Biochemical and Biophysical Research Communications 446 (2014) 656–662

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Liver X receptors interfere with the deleterious effect of diethylstilbestrol on testicular physiology

Abdelkader Oumeddour [a,b,c,d,e], Emilie Viennois [a,b,c,d], Françoise Caira [a,b,c,d], Clélia Decourbey [a,b,c,d], Salwan Maqdasy [a,b,c,d,f], Abdelkrim Tahraoui [e], Silvère Baron [a,b,c,d], David H. Volle [a,b,c,d,*], Jean-Marc A. Lobaccaro [a,b,c,d,*]

[a] Clermont Université, Université Blaise Pascal, Génétique Reproduction et Développement, BP 10448, F-63000 Clermont-Ferrand, France
[b] CNRS, UMR 6293, GReD, F-63171 Aubiere, France
[c] INSERM, UMR 1103, GReD, F-63171 Aubiere, France
[d] Centre de Recherche en Nutrition Humaine d'Auvergne, F-63000 Clermont-Ferrand, France
[e] Laboratoire de Neuroendocrinologie Appliquée, Université Badji Mokhtar Annaba, BP12, 23000 Annaba, Algeria
[f] Service d'endocrinologie, diabétologie et maladies métaboliques, CHU Clermont-Ferrand, F-63003 Clermont-Ferrand, France

ARTICLE INFO

Article history:
Received 22 November 2013
Available online 11 December 2013

Keywords:
Diethylstilbestrol
LXR
Nuclear receptors
Testis
Germ cells

ABSTRACT

Liver X receptors LXRα (NR1H3) and LXRβ (NR1H2) are transcription factors belonging to the nuclear receptor superfamily, activated by specific oxysterols, oxidized derivatives of cholesterol. These receptors are involved in the regulation of testis physiology. Lxr-deficient mice pointed to the physiological roles of these nuclear receptors in steroid synthesis, lipid homeostasis and germ cell apoptosis and proliferation. Diethylstilbestrol (DES) is a synthetic estrogen considered as an endocrine disruptor that affects the functions of the testis. Various lines of evidences have made a clear link between estrogens, their nuclear receptors ERα (NR3A1) and ERβ (NR3A2), and Lxrα/β. As LXR activity could also be regulated by the nuclear receptor small heterodimer partner (SHP, NR0A2) and DES could act through SHP, we wondered whether LXR could be targeted by estrogen-like endocrine disruptors such as DES. For that purpose, wild-type and Lxr-deficient mice were daily treated with 0.75 μg DES from days 1 to 5 after birth. The effects of DES were investigated at 10 or 45 days of age. We demonstrated that DES induced a decrease of the body mass at 10 days only in the Lxr-deficient mice suggesting a protective effect of Lxr. We defined three categories of DES-target genes in testis: those whose accumulation is independent of Lxr; those whose accumulation is enhanced by the lack of both Lxrα/β; those whose accumulation is repressed by the absence of Lxrα/β. Lipid accumulation is also modified by neonatal DES injection. Lxr-deficient mice present different lipid profiles, demonstrating that DES could have its effects in part due to Lxrα/β. Altogether, our study shows that both nuclear receptors Lxrα and Lxrβ are not only basally important for testicular physiology but could also have a preventive effect against estrogen-like endocrine disruptors.

1. Introduction

The nuclear receptors LXRα (NR1H3) [1] and LXRβ (NR1H2) [2,3] were first described in the mid 90' in the liver as orphan receptors. David Mangelsdorf's group "deorphanized" them [4] and oxidized or hydroxylated metabolites of cholesterol known as oxysterols have been described to be the *bona fide* ligands [5,6]. Their putative association with human diseases makes them promising pharmacological targets [7,8]. First in vivo analyses of Lxr-deficient mice [9,10] pointed out their pivotal roles in cholesterol homeostasis; Lxrα/β are hence considered as intracellular cholesterol sensors whose activation leads to decreased plasma cholesterol. Beyond cholesterol homeostasis, they modulate numerous physiological functions such as fatty acid synthesis, lipid metabolism, glucose homeostasis, steroidogenesis, immunity, and neurological homeostasis. Lxrα/β are also involved in the control of reproductive functions in both females [11,12] and males (for a review see [13]). Indeed, deficient mice for both isoforms become infertile during aging [14,15]. Several functions have been associated with both Lxr isoforms in mouse testis. Lxrα regulates steroid synthesis by Leydig cells through transcriptionally enhancing *Star* and *Cyp11a1*, respectively encoding the steroidogenic acute regulatory (StAR) protein whose function is to translocate cholesterol from the outer to the inner mitochondrial membrane in steroidogenic cells [16] and the cholesterol side-chain cleavage enzyme

* Corresponding authors at: Clermont Université, Université Blaise Pascal, Génétique Reproduction et Développement, BP 10448, F-63000 Clermont-Ferrand, France.
E-mail addresses: david.volle@inserm.fr (D.H. Volle), j-marc.lobaccaro@univ-bpclermont.fr (J.-M.A. Lobaccaro).

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.12.005

that catalyzes conversion of cholesterol to pregnenolone [15]. Lxrα-deficient mice also present an increased apoptosis of the germ cells. Next to this, Lxrβ has been identified in Sertoli cells as a major sensor of lipid homeostasis. Moreover Lxrβ-deficient mice show lower germ cell proliferation [15]. These data define the Lxrα/β as key factors to control testicular integrity and functions [13].

