Biochemical and Biophysical Research Communications 422 (2012) 327–332

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

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

Testicular steroidogenesis is locally regulated by androgen via suppression of Nur77

Chin-Hee Song [1], Eun-Yeung Gong [1], Ji soo Park, Keesook Lee *

Hormone Research Center, School of Biological Sciences and Technology, Chonnam National University, Gwangju 500-757, Republic of Korea

ARTICLE INFO

Article history:
Received 27 April 2012
Available online 7 May 2012

Keywords:
Steroidogenesis
Local regulation
Androgen
Nur77
AR
Leydig cell

ABSTRACT

Steroidogenesis in the testis is regulated by a negative feedback mechanism through the hypothalamus–pituitary–testis axis. Recent studies suggest that besides this long-loop regulation, testicular steroidogenesis is also locally regulated by androgen. However, the molecular mechanism behind this additional regulatory pathway has been poorly addressed. In the present study, we demonstrate that liganded androgen receptor (AR) suppresses the transcriptional activity of Nur77 on steroidogenic enzyme gene promoters, affecting testicular steroidogenesis. AR physically interacts and colocalizes with Nur77 in the nucleus in the presence of androgen. AR inhibits Nur77 transactivation by competing mainly with coactivators such as SRC-1 for Nur77 binding. These results suggest that androgen, through binding to AR, directly acts as a signal inhibiting the expression of steroidogenic enzyme genes in Leydig cells, eventually resulting in decreased testicular steroidogenesis. These findings strongly support the hypothesis that androgen acts locally to regulate testicular steroidogenesis, and may provide its action mechanism.

1. Introduction

Androgens, which are mainly produced in testicular Leydig cells, are essential for male sexual differentiation, maintenance of spermatogenesis, and expression of male secondary sex characteristics. The biosynthesis of testosterone is dependent on both acute and chronic stimulation of Leydig cells by the pituitary luteinizing hormone (LH). Testosterone can regulate release of the hypothalamic gonadotropin-releasing hormone (GnRH), the signal that initially stimulates the pituitary to synthesize and release LH. This negative feedback loop is referred to as the hypothalamic–pituitary–gonadal (HPG) axis. Previous studies, however, have shown that testosterone represses the cAMP-induced *de novo* synthesis of P450c17 protein and accumulation of its mRNA in mouse Leydig cells, which occurs in an AR-dependent manner [1,2].

Steroidogenesis in Leydig cells is initiated with cholesterol transfer into the mitochondria, which is mediated by the steroidogenic acute regulatory (StAR) protein. In the mitochondria, cholesterol is converted to pregnenolone by the cholesterol side chain cleavage enzyme (P450scc). Pregnenolone is then transported to the endoplasmic reticulum and converted sequentially to progesterone by 3β-hydroxysteroid dehydrogenase/Δ5−Δ4 isomerase (3β-HSD), to 17-hydoxyprogesterone, and then to androstenedione by 17α-hydroxylase/C_{17-20} lyase (P450c17), and finally to testosterone by 17-hydroxyateroid dehydrogenase.

The steroidogenic-enzyme genes are generally regulated at the transcriptional level. The orphan nuclear receptor Nur77 and SF-1 are major transcription factors which are known to regulate steroidogenic-enzyme genes [3,4]. Nur77 family members regulate the expression of steroidogenic-enzyme genes such as steroid 21-hydroxylase, P450c17, and 20-hydroxysteroid dehydrogenase [5]. Nur77 binding regions within the gene promoter of rat P450c17 [6], Star [7], and human 3β-HSD2 have been defined [8]. In addition, LH treatment has been shown to trigger the expression of Nur77 in Leydig cells [9].

Nur77 is a member of the Nur77 gene family, which also contains the orphan nuclear transcription factors Nurr-1 and NOR-1. These factors have similar structural features of the conserved DNA binding domain (DBD) and ligand binding domain (LBD), but retain a variable sequence in the N-terminal AF-1 domain. Nur77 family members behave as endpoint effectors of the protein kinase A (PKA) signaling pathway acting through dimmers, and the AF-1 domain of Nur77 plays a major role in transcriptional activation, cofactor recruitment, and intra- and intermolecular interactions. Although Nur77 has been well characterized as an immediate early response gene and for its posttranslational modifications [10,11], coregulators involved in Nur77 transactivation are not fully characterized. Some proteins including steroid receptor coactivator (SRC)-1, silencing mediator for retinoid and thyroid hormone receptors (SMRT) have been shown to regulate Nur77 transactivation through direct protein–protein interactions [12,13].

Androgen action is mediated by androgen receptor (AR), which is a ligand-inducible transcription factor [14]. AR consists of three

* Corresponding author. Fax: +82 62 530 0500.
E-mail address: klee@chonnam.ac.kr (K. Lee).
[1] These authors contributed equally to this work.

