na acetate buffer, pH 5.6), and 100 μl of substrate [5 mM 4-methylumbelliferyl sulfate (MUS) in assay buffer] in wells of a microplate [1,18,19]. The microplate was incubated for 30 min at 37 °C, and the reaction was stopped by 150 μl of stop buffer (Glycine-Carbonate buffer, pH 10.7), and fluorescence was measured at 360 nm (excitation) and 465 nm (emission) in a microplate reader (FLUOstar, BMG, Cary, North Carolina). Activity was expressed as nmol/mg protein/h, based on a standard curve for ARSB activity prepared with known quantities of 4-methylumbelliferyl at pH 5.6. Protein was determined by total protein assay kit (Pierce, Thermo Fisher Scientific, Inc., Rockford, IL).

For STS determination, six 20 μl prostate tissue homogenates were incubated with 80 μl of assay buffer (0.5 M Tris–Cl Buffer, pH 7.5), and 100 μl of substrate (0.5 mM 4-MUS in assay buffer), as previously detailed [1,18,20]. The reaction mixture was incubated for 4 h at 37 °C, at which time 100 μl of stop buffer (1 M Tris–Cl buffer at pH 10.4) was added, and fluorescence was measured in a microplate reader (BMG). Activity was expressed as nmol/mg protein/h, and was derived from a standard curve prepared with known quantities of 4-methylumbelliferyl at pH 7.5. Protein was determined by total protein assay kit (Pierce).

The measurement of galactose-6-sulfatase (GALNS) activity was performed using 5 μl of tissue homogenate made in ddH_2O by sonication with metal tip, combined with 5 μl 0.2% heat-inactivated BSA (or 10 μl of 0.2% heat-inactivated BSA for blank) and 20 μl of substrate [10 mM 4-methylumbelliferyl-ß-D-galactoside-6-sulfateNH_4 (MU-ßGal-6S)] in substrate buffer (0.1 M sodium acetate/0.1 M acetic acid at pH 4.3 with 0.1 M NaCl, 5 mM Pb-acetate (1.9 mg/ml) and 0.02% Na-azide) in wells of a microplate [1,18,21]. After incubation for 17 h at 37 °C, 5 μl 0.9 M Na-phosphate buffer at pH 4.3 with 0.02% Na-azide was added, as well as 10 μl of 10 U ß-D-galactoside galactohydrolase (Sigma)/ml 0.2% heat-inactivated BSA. After incubation for 2 h at 37 °C, 200 μl of stop buffer (0.5 M $NaHCO_3$/0.5 M Na_2CO_3 at pH 10.7 with 0.025% Triton-X-100) was added, and readings of fluorescence were taken at 360 nm and 465 nm in a plate reader (BMG). GALNS activity was expressed as nmol/mg protein/h, and protein was determined by total protein assay kit (Pierce).