The incidence of male fertility disorders is constantly increasing [17,18] since the 60's as proven by studies on semen quality performed on men from the Nordic countries, Germany, France, UK and the Baltic countries, as well as from Japan and the USA. This has been linked to multiple factors [19]. Among these, endocrine disruptors have been suspected to be involved [20]. These compounds have either estrogenic or anti-androgenic activities. Diethylstilbestrol (DES) is a synthetic estrogen that has been prescribed for 30 years until the 70's to pregnant women to prevent abortions and pregnancy complications. DES was banned from reproductive medication after it was proved to increase the incidence of cancer clear-cell adenocarcinoma of the vagina in pubertal girls who were exposed *in utero* [21] and cryptorchidism and abnormalities in the urogenital tract of boys [22]. This synthetic estrogen is hence considered as an endocrine disruptor that affects the functions of the testis. Interestingly, several types of evidences have linked the estrogens, their nuclear receptors ERα (NR3A1) and ERβ (NR3A2), and Lxrα/β [23–26].

Based on that, we hypothesized that Lxrα/β could be in part involved in the deleterious impact of estrogenic endocrine disruptors on testicular physiology. To analyze whether such a link exists, we neonatally treated wild-type or Lxr-deficient mice with a low dose of DES and measured the body and testis masses, as well as accumulation of lipids and DES-affected genes.

2. Materials and methods

2.1. Animals

The Lxr-deficient mice (*Lxr−/−*) were previously described [15], maintained on a mixed background (C57BL/129sv) and housed in a temperature-controlled room with 12-h light, 12-h dark cycles. Mice were fed *ad libitum* with water and Global-diet 2016S from Harlan (Gannat, France). Mice were daily injected subcutaneously from days 1 to 5 after birth with 0.75 μg diethylstilbestrol (DES, Sigma–Aldrich, L'Isle D'Abeau, France) diluted in 25 μl of corn oil as previously described [27]. At day 10 or day 45, mice were euthanized by decapitation less than 1.5 h after the beginning of the light cycle and bled before tissue collection. All aspects of animal care were approved by the Regional Ethics Committee (authorization CE2-04).

2.2. Histology

Hematoxylin/eosin staining was performed as described previously [27]. Testes from 10- or 45-days-old mice were collected, fixed with 4% paraformaldehyde and embedded in paraffin, and 5 μm-thick sections were prepared and stained with hematoxylin/eosin (Supplementary data 1).

2.3. Real-time PCR

Following testis RNA extraction (TRIzol®; Invitrogen, Cergy-Pontoise, France) and cDNA synthesis (SuperScript II First-Strand Synthesis System; Fisher Scientific, Illkirch, France), real-time PCR measurement of individual cDNAs was performed using SYBR green dye to measure duplex DNA formation. Primer sequences are shown in Table 1. Results were analyzed using the ΔΔCt method.

Table 1
qPCR primer sequences used for qPCR analyses.

Gene		Sequence
18S	fw	GGGAGCCTGAGAAACGGC
	rev	GGGTCGGGAGTGGGTAATTTT
Lxrα/nr1h3	fw	TGCCATCAGCATCTTCTCTG
	rev	GGCTCACCAGCTTCATTAGC
Lxrβ/nr1h2	fw	CGCTACAACCACGAGACAGA
	rev	TGTTGATGGCGATAAGCAAG
Erα/nr3a1	fw	CATATGATCAACTGGGCAAAGA
	rev	ACTCCGGAATTAAGCAAAATGA
Erβ/nr3a2	fw	TCTTTGCTCCAGACCTCGTT
	rev	CCAGGAACTTGAGACATACAAACTC
Lrh1/nr5a2	fw	CTCTTGATTCTCGATCACATTTACC
	rev	CCAGGAACTTGAGACATACAAACTC
Shp/nr0b2	fw	CGATCCTCTTCAACCCAGATG
	rev	AGGGCTCCAAGACTTCACACA
CyclinA1	fw	GATGTGTATGAAGTCGACACC
	rev	GTGGGGTCAACCAGCATTGG
CyclinA2	fw	CAAGGAGTGTGTGCATCAGGACT
	rev	CTGGCCAGAAGTGTCTGTTC
CyclinB2	fw	CTGGCCAGAAGTGTCTGTTC
	rev	TTTCTCGGATTTGGGAACTG
CyclinD1	fw	TCTCCTGCTACCGCACAAC
	rev	TTCCTCCACTTCCCCCTC
CyclinD2	fw	TCCCGCAGTGTTCCTATTTC
	rev	TCCCGCAGTGTTCCTATTTC
Stra8	fw	GTTTCCTGCGTGTTCCACAAG
	rev	CACCCGAGGCTCAAGCTTC
Oct3	fw	AAGTTGGAGAAGGTGGAACC
	rev	CTTCCTGCCACTTTTGGAAC
abca1	fw	CGTTTCCGGGAAGTGTCCTA
	rev	GCTAGAGATGACAAGGAGGAGGA
Star	fw	TGTCAAGGAGATCAAGGTCCTG
	rev	CGATAGGACCTGGTTGATGAT
cyp11a	fw	CTGCCTCCAGACTTCTTTCG
	rev	TTCTTGAAGGGCAGCTTGTT
3βhsd3	fw	ATGGTCTGCCTGGGAATGAC
	rev	ACTGCAGGAGGTCAGAGCT
cyp17	fw	CCAGGACCCAAGTGTGTTCT
	rev	CCTGATACGAAGCACTTCTCG
Cyp19	fw	CGGAAGAATGCACAGGCTCGAG
	rev	CGATGTACTTCCCAGCACAGC
Insl3	fw	ACTGATGCTCCTGGCTCTGG
	rev	GGAGATGTCTCTGCTCTAGC
Lhr	fw	AGCTAATGCCTTTGACAACC
	rev	GATGGACTCATTATTCATCC
Fshr	fw	GTGCTCACCAAGCTTCGAGTCAT
	rev	AAGGCCTCAGGGTTGATGTACAG

Fw, forward primer; rev, reverse primer. Sequences are given 5′ → 3′.