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.04.161

functional domains: the N-terminal transactivation domain (A/B domain), the central DBD, and LBD [15]. The precise function of AR in Leydig cell is unknown; however, several studies have demonstrated that androgens negatively regulate the cAMP-induced expression of P450c17 via an AR-mediated mechanism [1,2]. Recent studies also have shown that testicular StAR expression is inhibited by androgens *in vitro* and *in vivo*, likely via a local feedback mechanism [7,16].

In this study, to understand the mechanism by which androgen regulates the expression of steroidogenic enzymes in the testis, we investigated the effect of androgen on the transactivation of Nur77, a major transcription factor for steroidogenic enzyme gene expression. We have shown that androgen-bound AR physically interacts with Nur77, and inhibits Nur77 transactivation through competitive binding with Nur77 coactivators, which results in the down-regulation of steroidogenic enzyme gene expressions in mouse Leydig cells. These results strongly support the idea of androgen's local action on testicular steroidogenesis.

2. Materials and methods

2.1. Plasmids

The expression plasmids of Nur77, AR, SRC-1, NurRE-luc, NBRE-luc, GST-Nur77, GST-Nur77 domain mutants, and GST-mAR domain mutants were previously described [17–20]. The reporter plasmids (StAR (−2200/+3)-luc, WT (−4473 to 399) P450c17-luc, and 3β-HSD (−4470/+40)-luc) and cDNA probes (StAR, P450c17, and 3β-HSD) were previously described [21]. GFP-Nur77 and RFP-mAR were constructed by cloning the genes into XhoI/BamHI sites in pEGFP-C1 and pHcRed1-C1 vector (CLONTECH), respectively.

2.2. Cell culture

mLTC-1 cells were maintained in RPMI 1640 medium (Invitrogen) supplemented with 10% fetal bovine serum (FBS), and R2C cells were maintained in F-10 nutrient medium (Invitrogen) supplemented with 15% horse serum (HS) and 5% FBS. COS-7 cells were maintained in DMEM supplemented with 10% FBS. All cells were cultured at 37 °C in 5% CO_2 [22].

2.3. Preparation of primary Leydig cells

Purification of mouse Leydig cells was carried out according to the method previously described [23] with some modification. Animals were anesthetized and then decapitated. Eight testes per genotype were removed and testicular cells were dispersed by treating the decapsulated testis with collagenase (0.25 mg/ml, Sigma) in M199 media (Invitrogen) at room temperate for 20 min with gentle shaking. After incubation, the dispersed tissues were diluted with M199 and the solution was filtered. Interstitial cells were precipitated by centrifugation of the filtrate and washed once with M199 and twice with PBS. Cells were maintained in RPMI 1640 medium supplemented with 15% horse serum. Enrichment for Leydig cells was estimated by 3β-HSD cytochemistry [16].

2.4. Northern blot analysis and qRT-PCR

Total RNA was extracted from R2C, and primary Leydig cells using Tri reagent (Molecular Research Center, Inc.). Total RNA (20 μg) was separated on a 1.2% denaturing agarose gel, transferred onto a Zeta probe nylon membrane (Bio-Rad) in 10 X SSC, and immobilized under UV light. Northern blot analysis was conducted as previously described [24]. Quantitative analysis of StAR and P450c17 gene expression in primary Leydig cells was assessed by qRT-PCR as previously described [25].

2.5. Radioimmunoassay (RIA)

The levels of progesterone and testosterone were measured by radioimmunoassay. The assay procedure was performed as previously described [21], using labeled progesterone (1,2,6,7-3H-progesterone, 96 Ci/mol) and testosterone (1,2,6,7-3H-testosterone, 96 Ci/mole) obtained from NEN. R2C cells were seeded and cultured with 15% charcoal-stripped FBS (CSS). Two sets of standard and samples were routinely included in each assay.

2.6. Transient transfection assay

Transfections were carried out using SuperFect (Qiagen) and Lipofectamine Plus (Invitrogen) transfection reagent for COS-7 and mLTC-1 cells according to the manufacturers' specifications, respectively. Cells were plated in 24-well plates with CSS medium and transfected with the appropriate amounts of expression plasmids. The total amount of DNA was kept constant by adding appropriated amounts of pCDNA3 empty vector. After 24 h of transfection, cells were treated with ligand or vehicle for 24 h. The cells were lysated using lysis buffer for mLTC-1 [9] and COS-7 cells [24] as described previously. Luciferase and β-galactosidase activities were assayed as described previously [24]. Levels of luciferase activity were normalized to the β-gal expression.

2.7. Fluorescence

COS-7 cells were plated onto gelatin-coated coverslips the day before transfection in 6-well plates and incubated in 5% CSS DMEM. Cells were transfected with GFP-Nur77, RFP-mAR using SuperFect. After 18 h transfection, cells were treated with 10 nM DHT for 4 h. For fluorescent microscopy, the cells were fixed in 2% para-formaldehyde (pH 7.4) for 10 min [26]. Cells were visualized with a confocal microscope (ZEISS, Laser Scanning Microscope). Images were captured and analyzed using LSM 5 PASCAL software.

