Expression Pattern of Sulfated Glycoprotein-2 (SGP-2) mRNA in Rat Testes Exposed to Endocrine Disruptors

Jung-Min YON1), Dong Hoon KWAK1), Young Kwang CHO1), Se-Ra LEE1), Yan JIN1), In-Jeoung BAEK1), Jeung Eun LEE1), Sang-Soep NAHM2), Young-Kug CHOO3), Beom Jun LEE1), Young Won YUN1) and Sang-Yoon NAM1)

1)College of Veterinary Medicine and Research Institute of Veterinary Medicine, Chungbuk National University, 12 Gaeshin-dong, Heungduk-gu, Cheongju 361-763, 2)College of Veterinary Medicine, Konkuk University, 1 Hwayang-dong, Giwang-gu, Seoul 143-701 and 3)Department of Biological Science, College of Natural Sciences, Wonkwang University, Iksan, Jeonbuk 570-749, Korea

Abstract. Sulfated glycoprotein-2 (SGP-2) is secreted in Sertoli cells and epididymal epithelial cells and plays important roles in the regulation of spermatogenesis and sperm maturation. To investigate whether endocrine disruptors affect spermatogenesis through an SGP-2-dependent mechanism, daily oral doses of testosterone (50, 200 and 1,000 µg/kg), flutamide (1, 5 and 25 mg/kg), ketoconazole (0.2, 1, 5 and 25 mg/kg), diethylhexylphthalate (10, 50 and 250 mg/kg), nonylphenol (10, 50, 100 and 250 mg/kg), octylphenol (10, 50 and 250 mg/kg), diethylstilbestrol (10, 20 and 40 µg/kg) or corn oil (control) were administered to 5 week-old, male Sprague-Dawley rats for 3 weeks. Following treatment with these endocrine disruptors, testicular expression of SGP-2 mRNA was analyzed using reverse transcription-polymerase chain reaction. Compared with the control, the lowest dose of testosterone (50 µg/kg/day) significantly increased expression of SGP-2 mRNA, whereas 200 and 1,000 µg/kg/day testosterone significantly decreased the expression (P<0.05). Flutamide, ketoconazole, diethylhexylphthalate, nonylphenol, octylphenol and diethylstilbestrol significantly decreased SGP-2 mRNA expression in testes at all doses studied, with the exception of 1 mg/kg/day flutamide (P<0.05). These results suggest that endocrine disruptors might decrease spermatogenesis in testes by decreasing expression of SGP-2 mRNA.

Key words: Endocrine disruptors, Sertoli cells, Spermatogenesis, Sulfated glycoprotein-2, Testis

Spermatogenesis is a testosterone-dependent process [1]. In mammalian testes, spermatogenesis occurs in Sertoli cells [2]. Sertoli cells and epididymal epithelial principal cells have been shown to synthesize and secrete a number of glycoproteins that have been suggested to play important roles in the regulation of spermatogenesis and sperm maturation [3]. One of the major proteins secreted by both Sertoli cells and epididymal epithelial cells is sulfated glycoprotein-2 (SGP-2) [4]. SGP-2 is a heavily glycosylated protein composed of two monomers that arise from a proteolytically-cleaved, single chain polypeptide precursor [5]. In the reproductive tract, immunofluorescence has shown that SGP-2 is localized in the cytoplasm of Sertoli cells and in the heads and tails of spermatoozoa [6]. SGP-2 is a component of both bovine adrenal chromaffin granules and human neuroendocrine
secretary granules and is induced in porcine smooth muscles cells during differentiation in vitro [7, 8]. High levels of SGP-2 have also been found in the brain after biological insults such as Alzheimer’s disease and hippocampal lesions [9, 10]. The SGP-2 protein has been considered to be a possible participant in a number of biological activities, including inhibition of the C’ cascade, protection of cells from injurious environments, mediation of apoptosis, lipid metabolism and transport, remodeling of cell membranes, cell-cell interactions and differentiation and maturation of germ cells [11-13].

