The Effects of Exogenous Estrogens on Estrogen Receptors in Male Reproductive Organs

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Abstract:

There is an essential physiological role for estrogen in male reproduction. Conversely, exposure to exogenous sources of estrogen has negative effects on reproductive physiology and fertility in men. Infertility, affecting nearly 15% of couples, is defined as the inability to conceive after one year of unprotected sexual intercourse. In at least 20% of cases, male reproductive pathology is the major cause for a couple's infertility. Thus, it is essential to investigate potential causes of infertility in adult males. Evidence shows that exposure to certain endocrine disruptors is associated with reduced semen quality and impaired fertility in men. Bisphenol A and Diethylstilbestrol are endocrine disruptors that act as exogenous sources of estrogen and have been associated with male reproductive pathology. This review will examine the role of exogenous estrogens on changes in gene expression of estrogen receptors ERα, ERβ, and GPR30. Previous studies have had conflictive results, suggesting that the effects of exogenous estrogens on male reproduction are multi-faceted. Future studies should focus on determining whether exogenous estrogens have a stimulatory and/or inhibitory effect on gene expression and whether this relationship is dose-dependent or if it follows a more complex dosage pattern.

Keywords:

Estrogen, Bisphenol A (BPA, 2,2-bis(4-hydroxyphenyl)), propane, Diethylstilbestrol (DES), endocrine disruption, GPR30

Introduction

The purpose of this review is to examine the roles of exogenous estrogen on gene expression in the male reproductive system. The first section will briefly describe the function of estrogen and its receptors in regulating normal physiological processes in males. Next, the effects of exogenous estrogens on estrogen receptors ERα, ERβ, and GPR30 will be considered. This review will focus primarily on the effects of two endocrine disruptors, Bisphenol A and Diethylstilbestrol, as sources of exogenous estrogen affecting the male reproductive system.

Estrogen and the Male Reproductive System

Estrogen Ligand

Estrogens are a group of steroid compounds, which include estrone, estradiol, and estriol, that act as hormones regulating reproductive development and function. Although estrogen was historically believed to be a female hormone, there is growing evidence of a biological role of this steroid in male reproduction. Estrogen is said to play a regulatory role in the male reproductive tract because estrogen biosynthesis occurs in the testes, and also because the absence of estrogen receptors (ERs) causes adverse effects on both spermatogenesis and steroidogenesis (O'Donnell et al., 2001).

In males, estrogen may be produced by the liver, adrenal glands, adipose tissue, and in the testes. Aromatase, an enzyme involved the production of estrogen from androgens, has been localized in virtually every cell type within the adult testis, including Sertoli cells, Leydig cells, spermatocytes, spermatids, and spermatozoa. The presence of aromatase confers the ability to synthesize estrogen locally within the male reproductive system (O'Donnell et al., 2001).

Mice with deletion of the aromatase gene (ARKO) are unable to produce endogenous estrogen and show disturbances of spermatogenesis associated with increased apoptosis of developing germ cells. In contrast, overexpression of aromatase induces cryptorchidism as well as spermatogenic arrest in mice (Akingbemi, 2005).

Furthermore, studies have been conducted using mice with deletion of the ER gene (ERKO), which codes for a subtype of estrogen receptors. These mice are able to produce estrogen, but they are unable to carry out responses mediated by this estrogen receptor, which include the production and function of sperm. The ERKO mice exhibit reduced number of sperm in the epididymis, reduced motility, and reduced ability to fertilize oocytes/eggs, supporting the regulatory role of estrogen in male reproduction (Eddy et al., 1996).

Estrogen Receptors

1. Estrogen Receptors Alpha and Beta

Estrogen receptors (ERs) are classic steroid hormone receptors and are members of a ligand-activated nuclear receptor gene superfamily. It was originally believed that only one form of nuclear ER existed (now ERα), until in 1996 the second form (now ERβ) was localized in many species including humans. The two subtypes of ERs are synthesized from separate genes, making them structurally and functionally distinct proteins. However, these receptors share the same organization of a ligand-binding domain, DNA-binding domain, and two transcriptional activation function domains (O'Donnell et al., 2001).

Classic ER action involves the ligand-dependent regulation of gene expression in target tissues (Hall et al., 2001). Estrogen binding to the ER releases the receptor from an inhibitory complex associated with heat shock proteins and chaperone proteins and leads to receptor phosphorylation. Next, the receptor complex homodimerizes and translocates to the nucleus. The ER then binds to estrogen response element (ERE), a 15-bp sequence of DNA located on the promoter region of target genes (Ho & Liao, 2002).