2.8. Glutathione-S-transferase (GST) pull-down assay

GST alone and GST fusion proteins were expressed in Escherichia coli BL21 cells and isolated with Glutathione-Sepharose 4B beads (Amersham Biosciences). Immobilized GST fusion proteins were incubated with ^{35}S-methionine labeled proteins produced by *in vitro* translation using the TNT-coupled transcription–translation system (Promega). Binding reactions were carried out as previously described [17]. Following gel electrophoresis, radiolabeled proteins were visualized by autoradiography.

2.9. Co-immunoprecipitation (Co-IP) assay and Western blot analysis

In vivo co-immunoprecipitation assays were performed with R2C cells cultured in complete media as previously described [17]. The cells were harvested with RIPA cell lysis buffer. Lysates were incubated with anti-AR and anti-Nur77 polyclonal antibodies (Santa Cruz) overnight at 4 °C and subsequently with protein A agarose bead slurry. Agarose beads were washed with RIPA buffer at 4 °C and bound proteins were separated by SDS–PAGE. Proteins on the gels were transferred to a Protran nitrocellulose transfer membrane (Schleicher & Schuell Bioscience), and subjected to Western blot analysis with anti-AR and anti-Nur77 antibodies. Signals were detected with an ECL kit (Amersham Biosciences).

2.10. Statistical analysis

A Student's *t* test was used for statistical analyses. Data are shown as the means ± standard error of the mean (SEM). $P < 0.05$ was used as the criterion for determination of statistical significance.

3. Results

3.1. Androgens suppress the expression of steroidogenic enzyme genes in Leydig cells

cAMP-induced P450c17 gene expression was previously shown to be repressed by androgens in testicular Leydig cells [1,2]. In order to investigate the effect of androgens on the expression of other steroidogenic enzyme genes, R2C cells were treated with various concentrations of DHT for different time periods. The mRNA levels of all tested steroidogenic enzyme genes such as StAR, 3B-HSD, and P450c17 were decreased after 1 h treatment with 1 μM DHT, while such an effect was detected only after 6 h treatment with 0.1 μM DHT (Fig. 1A). Nur77 mRNA and protein levels were not altered by the treatment of androgen (Fig. 1A and data not shown). As expected, cAMP-induced StAR and P450c17 mRNA levels were decreased by DHT treatment also in mouse primary Leydig cells (Fig. 1B). In consistent with androgen effect on the expression of steroidogenic enzymes, the production of steroids, progesterone and testosterone, was reduced by DHT treatment in R2C cells (Fig. 1C). These results suggest that androgens suppress the expression of steroidogenic enzyme genes in Leydig cells, supporting previous findings [1,2,7,16].

3.2. Androgen/AR inhibits the transactivation of Nur77, decreasing the promoter activity of steroidogenic enzyme genes

Although androgen affects the expression of steroidogenic enzyme genes, no androgen response element has been defined in their promoters. Since Nur77 has been well demonstrated to regulate the promoter activity of steroidogenic enzyme genes, we checked the possibility of indirect action of androgen by investigating the cross-talk of androgen/AR with Nur77. As shown in Fig. 2A, AR suppressed the transactivation of Nur77 in a dose-dependent manner. Interestingly, there was a ligand-independent effect of AR on Nur77 transactivation, although it was much less compare to its ligand-dependent effect. This was probably due to minor translocation of AR to the nucleus in the absence of ligand because of overexpression, or residual androgens left in the charcoal-stripped serum. Otherwise, some Nur77 might be retained in the cytoplasm by interacting with AR localized in the cytoplasm in the absence of ligand. AR specificity of Nur77 suppression was addressed by transient transfection assays. As shown in Fig. 2B, AR and ER, but not GR and RXR, were able to inhibit the transactivation of Nur77. These results suggest that Nur77 cross-talks with AR with a certain level of specificity.

AR was also able to suppress the transactivation of Nur77 on the natural steroidogenic enzyme promoters, StAR (Fig. 2C) and P450c17 (Fig. 2D), in a dose-dependent manner. To confirm the AR action through Nur77 on the P450c17 promoter, a reporter fused to a defined Nur77 binding site of P450c17 promoter and minimal thymidine kinase promoter was tested. As shown in Fig. 2E, AR suppressed the transactivation of Nur77 on WT (−447/−399) P450c17-luc promoter, which contained only a Nur77/SF-1 binding site. There results imply that transcription of steroidogenic enzyme genes enhanced by Nur77 is suppressed by androgen/AR in Leydig cells.

