Life Sciences 85 (2009) 11–18

Contents lists available at ScienceDirect

Life Sciences

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

Impairment in protein expression profile of testicular steroid receptor coregulators in male rat offspring perinatally exposed to Bisphenol A

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ARTICLE INFO

Article history:
Received 10 October 2008
Accepted 14 April 2009

Keywords:
Endocrine disruptor
Bisphenol A
Perinatal exposure
Steroid receptors
Coactivators
Corepressors

ABSTRACT

Aims: Steroid hormones and steroid receptors (SRs) play a crucial role in spermatogenesis. Steroid receptor coregulators are the major determinants of SR functioning, and any alteration in their expression is known to be associated with impaired spermatogenesis. Since Bisphenol A (BPA) exposure leads to an impairment of spermatogenesis, we hypothesized that this effect could be associated with the altered expression of steroid receptors and their coregulators in the testes. The present study describes the effect of perinatal exposure of rats to BPA on the expression profile of testicular steroid receptor coregulators in the F_1 generation. These effects were further studied in the F_2 and F_3 generations to determine vertical transmission.
Main methods: Pregnant female rats (F_0) were gavaged daily with BPA (1.2 and 2.4 μg/kg bw) (or vehicles for controls) from gestation day 12 through postnatal day (PND) 21 to obtain the F_1 and subsequent F_2 and F_3 generations. Immunohistochemical localization of steroid receptor coactivator-1 (SRC-1), G-receptor integrating protein-1 (GRIP-1), p300/CBP/cointegrator-associated protein (p/CIP) and nuclear corepressor (NCoR) was carried out in the testes of F_1, F_2 and F_3 generation adult rats.
Key findings: A significant reduction in the expression of SRC-1 and NCoR, with a parallel increase in the expression of p/CIP and GRIP-1, was observed in the testes of rats exposed perinatally to BPA. Surprisingly, a similar pattern was observed in the testes of F_2 and F_3 rats.
Significance: Perinatal exposure of male rats to BPA leads to transgenerational perturbations in the expression profile of testicular steroid receptor coregulators.

Introduction

Bisphenol A (BPA), an endocrine disruptor (ED), is used in the manufacture of polycarbonate plastic and epoxy resins. Sources of BPA exposure are numerous, as it easily leaches from the inner lining of tin cans and microwave containers during heating, from dental sealant into saliva, and into beverages from repeated usage or contact with any acidic/alkaline content in polycarbonate bottles (Brotons et al. 1995; Olea et al. 1996).

There is a plethora of evidence indicating that EDs can act on target tissues (mainly steroid responsive tissues), thereby disrupting their normal physiological function, development and reproduction (Nishihara et al. 2003; Vom saal and Hughes 2005). Studies of perinatal exposure of BPA have documented its effect on target organs like the mammary gland and brain, resulting in changes in morphogenesis and disturbances in sexual and mating behavior (Farabollini et al. 2002; Negishi et al. 2004; Muñoz-de-Toro et al. 2005). Thus, the perinatal period is one of the critical stages for organ differentiation and development, and any disturbance following exposure to BPA can influence development and adult function. However, no information is currently available on the effects of BPA in the male offspring of rats exposed to environmentally relevant doses during the critical perinatal period of sexual differentiation and development of the reproductive tract. Studies in our lab have indicated that perinatal exposure of male rats to BPA leads to an impairment in fertility, spermatogenesis and alterations in the testicular steroid receptor expression profile in the adult F_1 generation males (Unpublished data; manuscript submitted).

Steroid and steroid receptor (SR) interactions play a crucial role in spermatogenesis. Coregulators are the bridging apparatus between the steroid receptors and transcriptional machinery, and play a pivotal biological role in regulating the reproductive axis (Mckenna et al. 1999). In a ligand dependent manner, the steroid receptor coregulators can either activate (coactivators) or repress (corepressors) gene expression (O'Malley 2007). The coactivators include the steroid receptor coactivator (SRC) family and the corepressors, including the nuclear corepressor (NCoR), which coordinately regulates the function of the SRs (Xu and O'Malley 2002). SRC knockouts have exhibited hypofertility, with impairment in spermatogenesis. These results document the pivotal role played by the SRC family in spermatogenesis (Xu and Li 2003; Molenda et al. 2003). Spermatogenesis is

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doi:10.1016/j.lfs.2009.04.005

morphologically represented as a spermatogenic cycle, and based on the association of various cell types within seminiferous tubules, a particular development stage is assigned to that tubule (I–XIV). Staging represents the dynamic changes in the spermatogenic cycle. Each testicular stage represents a different combination of germ cell development and is believed to be under the regulatory action of hormonal control. Determining the stage-specific expression of any protein is necessary for understanding its role in the regulation of spermatogenesis. Thus, staging plays a pivotal role in revealing the effects of any toxicant (Lanning et al. 2002).

The growing literature on the reproductive toxic effects of BPA, and especially on its influence on spermatogenesis, lead us to hypothesize that BPA exposure could be associated with an impairment in the expression profile of steroid receptors and their coregulators in the testes. Thus, the objective of the present study was to evaluate the effect of perinatal exposure of rats to environmentally relevant doses of BPA on the stage-specific expression profile of steroid receptor coregulators in the adult testes of F_1 male offspring. Additionally, we aimed to study whether these effects are transmitted vertically in subsequent F_2 and F_3 generations. Perturbations in the expression profile of steroid receptor coregulators will have a significant influence on the functions of the steroid responsive organs during adulthood, and will eventually have an impact on fertility.