Quantitative PCR experiments were performed as previously described [15]. 18S was used as housekeeping gene.

2.4. TUNEL analysis

TUNEL experiment was performed as described previously [27] on 5 μm of testis fixed in 4% PFA. Briefly 5-μm-thick paraffin-embedded sections were deparaffined with toluol followed by rehydratation. The slides of each group were incubated for 5 min in unmasking buffer (citrate acetate 1.8 mM, sodium citrate 8.2 mM, pH 6.0) at 86 °C. Then the slides were incubated with 0.3 U/μl terminal deoxynucleotidyl transferase (Euromedex, Mundolsheim, France), 6.7 mM biotin-11-dUTP (Euromedex), and 26.7 mM dATP (Promega, Charbonnières, France) in terminal deoxynucleotidyl transferase buffer 1 h at 37 °C. Counterstain was performed with Mayer's hematoxylin solution (Sigma–Aldrich) for 30 s. In each testis, at least 100 random seminiferous tubules were counted.

2.5. Ki67 staining

Five-micrometer cryosections of testis were fixed 10 min in 4% paraformaldehyde. Slides were incubated with anti Ki67 1/500

(Tebu-bio, Le Perray en Yvelines, France) overnight at 4 °C and then washed three times in 1 × PBS. Slides were incubated for 1 h at room temperature with a goat antirabbit secondary antibody labeled with Alexa 488 (1/250; from Invitrogen). In each testis, at least 100 random seminiferous tubules were counted.

2.6. Lipid analysis

Lipids were extracted as described previously [15]. High-performance thin-layer chromatography plates (Silica gel 60; Merck) were used after being prewashed with a mixture of methanol/chloroform (1:1, vol/vol) followed by heating at 125 °C for 5 min. Plates were then developed with hexane, diethylether, and glacial acetic acid (80:20:2, vol/vol) and analyzed by densitometry (Sigma Scan Pro; Sigma–Aldrich) using standards.

2.7. Testosterone measurement

Testosterone levels were measured in testis from 45 days old mice using the direct ELISA Kit EiAsyTM WayTESTOSTERONE (Diagnostics Biochem Canada Inc, London, Canada) according the manufacturer's instruction. Briefly, fresh testes were dounced-homogenized in PBS-BSA 0.1 mg/ml and aliquots were used for the testosterone as well protein assays.

2.8. Statistics

For statistical analysis, 2-way ANOVA was performed using the statistical software package SigmaStat 3.0. When significant effects of treatment or genotype or their interactions were obtained, multiple comparisons were made with Holm–Sidak method. All numerical data are mean ± SEM. A p value less than 0.05 was considered significant.

3. Results

As the activity of DES on testis function has been extensively studied and the effects reported in numerous articles ([27]; for a review see [28]), and in order to clarify the role of the nuclear receptors Lxrα/β in the development of these phenotypes, we chose to compare the effect of DES *vs.* oil treatments in each studied genotype.

3.1. Neonatal treatment with DES decreases body mass and testis somatic index of LXR deficient-mice at 10 days

Neonatal DES treatment has been known to alter adult body mass [29], as shown by the significant decreased body weights at 45 days for wild-type and *Lxr*−/− mice (Fig. 1A, left panel) demonstrating the efficient effect of DES. Interestingly, at 10 days, a significant 25% lower body weight was observed in the *Lxr*−/− mice treated with DES, while this effect was not found in wild-type animals. This suggests that Lxr-deficient mice are more prone to DES effect compared to wild-type animals. A similar DES-effect was also found for testis somatic index, defined by the calculation of the testis mass as a proportion of the total body mass (testis weight/body weight × 100), (Fig. 1A, right panel): neonatal DES treatment induced a higher decrease of the somatic index only in *Lxr*−/− mice (20% at 10 and 45 days), compared to the *Lxr*−/− mice treated with vehicle (oil). Conversely, DES did not alter the testis weight of wild-type animals at these ages.

As many physiological functions of the testis are regulated by nuclear receptors, we investigated whether neonatal DES treatment could affect their mRNA levels (Fig. 1B). Both Lxr levels were not modified by the treatment in wild-type animals. Estrogen receptor Erα accumulation was induced as previously shown [27], demonstrating that DES was efficient. Likewise, Erβ level was increased by DES in wild-type mice. Two downstream Erα