Many studies have shown that exposure to endocrine disruptors during prenatal or early postnatal life can disturb development of the endocrine system and organs that respond to endocrine signals [14]. Anti-androgenic endocrine disruptors inhibit spermatogenesis and exhibit other antigonadotropic effects. Flutamide (FM) is an antiandrogen that has been shown to have a relatively small and transient influence on spermatogenesis. FM inhibits the initial step of spermatogenesis, resulting in reduced sperm counts in rats [15]. Ketoconazole (KC) has less of an effect on spermatogenesis, but more readily decreases epididymal sperm number and motility, increases the proportion of abnormal sperm [16] and induces reversible immobilization of mature spermatozoa in the post-testicular sex organs [17]. Diethylhexylphthalate (DEHP) has been shown to produce seminiferous tubule degeneration, resulting in testicular atrophy [18]. Octylphenol (OP) has been shown to profoundly suppress testicular function in adult male rats, as evidenced by low levels of circulating testosterone, suppression of spermatogenesis [19], reduced sperm counts and testicular atrophy [20] following treatment with OP. Nonylphenol (NP) has been shown to act directly on male reproductive tissues [21] to decrease testicular size [22]. Diethylstilbestrol (DES) has been shown to inhibit testicular formation in rats, resulting in abnormal semen production and infertility [23, 24].

The Sertoli cell is the only cell type shown to have a significant level of SGP-2 mRNA in the testes by in situ hybridization [25]. Plotton et al. (2005) reported that the amount of SGP-2 (clusterin) mRNA is a good marker of the amount of mRNA of Sertoli cell origin in the testes of post pubertal rats [26]. In the present study, we examined the effects of various endocrine disruptors on spermatogenesis using SGP-2 mRNA expression as a biomarker for spermatogenesis in the Sertoli cells of rat testes.

Materials and Methods

Animals and pharmacological treatments

Four-week-old Sprague-Dawley rats (Samtaco Inc., Gyeonggido, Korea) were housed for 1 week in polycarbonate cages with a temperature of 21 ± 2 C, a relative humidity of 50 ± 10% and a 12-h light/dark cycle. The animals were fed standard rat pellets (Purina Inc., Gyeonggido, Korea), and water ad libitum. All experiments were approved and carried out according to the “Guide for Care and Use of Animals” (Chungbuk National University Animal Care Committee according to NIH #86-23).

The endocrine disruptors testosterone propionate (50, 200 and 1,000 µg/kg), ketoconazole (KC; 0.2, 1, 5 and 25 mg/kg), diethylhexylphthalate (DEHP; 10, 50 and 250 mg/kg), octylphenol (OP; 10, 50 and 250 mg/kg), diethylstilbestrol (DES; 10, 20 and 40 µg/kg), nonylphenol (NP; 10, 50, 100 and 250 mg/kg) and flutamide (FM; 1, 5 and 25 mg/kg) were orally-administrated to the rats daily for 3 weeks. The control group was treated with corn oil daily. The rats were then sacrificed at 8 weeks of age under pentobarbital anesthesia. Each group contained 10 rats. Testes were dissected, immediately frozen with liquid nitrogen and stored at –70 C. All chemicals were obtained from Sigma-Aldrich (St.Louis, MO, USA).

Total RNA extraction and reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was extracted from testes using TRIzol Reagent (Life Technologies, Gaithersburg, MD, USA) according to the manufacturer’s instructions. The RNA pellet obtained in the final step was dissolved in 50 µl of sterile diethylpyrocarbonate (DEPC) treated water, and its concentration was determined using a UV spectrophotometer at 260 nm. The RNA was kept in DEPC-treated water at –70 C until use. Reverse transcription of mRNA and amplification of cDNA were performed using a Peltier Thermal Cycler (MJ Research, Waltham, MA, USA).

A cDNA Synthesis Kit (Roche Diagnostics, Mannheim, Germany) was used to generate cDNA from 1.0 µg total RNA according to the manufacturer’s instructions. The primer sets used for amplification of SGP-2 (GenBank accession number: X13231)
EFFECTS OF ENDOCRINE DISRUPTORS ON SGP-2 mRNA EXPRESSION

Reverse transcription-polymerase chain reaction (RT-PCR) experiments showing the expression of sulfated glycoprotein-2 (SGP-2) and GAPDH (internal control) mRNA in the testes of testosterone-treated Sprague-Dawley rats. Values represent means ± SD (n=10). *P<0.05: compared with the control.