3.3. AR physically interacts with Nur77 in vitro and in vivo

To investigate whether the functional interaction of AR with Nur77 is due to physical interaction, we performed GST pull-down assays. ^{35}S-methionine labeled Nur77 was incubated with GST-AR deletion mutants (TAU, AF-1+DBDh, DBDh, and LBD) (Fig. 3A, *left*), or ^{35}S-methionine labeled full-length Nur77 and Nur77 deletion mutants (N-term, C-term, and ΔAF-2) were incubated with the GST-AR DBDh (Fig. 3A, *right*). As shown in Fig. 3A, full-length Nur77 bound AR AF-1+DBDh and DBDh, but not TAU and LBD. Full-length Nur77, Nur77 N-term, and Nur77 ΔAF-2, but not Nur77 C-term, interacted with AR DBDh. The data suggest that Nur77 directly interacts with AR through Nur77 N-term and AR DBDh.

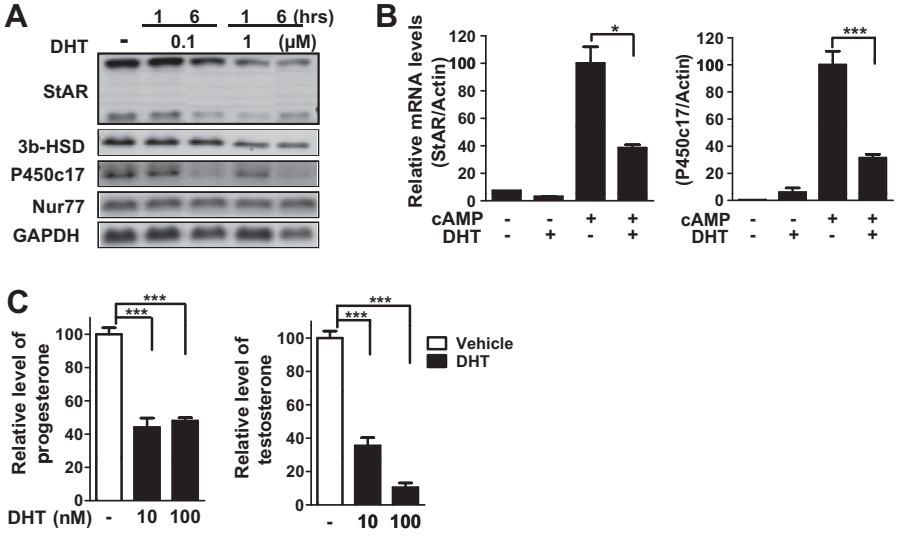

Fig. 1. Androgen suppresses the expression of steroidogenic enzyme genes in Leydig cells. Rat Leydig cell line R2C (A and C) and mouse primary Leydig cells (B) cultured in CSS medium were collected at the indicated time after treatment with 0.1 or 1 μM DHT. Northern blot (A) and real-time PCR analysis (B) were performed with total RNAs using a specific cDNA probe and primer set, respectively. The expression of GAPDH and β-actin were used as an internal control. Progesterone and testosterone levels in cell culture media were measured by RIA. $^{*}P < 0.05$; $^{***}P < 0.001$.