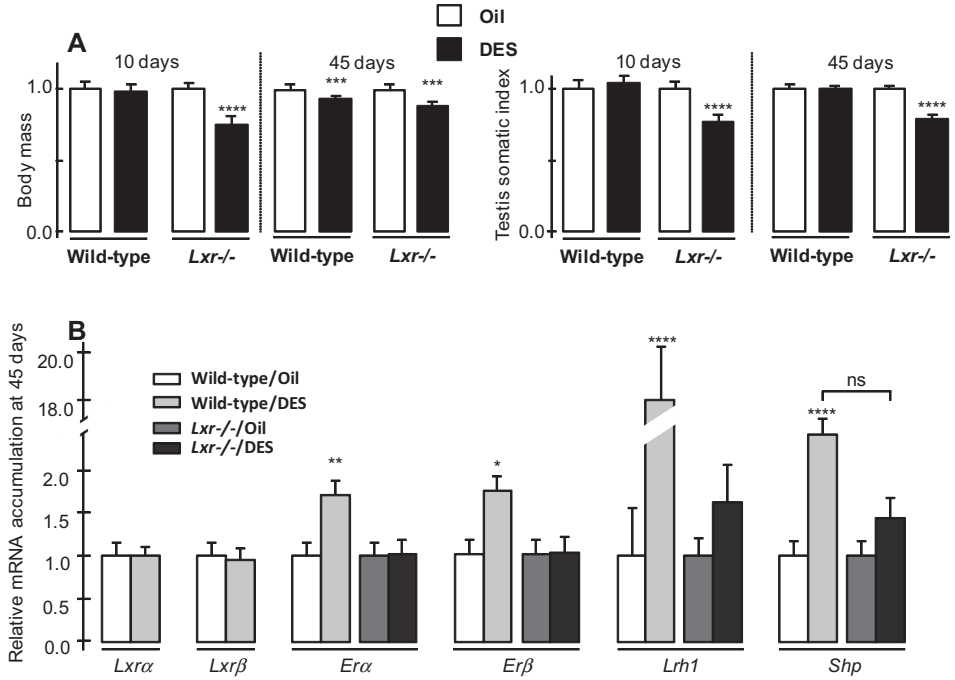

Fig. 1. Effects of neonatal DES treatment on body mass, testis somatic index and nuclear receptors mRNA accumulation. (A) Body mass and somatic index have been arbitrarily fixed at 1 for vehicle-treated wild-type and *Lxr*−/− mice. Measurements were done at 10 and 45 days of age. Histograms represent mean ± SEM; N = 8–15 animals per group. Statistical analysis: $^{***}p < 0.001$ *vs.* vehicle treated mice; $^{****}p < 0.0001$ *vs.* vehicle-treated mice. (B) Nuclear receptor encoding mRNAs were measured by qPCR. Vehicle-treated wild-type and *Lxr*−/− mice were arbitrarily fixed at 1. Measurements were done on testis collected at 45 days of age. Histograms represent mean ± SEM; N = 8–15 animals per group. Statistical analysis: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; $^{****}p < 0.0001$ *vs.* vehicle-treated mice.

genes were also accumulated, namely the encoding nuclear receptors *Lrh1* (NR5A2) and *Shp* (NR0B2) involved in lipid and steroid homeostasis in testis [27], respectively 18- and 2.5-fold. Interestingly, none of these genes was significantly accumulated following the neonatal DES treatment in Lxr-deficient mice, suggesting that part of the DES effect needs Lxr receptors.

3.2. Effect of DES on cell apoptosis is increased in LXR deficient mice at 10 days

As already reported [27], 0.75 μg DES did not induce a significant cell apoptosis in testis at 10 days of life (Fig. 2A, left panel), conversely to what was observed at 45 days. The same higher apoptosis rate (2-fold, $p < 0.001$) was present in Lxr-deficient mice at 45 days. Interestingly, a significant 3-fold increase ($p < 0.0001$) is present at 10 days in the DES-treated *Lxr−/−* mice, suggesting they are more sensitive to neonatal DES. While DES had no effect on cell proliferation in wild-type mice, a slight (15%) but significant lower proliferation was observable in DES-treated *Lxr−/−* mice at 45 days (Fig. 2A, right panel).

At 45 days, lower *Cyclin A1* (marker of meiosis of germ cells), *Cyclin B2* (mitosis maker), and *Oct3* (germ cell marker) mRNA accumulation was shown in *Lxr−/−* mice neonatally treated with 0.75 μg DES. In wild-type mice, DES induced a higher accumulation level of *Cyclin A2* (mitosis marker) and a decreased level of *Stra8* (meiosis marker) [27]. Altogether, the decreased meiosis marker together with the increased cell apoptosis and the lower cell proliferation rates could participate to the decreased somatic index of the testis.

3.3. DES affects accumulation of lipids in testis at 45 days of age

Lipids have an important role in male fertility [30] and Lxrα/β regulate testis lipid homeostasis [14,15,31]. Besides, endocrine disruptors are known to alter lipid composition in testis [32]. We thus wondered whether DES could interfere with the lipid defects observed in *Lxr−/−* mice, such as the higher cholesterol ester accumulation [14,15] (Supplementary data 2). As shown in Fig. 3A, cholesterol ester levels were not differentially affected by DES (means of 0.14 μg/mg tissue for vehicle *vs.* 0.12 for DES in wild-type animals; 0.35 μg/mg tissue *vs.* 0.24 for *Lxr−/−* mice, non-significant). As already described, testis total cholesterol was decreased by DES-treatment in wild-type animals. This effect was not observed in Lxr-deficient mice. Conversely a slight increase of *Abca1* (encoding the membrane cholesterol transporter ATP-binding cassette 1) was observed in DES-treated *Lxr−/−* mice (Fig. 3B, $p = 0.1$). Neonatal treatment with DES significantly decreased the amount of phosphatidyl-ethanolamine (55% decrease, $p < 0.05$), phosphatidyl-choline (35% decrease, $p < 0.05$) and sphingosine (25% decrease, $p < 0.05$). Note that these alterations were not seen in *Lxr−/−* mice (Fig. 3A).