Materials and methods

Antibodies and immunohistochemistry reagents

Goat polyclonal antibodies against steroid receptor coactivator-1 (SRC-1, Sc-6098), G-receptor integrating protein (GRIP-1, also known as SRC-2, Sc-8996), p300/CBP/cointegrator-associated protein (p/CIP, also known as SRC-3; Sc-6974) and nuclear corepressor (NCoR, Sc-816) were purchased from Santacruz (Burlingame, CA, USA). Blocking solution and secondary antibodies were used from the Immunocruz goat staining system (Sc-2053) from Santacruz Biotechnology Inc., Burlingame, CA. Antigenic peptides of SRC-1, GRIP-1, p/CIP and NCoR were purchased from Santacruz (Burlingame, CA, USA).

Dose selection and preparation

The selection of BPA doses (2.4 and 1.2 μg) for the current study were based on a paper published by Akingbemi et al. (2004), as well as our own study wherein a range of lower doses (0.6–10 μg) of BPA were evaluated for reproductive toxicity. It was observed that 1.2 and 2.4 μg doses of BPA were the lowest effective doses for impairing male fertility (unpublished data, manuscript submitted).

BPA (>99.8% Sigma Chemical Co., St. Louis, MO) was dissolved in ethyl alcohol (99% pure) and was then diluted in sesame oil to obtain the desired concentrations of 2.4 and 1.2 μg/ml of BPA. The final concentration of alcohol was kept below 0.15% of the final solution, as this was previously found to cause no adverse events in animals. Control groups received sesame oil with the same alcohol concentration. An additional control group that had received sesame oil without ethanol had been incorporated into the present study. Dose formulations were stored in an amber colored bottle at 37 °C overnight, and were subsequently kept at room temperature throughout the study and mixed thoroughly before use.

Animal handling and care

Holtzman strain male and female rats (9 weeks of age, weighing ~250 g) that were randomly bred in our animal house were used in the present study. The animals were kept in cages with autoclaved paddy husk for bedding and maintained under controlled temperature (23 ± 1 °C) and humidity (55 ± 5%), and in a 14 h light/10 h dark cycle. Animals were fed a diet of soy-free, in-house-prepared rat pellets (consisting of crude protein, fiber and nitrogen free extract) and water (purified by UV and reverse osmosis) ad libitum throughout the study. The quality of food and water provided was routinely monitored by qualitative and quantitative proximal analysis. Ethical clearance for the use of animals in the study was obtained from the Institutional Animal Ethics Committee prior to the initiation of the study, and experiments were performed in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA), India.

Experimental design

Experimental rats were bred by cohabitating one proven fertile male rat with two proestrous female rats. The presence of sperm in the vaginal smear of the female was considered as day 0 of gestation (GD 0). The copulated female rats (F_0) were randomly distributed into the following three groups (8 rats/group). Group I: vehicle control, Group II: BPA 1.2 μg/kg/day and Group III: BPA 2.4 μg/kg/day. These F_0 dams were gavaged once daily from gestation day 12 through postnatal day (PND) 21. Doses were administered according to daily changes in body weight. After parturition, F_1 litters were weighed and sexed, according to anogenital distance (AGD). To further ensure that each dam shared the same lactational burden, 4–5 male pups/litter of the F_1 generation were left with lactating F_0 mothers in each group until PND 21. All animals were weaned on PND 22 and subsequently housed separately.

On PND 75, randomly selected F_1 males ($n = 24$ from each group) were cohabitated with normal cycling adult females during the proestrous phase. These copulated females were then allowed to deliver, and F_2 male offspring were weaned and housed separately until adulthood. A similar experimental protocol for mating was followed for these F_2 generation males to obtain the subsequent F_3 generation males. In the experimental design, only F_1 generation offspring received direct BPA exposure during the gestation and lactation periods, whereas the F_2 and F_3 generation did not receive any BPA treatment. Male rats from the F_1, F_2, and F_3 generations ($n = 24$ per group/generation) from each of the experimental and control groups were sacrificed on PND 125. The testes were dissected out and fixed in 10% neutral buffered formalin for 48 h and processed in an automatic tissue processor (Leica ASP 200, Germany). To determine stages of spermatogenesis in the testicular sections, paraffin-embedded tissue blocks were sectioned (5 μm) and utilized for Hematoxylin–Eosin staining.

Staging of seminiferous tubules

Due to the pivotal role played by staging, any perturbations in the stage-dependent expression pattern of specific proteins would indicate the impairment of its function associated with that stage. Thus, we have performed this study based on the stage-specific expression of key regulatory molecules involved in the process of spermatogenesis. The staging of each tubule was carried out on Hematoxylin–Eosin stained testicular sections according to the standard published criteria (Hess 1990). Concurrent serial sections from paraffin-embedded testicular blocks wherein stages were determined were utilized for immunohistochemical localization studies.

Immunohistochemical localization studies

Immunostaining for the expression of steroid receptor coactivators (SRC-1, GRIP-1 and pCIP) and corepressors (NCoR) was performed on testicular sections mounted on poly-L-lysine-coated slides using the Immunocruz goat staining system. Testicular sections were dewaxed in xylene and rehydrated in a series of ethanol grades. After initial blocking for endogenous peroxidase activity, all slides were subjected to antigen retrieval. Antigen retrieval was carried out by heating the sections in 0.1 M Citrate buffer (pH 6.0) in a microwave for 15 min (750 W). The slides were then left to stand undisturbed for 20 min,