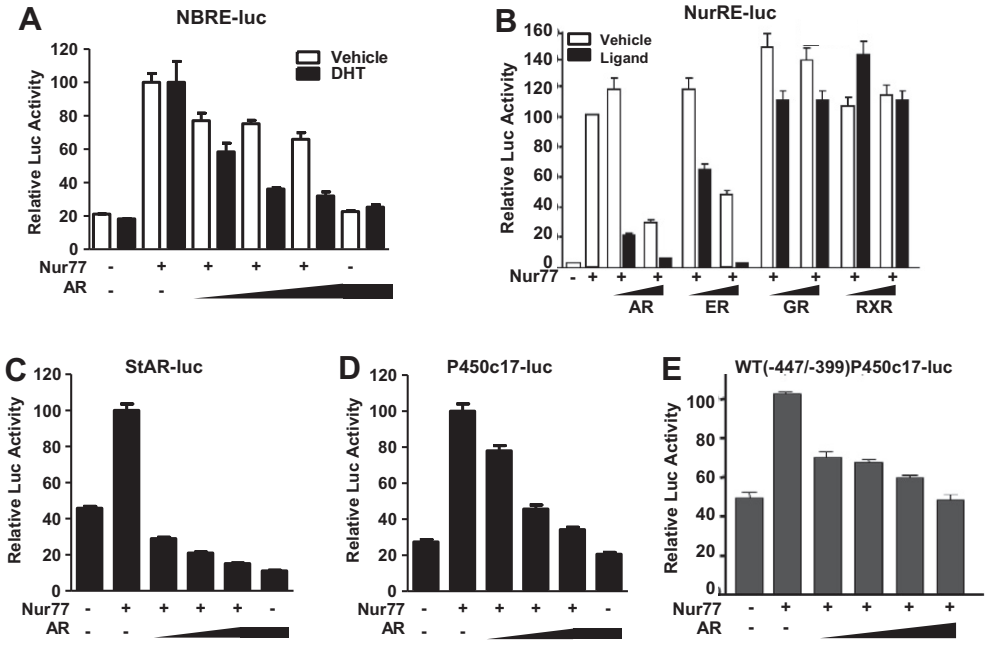

Fig. 2. Inhibition of Nur77 transactivation by AR in Leydig cells. mLTC-1 cells were cotransfected with Nur77 expression plasmid and the indicated reporter, together with increasing amounts of indicated nuclear receptor plasmid. Ligand (10 nM DHT for AR, 100 nM estradiol for ER, 100 nM dexamethasone for GR, and 100 nM retinoic acid for RXR) was added and luciferase activity was measured after 24 h of ligand treatment. Error bars indicate standard deviation.

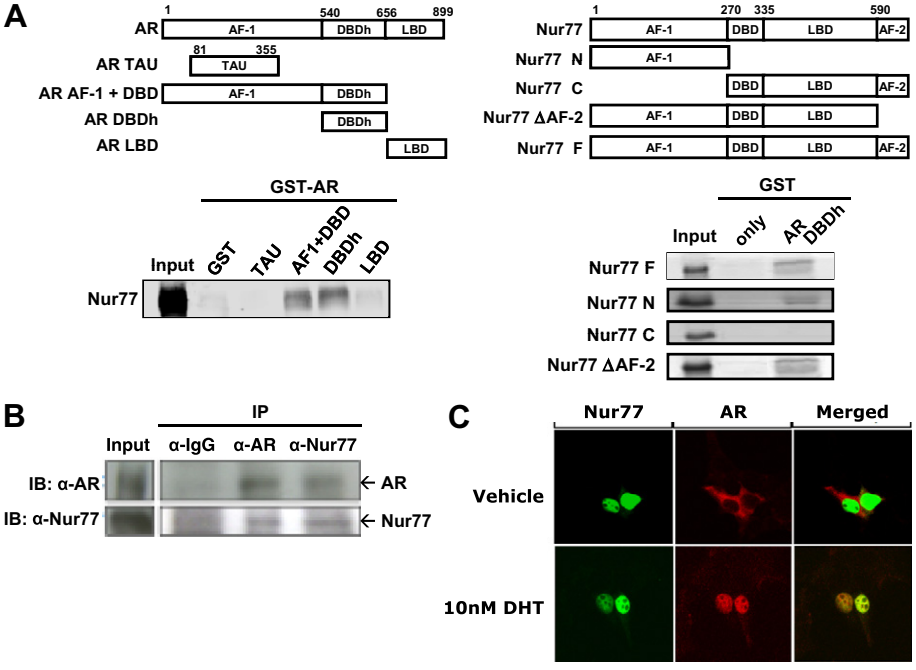

Fig. 3. Physical interaction between Nur77 and AR *in vitro* and *in vivo*. (A, left) ^{35}S-methionine-labeled Nur77 produced by *in vitro* translation was allowed to interact with GST fusion proteins of AR deletion mutants (TAU, AF-1 + DBD, DBDh and LBD) in the presence of 10 nM DHT. (A, right) ^{35}S-methionine-labeled Nur77 full-length and deletion mutant (N-term, C-term, ΔAF-2) proteins produced by *in vitro* translation were allowed to interact with GST fusion proteins of AR DBDh. Labeled protein (20%) used in the binding reaction was loaded as input. (B) R2C cell extracts were used for co-immunoprecipitation experiments with anti-AR and anti-Nur77 antibodies. Immunoprecipitations and Western blot analysis were performed as described in Section 2. Inputs are shown for the expression level of each protein. (C) Nur77 colocalizes with liganded AR in the nucleus. COS-7 cells transfected with GFP-Nur77 and RFP-AR were treated with or without 10 nM DHT for 4 h. Fluorescence was analyzed with a laser scanning confocal microscope.

To examine *in vivo* interaction between Nur77 and AR, co-immunoprecipitation assays were performed using R2C cells, which express both Nur77 and AR endogenously (Fig. 3B). The data revealed that AR was efficiently co-immunoprecipitated with Nur77. These results suggest that Nur77 interacts with AR *in vivo*.

3.4. AR is colocalized with Nur77 in the nucleus in an androgen-dependent manner

Distribution of transcriptional coregulators may give an insight into their mechanism of action. To establish subcellular localization of Nur77 and AR under different cellular conditions,

GFP-Nur77 and RFP-AR were transiently transfected into COS-7 cells. Nur77 was predictably localized in the nucleus, and the presence of 10 nM DHT did not affect its localization (Fig. 3C, *left*). On the other hand, AR was present in the cytoplasm without androgen and translocated into the nucleus upon addition of androgen (Fig. 3C, *middle*). The overlay revealed a nice colocalization of Nur77 and AR in the nucleus in an androgen-dependent fashion (Fig. 3C, *right*). These results support the possibility that AR protein, translocated into the nucleus in the presence of androgen, modulates Nur77 transactivation.