3.4. Lxrα/β modify neonatal effects of DES on Leydig and Sertoli cells markers

Lxrα and Lxrβ have been shown to regulate Leydig and Sertoli cell physiology [15], by the transcriptional regulation of genes such as *Star*, *Cyp11a1*, *Cyp17* (encoding P450c17 enzyme) and *3βHSD* (encoding 3-beta-hydroxysteroid dehydrogenase), or the decrease of the basal anti-Müllerian hormone (AMH) mRNA, respectively. Neonatal treatment with DES induced a decreased accumulated level *Star*, *Cyp11a1* and *Cyp17* in wild-type mice at 45 days. Conversely *3βHSD*, *Cyp19* (encoding aromatase), *Lhr* (encoding LH-receptor) and *Insl3* (encoding insulin-like 3) were not significantly affected. Interestingly, lack of both Lxr abolished DES effect on *Star* level while *3βHSD* was highly accumulated (Fig. 4A). The levels of testosterone were significantly ($p = 0.03$) decreased by 30% at 45 days in *Lxr−/−* testis neonatally treated with DES

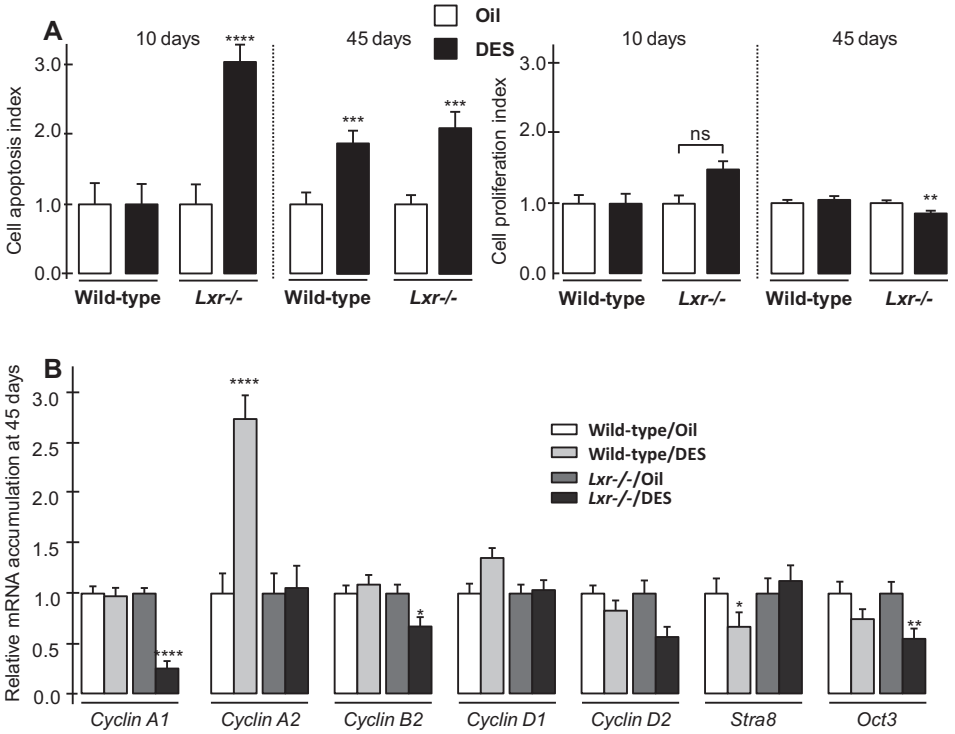

Fig. 2. Effects of neonatal DES treatment on cell apoptosis and proliferation and on meiosis and mitosis markers. (A) Cell apoptosis index (left panel) and cell proliferation index (right panel) determined by TUNEL analysis and Ki67 staining, respectively. Vehicle-treated wild-type and *Lxr−/−* mice were arbitrarily fixed at 1. Measurements were done at 10 and 45 days of age. Histograms represent mean ± SEM; $N = 3$–5 animals per group. Statistical analysis: $^{**}p < 0.01$; $^{***}p < 0.001$; $^{****}p < 0.0001$ *vs.* vehicle-treated mice. (B) Meiosis and mitosis marker encoding mRNA were measured by qPCR. Vehicle-treated wild-type and *Lxr−/−* mice were fixed at 1. Histograms represent mean ± SEM; $N = 6$-8 animals per group. Statistical analysis: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{****}p < 0.0001$ *vs.* vehicle-treated mice.