Results

Daily treatment with endocrine disruptors resulted in a general decrease in SGP-2 expression, except for treatment with testosterone. A dose of 50 µg/kg/day testosterone increased the expression of SGP-2 mRNA. However, larger doses of testosterone (200 and 1,000 µg/kg/day) significantly decreased SGP-2 mRNA expression by 80 and 50%, respectively (Fig. 1). FM decreased the SGP-2 mRNA level to 35-40% of the control level (Fig. 2), whereas KC dose-dependently decreased SGP-2 mRNA expression to 70-15% of the control level (Fig. 3). Treatment with DEHP significantly decreased SGP-2 mRNA expression to 10-40% of the control level (Fig. 4). SGP-2 mRNA expression was significantly decreased (50–85%) after treatment with OP. Specifically, 50 mg/kg of OP decreased SGP-2 mRNA expression to 10–40% of the control level (Fig. 5). In the NP-treated rats, SGP-2 mRNA expression was significantly reduced to 65–90% of the control level (Fig. 6). SGP-2 mRNA expression in the rats treated with DES was 15–38% lower than that of the control animals (Fig. 7).
The aim of this study was to examine the changes of SGP-2 mRNA expression in rat testes after treatment with various endocrine disruptors, including testosterone, FM, KC, DEHP, NP, OP and DES. The results presented here demonstrate that endocrine disruptors have different effects on SGP-2 mRNA expression in rat testes. SGP-2 mRNA is a well-established biomarker of steroidogenesis in Sertoli cells. Testosterone, the principal circulating androgen in the adult male, is essential for maintenance of spermatogenesis and expression of secondary sex characteristics. Thus,
production of testosterone is highly regulated by a negative feedback system in the testes [28].

This study showed that treatment with high doses of testosterone (200 and 1,000 µg/kg/day) decreased SGP-2 mRNA expression, while treatment with a lower dose of testosterone (50 µg/kg/day) increased SGP-2 mRNA expression compared with the control. This finding is consistent with previous reports that perinatal exposure of male rats to DEHP produces adverse effects in androgen-responsive tissues [32]. Specifically, 2,000 mg/kg/day DEHP has been previously shown to cause testicular atrophy [24], damage to the seminiferous epithelium [33] and reduced epididymal sperm density and motility [34] in rats.

OP is an alkylphenolic compound that is formed from some nonionic surfactants widely used in industrial detergents, such as plastic and petroil additives and dispensers for insecticides [35]. OP has been shown to decrease the expression of steroidogenesis factor-1 (SF-1) mRNA in fetal testes [36], to suppress gonadotropin secretion [37], and to have toxic effects on seminiferous cells in vitro [35]. In the present study, treatment with OP (10, 50, 100 and 250 mg/kg/day) resulted in a significant decrease in SGP-2 mRNA expression.

NP is an estrogenic compound that can be generated from alkylphenol ethoxylates widely used in the production of plastics, textiles, agricultural chemicals, household chemicals and cosmetics [38]. Several studies have reported that animals parenterally exposed to NP exhibit atrophied seminiferous tubules and decreased spermatogenesis [39]. In the present study, NP significantly decreased SGP-2 mRNA expression at all doses studied (10, 50, 100 and 250 mg/kg/day), suggesting that inhibition of SGP-2 mRNA expression by NP and OP might be due to functional defects in the testes, such as atrophied seminiferous and spermatogenic cells.

DES is an antiandrogenic compound that has been used therapeutically to prevent miscarriage and other pregnancy complications. It has been reported to reduce the activity of SF-1 in 17.5-day-old fetuses [40]. Other studies have reported that exposure of rats to DES results in a significant reduction in epididymal sperm count, atrophy of sperm motion, decrease in Sertoli cell number and increase in spermatogenic cell apoptosis [41]. In the present study, DES treatment inhibited SGP-2 mRNA expression in the testes, suggesting that decreased SGP-2 expression might inhibit spermatogenesis and reduce testosterone production in the testes.
In conclusion, this study showed that several endocrine disruptors changed the expression pattern of SGP-2 mRNA in the testes as determined by RT-PCR. These results suggest that the effects of endocrine disruptors on spermatogenesis in the testes might be mediated by an abnormal change in SGP-2 mRNA expression.

Acknowledgements

This work was supported by Korea Research Foundation Grants (KRF-2004-005-E00168 and KRF-2005-005-J15002) funded by the MOEHRD of Korea (Basic Research Promotion Fund).

References

19. Blake CA, Boockfor FR. Chronic administration of the environmental pollutant 4-tert-octylphenol to adult male rats interferes with the secretion of luteinizing hormone, follicle-stimulating hormone, prolactin, and testosterone. Biol Reprod 1997; 57: 255–266.
22. Lee PC, Arndt P, Nickels KC. Testicular abnormali-
EFFECTS OF ENDOCRINE DISRUPTORS ON SGP-2 mRNA EXPRESSION