3.5. AR inhibits Nur77 transactivation through competition with coactivators for Nur77 binding

To explore the mechanism by which AR functions as a corepressor of Nur77, we first investigated the effect of AR on the DNA binding activity of Nur77 by electrophoretic mobility shift assay (EMSA) using purified GST-Nur77 protein, *in vitro* translated AR protein, and the NurRE oligonucleotide as a probe. AR protein did not interfere with the formation of NurRE-Nur77 complex (data not shown), implying no effect of AR on the DNA binding activity of Nur77.

We then examined the possibility that AR interferes with the interaction between Nur77 and its coactivators. As shown in Fig. 4A, SRC-1, known as a Nur77 coactivator [12], relieved the AR-mediated suppression of Nur77 transactivation in a dose-dependent manner, suggesting that AR and Nur77 coactivators compete for the modulation of Nur77 transactivation. Furthermore, AR was able to repress the SRC-1-mediated enhancement of Nur77 transactivation (Fig. 4B). A similar competition between AR and SRC-2 (GRIP-1) coactivator for the modulation of Nur77 transactivation was also observed although the effect was less than with SRC-1 (data not shown).

To investigate whether the functional competition between AR and coactivators for the modulation of Nur77 transactivation is due to the competition for the physical interaction with Nur77, we performed GST pull-down assays using GST-Nur77 fusion protein and ^{35}S-methionine labeled SRC-1 and AR proteins. As shown in Fig. 4C, the amount of SRC-1 protein bound to GST-Nur77 was decreased, while the amount of AR protein bound to GST-Nur77 was increased, with the addition of an increased amount of AR protein. The *in vivo* competition between SRC-1 and AR for Nur77 binding was also investigated by co-immunoprecipitation using COS-7 cells transfected with different amounts of AR expression plasmid. As expected, the amount of SRC-1 protein bound to Nur77 decreased with an increased amount of AR protein bound to Nur77 (Fig. 4D). All together, these results suggest that AR inhibits Nur77 transactivation by interfering with the interaction between Nur77 and its coactivators.

4. Discussion

Androgens have been suggested to regulate testicular steroidogenesis locally in the testis, in addition to long-loop negative feedback regulation through the hypothalamus–pituitary–testis axis. In this study, we speculated that the local androgen action on testicular steroidogenesis might be accomplished by cross-talk between androgen signaling and major transcription factors responsible for the expression of steroidogenic enzyme genes. We demonstrate that androgen represses the expression of steroidogenic enzyme genes, including P450c17 and StAR (Fig. 1), which results from androgen/AR-mediated suppression of Nur77 transactivation on steroidogenic enzyme gene promoters. We also show that AR physically competes with Nur77 coactivators to modulate Nur77 transactivation. This study strongly supports a functional role of androgen/AR signaling in testicular steroidogenesis.

In view of the AR-suppression of Nur77 transactivation, AR may influence Nur77 expression, DNA binding activity, protein stability, or interaction with coactivators. To examine whether AR affects Nur77 expression, we did transient transfection assays using a Nur77 promoter fused to a reporter. The results showed that AR did not significantly alter the Nur77 promoter activity (data not shown). The specific binding of Nur77 to NurRE was neither interfered by AR coexpression in EMSA (data not shown). In addition, androgen treatment of R2C cells or coexpression of AR with Nur77 showed no AR effect on Nur77 protein stability (Fig. 1 and data not shown). Instead, AR blocks the recruitment of coactivators to Nur77 through physical interaction. The DBD domain of AR (AR-DBD) participated in the protein–protein interaction with Nur77. This is consistent with the previous observation that AR-DBD is