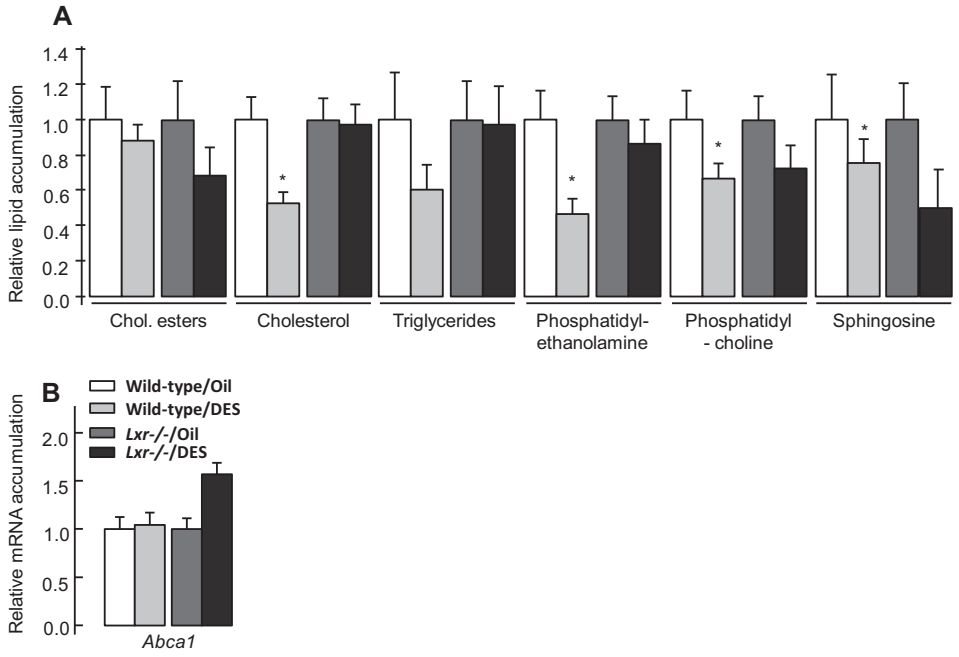

Fig. 3. Effects of neonatal DES treatment on lipid accumulation on testis from 45 days old animals. (A) Cholesterol esters, total cholesterol, triglycerides, phosphatidyl-ethanolamine, phosphatidyl-choline and sphingosine were measured by thin chromatography layer. Vehicle-treated wild-type and *Lxr*−/− mice were arbitrarily fixed at 1. Histograms represent mean ± SEM; $N = 5–15$ animals per group. Statistical analysis: $^{**}p < 0.01$ *vs.* vehicle-treated mice. (B) *Abca1* encoding mRNA was measured by qPCR. Vehicle-treated wild-type and *Lxr*−/− mice were fixed at 1. Histograms represent mean ± SEM; $N = 8–12$ animals per group. Statistical analysis: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{****}p < 0.0001$ *vs.* vehicle treated mice.

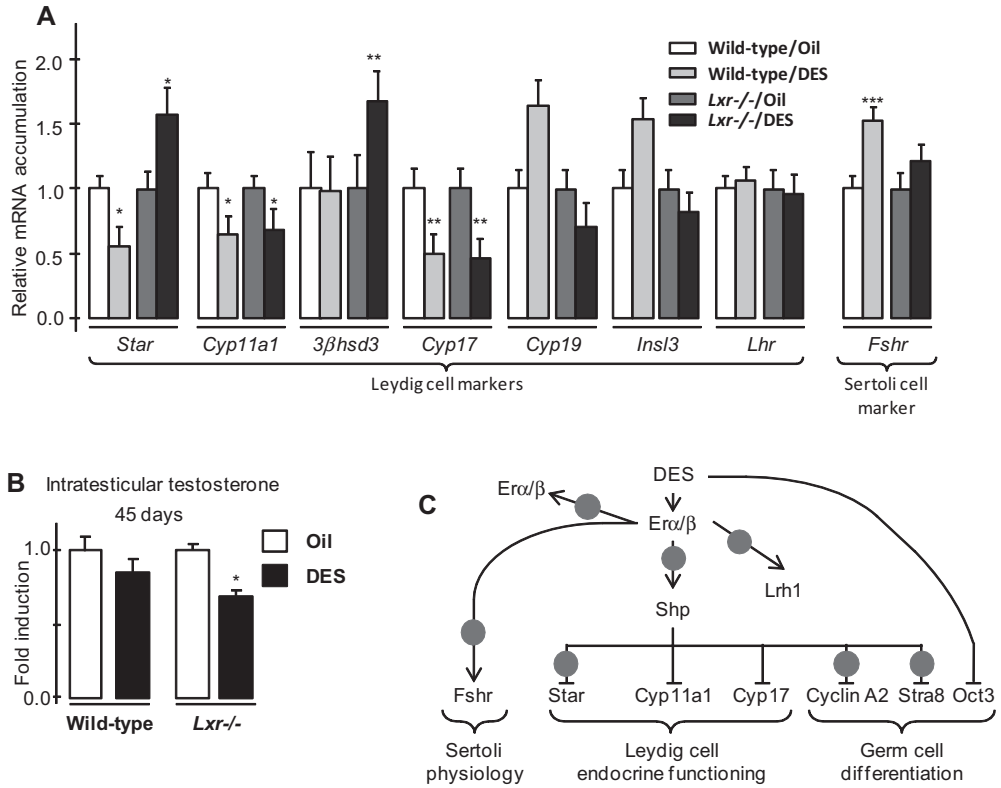

Fig. 4. Effects of neonatal DES treatment on Leydig and Sertoli cell markers from 45 days old animals. (A) Leydig and Sertoli cell markers were measured by qPCR. Vehicle-treated wild-type and *Lxr*−/− mice were arbitrarily fixed at 1. Histograms represent mean ± SEM; $N = 6–8$ animals per group. Statistical analysis: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ *vs.* vehicle treated mice. (B) Testosterone levels were measured in testis from 45 days old animals. Vehicle-treated wild-type and *Lxr*−/− mice were arbitrarily fixed at 1. Histograms represent mean ± SEM; $N = 5–10$ animals per group. Statistical analysis: $^{*}p < 0.05$ *vs.* vehicle treated mice. (C) Proposed model for the role of Lxrα/β in DES-induced gene variations. Adapted from Volle et al. [27]. Grey disk, Lxrα/β; arrow, induction; broken arrow, inhibition.

(Fig. 4B). Besides, DES significantly enhanced the accumulation of *Fshr* (FSH-receptor; 1.5-fold; Fig. 4B) as well as *InhA* (Inhibin A; 2-fold; data not shown), two Sertoli cell markers, in wild-type but not in *Lxr*−/− mice.

4. Discussion

In a previous study Volle et al. [27] demonstrated that mice treated with 0.75 μg DES induced the neonatal germ cell differentiation without gross abnormalities in the male genital tract, nor alteration in epididymis, vesicle seminals, and testis weights. The authors showed that Shp-deficient mice were protected from DES effects, which acts through several signaling pathways, such as estrogen receptors Erα and Erβ. Our study points out that DES does not always have a canonical effect on Lxr-deficient mice, i.e. repression of the steroidogenic encoding genes, modification of germ cell differentiation markers, and Sertoli cell physiology alteration. This has been observed on the testicular somatic index as well as on the accumulation of various transcripts involved in endocrine functions, cell differentiation and lipid accumulation, and encoded by Leydig, germ and Sertoli cells, respectively. Our data could potentially establish a link between DES, Shp, Erα/β and Lxrα/β.