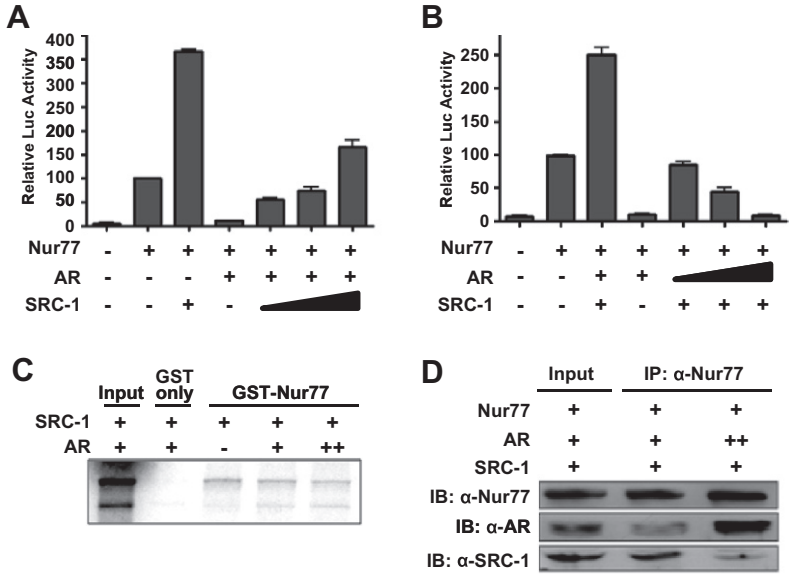

Fig. 4. AR competes with coactivators for the modulation of Nur77 transactivation. (A) Nur77 coactivator, SRC-1, relieves AR-mediated repression of Nur77 transactivation in a dose-dependent manner. COS-7 cells were cotransfected with AR, Nur77, and SRC-1 expression vectors. The expression level of each protein was monitored by Western blot analysis (data not shown). (B) AR repressed SRC-1-elevated Nur77 transactivation in a dose-dependent manner. (C) *In vitro* translated SRC-1 and AR bind competitively to Nur77 in GST-pull down experiments. (D) AR competed with SRC-1 for Nur77 binding *in vivo*.

necessary for AR-mediated repression of cAMP-induced P450c17 expression [2]. Interestingly, Nur77 interacts with AR via its N-terminus, which has a transactivation domain identified as AF-1. The N-terminal AF-1 domain of Nur77 is known to be necessary for the recruitment of cofactors such as SRC-1; thus, it is reasonable that AR and SRC-1 compete for Nur77 binding.

AR has been reported to suppress the promoter activity of bovine LHβ in a gonadotrope-derived cell line [27]. The LHβ promoter contains several *cis*-acting elements that are highly conserved across all mammals including the regulatory element for steroidogenic factor 1 (SF-1). Through additional studies, AR has been shown to suppress activity of the bovine LHβ promoter through protein–protein interaction with SF-1 [27]. We also tested the functional interaction between AR and SF-1, another major transcription factor that regulates the expression of steroidogenic enzyme genes in Leydig cells. The results showed that AR inhibited the transactivation of SF-1 in Leydig cells (data not shown) as in gonadotrope-derived cells [27]. Therefore, AR is likely to inhibit both Nur77 and SF-1 transactivation to suppress the expression of steroidogenic enzymes in testicular Leydig cells.

The endocrine system consists of dynamic biological processes involved in the regulation of a complex array of physiologic activities. The synthesis of sex steroids in male is elaboratively regulated by various local factors including testicular paracrines/autocrines in addition to the negative feedback through the hypothalamic–pituitary–gonadal (HPG) axis [21,24]. Through this study as well as previous studies, androgen has been proposed to have autocrine effect on the steroidogenesis in Leydig cells. Since the change of androgen level is associated with various physiological activities, the acute response may be necessary to keep hormone level rapidly within normal ranges.

Taken together, AR physically interacts with Nur77, and inhibits the transactivation of Nur77 on steroidogenic enzyme gene promoters, eventually resulting in decreased steroidogenesis in Leydig cells. These findings strongly support the local action of androgen/AR on testicular steroidogenesis, and may provide an insight into its regulatory mechanism with regard to the steroidogenesis in Leydig cells.

Acknowledgments

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2009-0070851).