The analysis of the transcript accumulation allowed classifying the genes sensitive to neonatal treatment by DES in 3 categories (Fig. 4C): (i) genes whose DES-variations are independent of Lxrα/β, such as *Cyp11a1* and *Cyp17*; (ii) genes whose DES-variations are countered by Lxrα/β, such as *Oct3* and *3βhSD*; (iii) genes whose induction by DES needs the presence of Lxrα/β, such as *Erα*, *Shp*, *Lrh1*, *Star*, and *Stra8*.

DES has been considered for years as the paradigm of the environmental endocrine disruptors with an estrogen-like activity. Indeed DES has been described to act through the estrogen receptors.

Regarding the results it appears that the nuclear receptors Lxrα and Lxrβ not only are basally important for testicular physiology but could also have a protective effect against estrogen-like endocrine disruptors as their absence induces a stronger variation of important testis target genes in Lxr-deficient mice.

Acknowledgments

We thank J.-P. Saru and A. De Haze for their excellent technical help; S. Plantade, K. Ouchen and P. Mazuel for expert technical assistance in breeding the transgenic mice; the members of the Chester's lab for critically reading the manuscript, assistance in animal dissections and discussions; Dr. D.J. Mangelsdorf (Howard Hughes Medical Institute, Dallas, TX) for providing the mice; AO is recipient of a Grant from Algeria government. EV was recipient of Fond Européen de DEveloppement Régional (FEDER) and Région Auvergne Grants. JMAL is supported by Fondation pour la Recherche Médicale and Fondation BNP-Paribas, Association de Recherche sur les Tumeurs Prostatiques, Ligue contre le Cancer (Comités Allier et Puy-de-Dôme), Association de Recherche contre le Cancer, Région Auvergne grants. J.M.A.L. is Professor at the Université Blaise Pascal. DHV is supported by Grants from Agence National de la Recherche "Programme Jeune Chercheur". DHV and SB are recipients of grants from Région Auvergne.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.12.005.

References

[1] P.J. Willy, K. Umesono, E.S. Ong, R.M. Evans, R.A. Heyman, D.J. Mangelsdorf, LXR, a nuclear receptor that defines a distinct retinoid response pathway, Genes Dev. 9 (1995) 1033–1045.
[2] C. Song, J.M. Kokontis, R.A. Hiipakka, S. Liao, Ubiquitous receptor: a receptor that modulates gene activation by retinoic acid and thyroid hormone receptors, Proc. Natl. Acad. Sci. USA 91 (1994) 10809–10813.
[3] D.M. Shinar, N. Endo, S.J. Rutledge, R. Vogel, G.A. Rodan, A. Schmidt, NER, a new member of the gene family encoding the human steroid hormone nuclear receptor, Gene 147 (1994) 273–276.
[4] D.J. Peet, B.A. Janowski, D.J. Mangelsdorf, The LXRs: a new class of oxysterol receptors, Curr. Opin. Genet. Dev. 8 (1998) 571–575.
[5] B.A. Janowski, P.J. Willy, T.R. Devi, J.R. Falck, D.J. Mangelsdorf, An oxysterol signalling pathway mediated by the nuclear receptor LXR alpha, Nature 383 (1996) 728–731.
[6] B.A. Janowski, M.J. Grogan, S.A. Jones, G.B. Wisely, S.A. Kliewer, E.J. Corey, et al., Structural requirements of ligands for the oxysterol liver X receptors LXRα and LXRβ, Proc. Natl. Acad. Sci. USA 96 (1999) 266–271.
[7] E. Viennois, K. Mouzat, J. Dufour, L. Morel, J.-M. Lobaccaro, S. Baron, Selective liver X receptor modulators (SLiMs): what use in human health?, Mol Cell. Endocrinol. 351 (2012) 129–141.
[8] E. Viennois, A.J.C. Pommier, K. Mouzat, A. Oumeddour, F.-Z. El Hajjaji, J. Dufour, et al., Targeting liver X receptors in human health: deadlock or promising trail?, Expert Opin Ther. Targets 15 (2011) 219–232.
[9] D.J. Peet, S.D. Turley, W. Ma, B.A. Janowski, J.M. Lobaccaro, R.E. Hammer, et al., Cholesterol and bile acid metabolism are impaired in mice lacking the nuclear oxysterol receptor LXR alpha, Cell 93 (1998) 693–704.
[10] S. Alberti, G. Schuster, P. Parini, D. Feltkamp, U. Diczfalusy, M. Rudling, et al., Hepatic cholesterol metabolism and resistance to dietary cholesterol in LXRbeta-deficient mice, J. Clin. Invest. 107 (2001) 565–573.
[11] K. Mouzat, M. Prod'homme, D.H. Volle, B. Sion, P. Déchelotte, K. Gauthier, et al., Oxysterol nuclear receptor LXRbeta regulates cholesterol homeostasis and contractile function in mouse uterus, J. Biol. Chem. 282 (2007) 4693–4701.
[12] K. Mouzat, F. Volat, S. Baron, G. Alves, A.J.C. Pommier, D.H. Volle, et al., Absence of nuclear receptors for oxysterols liver X receptor induces ovarian hyperstimulation syndrome in mice, Endocrinology 150 (2009) 3369–3375.
[13] F.-Z. El-Hajjaji, A. Oumeddour, A.J.C. Pommier, A. Ouvrier, E. Viennois, J. Dufour, et al., Liver X receptors, lipids and their reproductive secrets in the male, Biochim. Biophys. Acta 2011 (1812) 974–981.
[14] K.M. Robertson, G.U. Schuster, K.R. Steffensen, O. Hovatta, S. Meaney, K. Hultenby, et al., The liver X receptor-{beta} is essential for maintaining cholesterol homeostasis in the testis, Endocrinology 146 (2005) 2519–2530.
[15] D.H. Volle, K. Mouzat, R. Duggavathi, B. Siddeek, P. Déchelotte, B. Sion, et al., Multiple roles of the nuclear receptors for oxysterols liver X receptor to maintain male fertility, Mol. Endocrinol. Baltim. Md. 21 (2007) 1014–1027.
[16] D.M. Stocco, Tracking the role of a star in the sky of the new millennium, Mol. Endocrinol. Baltim. Md. 15 (2001) 1245–1254.
[17] T.K. Jensen, A.-M. Andersson, N.E. Skakkebæk, U.N. Joensen, M.B. Jensen, T.H. Lassen, et al., Association of sleep disturbances with reduced semen quality: a cross-sectional study among 953 healthy young Danish men, Am. J. Epidemiol. 177 (2013) 1027–1037.
[18] T.K. Jensen, T. Sobotka, M.A. Hansen, A.T. Pedersen, W. Lutz, N.E. Skakkebaek, Declining trends in conception rates in recent birth cohorts of native Danish women: a possible role of deteriorating male reproductive health, Int. J. Androl. 31 (2008) 81–92.
[19] C. Wohlfahrt-Veje, K.M. Main, N.E. Skakkebaek, Testicular dysgenesis syndrome: foetal origin of adult reproductive problems, Clin. Endocrinol. (Oxford) 71 (2009) 459–465.
[20] K. Bay, C. Asklund, N.E. Skakkebaek, A.-M. Andersson, Testicular dysgenesis syndrome: possible role of endocrine disrupters, Best Pract. Res. Clin. Endocrinol. Metab. 20 (2006) 77–90.
[21] A.L. Herbst, H. Ulfelder, D.C. Poskanzer, Adenocarcinoma of the vagina. Association of maternal stilbestrol therapy with tumor appearance in young women, N. Engl. J. Med. 284 (1971) 878–881.
[22] B.E. Henderson, B. Benton, M. Cosgrove, J. Baptista, J. Aldrich, D. Townsend, et al., Urogenital tract abnormalities in sons of women treated with diethylstilbestrol, Pediatrics 58 (1976) 505–507.
[23] T. Ishikawa, I.S. Yuhanna, J. Umetani, W.-R. Lee, K.S. Korach, P.W. Shaul, et al., LXRβ/estrogen receptor-α signaling in lipid rafts preserves endothelial integrity, J. Clin. Invest. 123 (2013) 3488–3497.
[24] L.-L. Vedin, S.A. Lewandowski, P. Parini, J.-A. Gustafsson, K.R. Steffensen, The oxysterol receptor LXR inhibits proliferation of human breast cancer cells, Carcinogenesis 30 (2009) 575–579.
[25] L. Lundholm, S. Movérare, K.R. Steffensen, M. Nilsson, M. Otsuki, C. Ohlsson, et al., Gene expression profiling identifies liver X receptor alpha as an estrogen-regulated gene in mouse adipose tissue, J. Mol. Endocrinol. 32 (2004) 879–892.
[26] H. Gong, P. Guo, Y. Zhai, J. Zhou, H. Uppal, M.J. Jarzynka, et al., Estrogen deprivation and inhibition of breast cancer growth in vivo through activation of the orphan nuclear receptor liver X receptor, Mol. Endocrinol. Baltim. Md. 21 (2007) 1781–1790.
[27] D.H. Volle, M. Decourteix, E. Garo, J. McNeilly, P. Fenichel, J. Auwerx, et al., The orphan nuclear receptor small heterodimer partner mediates male infertility induced by diethylstilbestrol in mice, J. Clin. Invest. 119 (2009) 3752–3764.

[28] H.E. Virtanen, A. Adamsson, Cryptorchidism and endocrine disrupting chemicals, Mol. Cell. Endocrinol. 355 (2012) 208–220.
[29] K. Warita, K. Okamoto, K.-I. Mutoh, Y. Hasegawa, Z.-P. Yue, T. Yokoyama, et al., Activin A and equine chorionic gonadotropin recover reproductive dysfunction induced by neonatal exposure to an estrogenic endocrine disruptor in adult male mice, Biol. Reprod. 78 (2008) 59–67.
[30] A. Lenzi, M. Picardo, L. Gandini, F. Dondero, Lipids of the sperm plasma membrane: from polyunsaturated fatty acids considered as markers of sperm function to possible scavenger therapy, Hum. Reprod. Update 2 (1996) 246–256.
[31] B. Mascrez, N.B. Ghyselinck, M. Watanabe, J.-S. Annicotte, P. Chambon, J. Auwerx, et al., Ligand-dependent contribution of RXRbeta to cholesterol homeostasis in Sertoli cells, EMBO Rep. 5 (2004) 285–290.
[32] M. Chen, B. Xu, W. Ji, S. Qiao, N. Hu, Y. Hu, et al., Bisphenol A alters n-6 fatty acid composition and decreases antioxidant enzyme levels in rat testes: a LC-QTOF-based metabolomics study, PLoS ONE 7 (2012) e44754.