References

[1] A.H. Payne, G.L. Youngblood, Regulation of expression of steroidogenic enzymes in Leydig cells, Biol. Reprod. 52 (1995) 217–225.
[2] M. Burgos-Trinidad, G.L. Youngblood, M.R. Maroto, et al., Repression of cAMP-induced expression of the mouse P450 17 alpha-hydroxylase/C17-20 lyase gene (Cyp17) by androgens, Mol. Endocrinol. 11 (1997) 87–96.
[3] K.L. Parker, B.P. Schimmer, Steroidogenic factor 1: a key determinant of endocrine development and function, Endocr. Rev. 18 (1997) 361–377.
[4] S.H. Mellon, N.A. Compagnone, P. Zhang, Orphan receptors, proto-oncogenes and other nuclear factors regulate P450C17 gene transcription, Endocr. Res. 24 (1998) 505–513.
[5] T.E. Wilson, A.R. Mouw, C.A. Weaver, et al., The orphan nuclear receptor NGFI-B regulates expression of the gene encoding steroid 21-hydroxylase, Mol. Cell. Biol. 13 (1993) 861–868.
[6] P. Zhang, S.H. Mellon, Multiple orphan nuclear receptors converge to regulate rat P450c17 gene transcription: novel mechanisms for orphan nuclear receptor action, Mol. Endocrinol. 11 (1997) 891–904.
[7] C.P. Houk, E.J. Pearson, N. Martinelle, et al., Feedback inhibition of steroidogenic acute regulatory protein expression in vitro and in vivo by androgens, Endocrinology 145 (2004) 1269–1275.
[8] L.J. Martin, J.J. Tremblay, The human 3beta-hydroxysteroid dehydrogenase/Delta5-Delta4 isomerase type 2 promoter is a novel target for the immediate early orphan nuclear receptor Nur77 in steroidogenic cells, Endocrinology 146 (2005) 861–869.
[9] K.H. Song, J.I. Park, M.O. Lee, et al., LH induces orphan nuclear receptor Nur77 gene expression in testicular Leydig cells, Endocrinology 142 (2001) 5116–5123.
[10] K.D. Wansa, J.M. Harris, G. Yan, et al., The AF-1 domain of the orphan nuclear receptor NOR-1 mediates trans-activation, coactivator recruitment, and activation by the purine anti-metabolite 6-mercaptopurine, J. Biol. Chem. 278 (2003) 24776–24790.
[11] T. Hauet, J. Liu, H. Li, et al., PBR, StAR, and PKA: partners in cholesterol transport in steroidogenic cells, Endocr. Res. 28 (2002) 395–401.
[12] M. Maira, C. Martens, E. Batsche, et al., Dimer-specific potentiation of NGFI-B (Nur77) transcriptional activity by the protein kinase A pathway and AF-1-dependent coactivator recruitment, Mol. Cell. Biol. 23 (2003) 763–776.
[13] Y.C. Sohn, E. Kwak, Y. Na, et al., Silencing mediator of retinoid and thyroid hormone receptors and activating signal cointegrator-2 as transcriptional coregulators of the orphan nuclear receptor Nur77, J. Biol. Chem. 276 (2001) 43734–43739.
[14] J.M. Kokontis, S. Liao, Molecular action of androgen in the normal and neoplastic prostate, Vitam. Horm. 55 (1999) 219–307.
[15] A.O. Brinkmann, P. Klaasen, G.G. Kuiper, et al., Structure and function of the androgen receptor, Urol. Res. 17 (1989) 87–93.
[16] S.M. Eacker, N. Agrawal, K. Qian, et al., Hormonal regulation of testicular steroid and cholesterol homeostasis, Mol. Endocrinol. 22 (2008) 623–635.
[17] J.H. Suh, E.Y. Gong, J.B. Kim, et al., Sterol regulatory element-binding protein-1c represses the transactivation of androgen receptor and androgen-dependent growth of prostatic cells, Mol. Cancer Res. 6 (2008) 314–324.
[18] C.Y. Hong, E.Y. Gong, K. Kim, et al., Modulation of the expression and transactivation of androgen receptor by the basic helix-loop-helix transcription factor Pod-1 through recruitment of histone deacetylase 1, Mol. Endocrinol. 19 (2005) 2245–2257.
[19] B.C. Jeong, C.Y. Hong, S. Chattopadhyay, et al., Androgen receptor corepressor-19 kDa (ARR19), a leucine-rich protein that represses the transcriptional activity of androgen receptor through recruitment of histone deacetylase, Mol. Endocrinol. 18 (2004) 13–25.
[20] K.H. Song, Y.Y. Park, K.C. Park, et al., The atypical orphan nuclear receptor DAX-1 interacts with orphan nuclear receptor Nur77 and represses its transactivation, Mol. Endocrinol. 18 (2004) 1929–1940.
[21] C.Y. Hong, J.H. Park, R.S. Ahn, et al., Molecular mechanism of suppression of testicular steroidogenesis by proinflammatory cytokine tumor necrosis factor alpha, Mol. Cell. Biol. 24 (2004) 2593–2604.
[22] S. Chattopadhyay, E.Y. Gong, M. Hwang, et al., The CCAAT enhancer-binding protein-alpha negatively regulates the transactivation of androgen receptor in prostate cancer cells, Mol. Endocrinol. 20 (2006) 984–995.
[23] P.F. Moraga, M.N. Llanos, A.M. Ronco, Arachidonic acid release from rat Leydig cells depends on the presence of luteinizing hormone/human chorionic gonadotrophin receptors, J. Endocrinol. 154 (1997) 201–209.
[24] S.Y. Lee, E.Y. Gong, C.Y. Hong, et al., ROS inhibit the expression of testicular steroidogenic enzyme genes via the suppression of Nur77 transactivation, Free Radic. Biol. Med. 47 (2009) 1591–1600.
[25] Y. Kim, J.C. Ryu, H.S. Choi, et al., Effect of 2,2′,4,4′-tetrahydroxybenzophenone (BP2) on steroidogenesis in testicular Leydig cells, Toxicology 288 (2011) 18–26.
[26] E.Y. Gong, E. Park, H.J. Lee, et al., Expression of Atp8b3 in murine testis and its characterization as a testis specific P-type ATPase, Reproduction 137 (2009) 345–351.
[27] J.S. Jorgensen, J.H. Nilson, AR suppresses transcription of the LHbeta subunit by interacting with steroidogenic factor-1, Mol. Endocrinol. 15 (2001) 1505–1516.