There is considerable tissue specificity with respect to the expression of ERα and ERβ. There has been extensive research on the expression and localization of the two ER subtypes within the adult testes in several mammals including humans. The localization of these subtypes in the Leydig cells, in humans especially, is extremely controversial with some studies showing and others not detecting immunoexpression. Sertoli cells in humans contain ERβ, but do not contain ERα. Furthermore, germ cells have been found to contain both ERs, with ERβ being more predominant (O'Donnell et al., 2001). The considerable conflict in the literature with respect to the expression of ER subtypes arises when comparing studies that use different antibodies in their immunohistochemical techniques. In order to clarify the exact location of ER subtypes in the testes, numerous well-characterized antibodies are required (O'Donnell et al., 2001).

In addition to the classic genomic pathway of ER interaction with target genes, estrogen can have non-genomic effects, presumably through interaction with receptors on the plasma membrane. In various cell types, both ERs can also elicit rapid cellular effects that peak within seconds to minutes after stimulation, making them too rapid to be induced transcriptionally. Furthermore, inhibition of RNA or protein synthesis does not block these effects, supporting the non-nuclear actions of ERs (Ho & Liao, 2002).

These signaling pathways may recruit second messengers including Ca2+ and cAMP, receptor tyrosine kinases including insulin-like growth factor (IGF)-1 receptors and G-Protein Coupled Receptors (GPCRs), serine/threonine kinase Akt, mitogen-activated protein kinases (MAPKs), non -receptor tyrosine kinases, as well as protein Kinases A and C (Ho & Liao, 2002).

The precise nature of these non-genomic receptors is unknown, but many of the pathways involve receptors at the plasma membrane. Thus, investigators are looking to determine if classical estrogen receptors are also present on the plasma membrane. Although the structure of ERα itself does not suggest that it can be anchored to the membrane, there is some evidence to support this theory (Ho & Liao, 2002).

Table 1

Summary of the likely localization of ERα, ERβ, and aromatase in the adult testis. The localization has been obtained from a literature view looking at estrogen and spermatogenesis (see review O'Donnell et al., 2001). The marker (X) indicates the potential presence of each protein in the specified cell type. It is important to note that several inconsistencies still exist regarding the localization of these proteins. This data suggests that estrogen production and action are present in somatic testicular cells as well as germ cells.

Cell Type	ERα	ERβ	Aromatase
Leydig cell	X	X	X
Sertoli cell		X	X
Spermatogonia		X	
Pachytene spermatocyte		X	X
Round spermatid		X	
Elongated spermatid			X
Epididymis	X	X	X

In summary, estrogen can act as a ligand, binding to ERα and ERβ located within the nucleus and inducing its effects through gene transcription. Furthermore, estrogen can also bind to non-nonnuclear ERs, which may be located at the plasma membrane, inducing its effects in a non-genomic manner, such as through the use of secondary messengers or through protein phosphorylation.

2. GPR30

In many reports, the non-genomic estrogen-responsive receptor is proposed to be ERα and/or ERβ, or a modified form of the protein. (Prossnitz et al., 2007). In various organs, however, non-genomic effects of estrogen are not regulated by either membrane-bound ERα or ERβ. Furthermore, aromatase knockout mice show a more severe testicular phenotype than ER knockouts, suggesting that estrogen is still functioning in the absence of ERα and ERβ (Murata et al., 2002). Thus, there is the potential for estrogen action that is unrelated to the ER family of steroid receptors. The same conclusion can be reached by combining findings of studies that have shown the existence of G protein-regulated signaling by estrogen as well the localization of estrogen binding sites to the plasma membrane (Prossnitz et al., 2008).

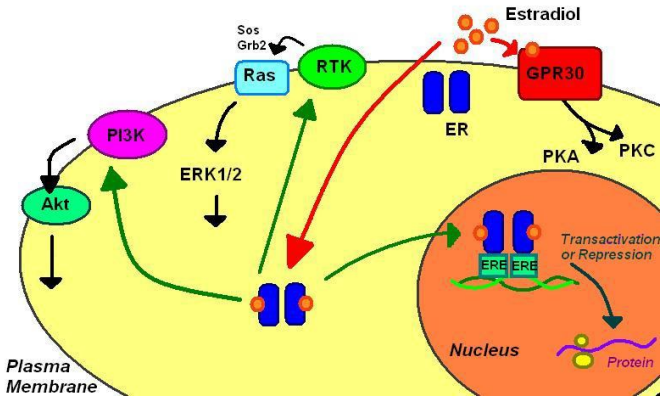
G protein-coupled receptors (GPCRs) are membrane proteins that are activated by ligands such as hormones, neurotransmitters, chemokines, and small peptides. (Funakoshi et al., 2006). The two principal signal transduction pathways involving GPCRs are the cAMP pathway and the phosphatidylinositol signal pathway (Gilman, 1987).

In the late 1990's, a novel G protein-coupled receptor (GPCR) was found which was distinct from any other GPCR. GPR30, a novel 7-transmembrane G protein-coupled receptor, responds to estrogen stimulation and is involved in the regulation of cell growth, including proliferation and apoptosis. There is evidence of GPR30 localization at the plasma membrane and at the endoplasmic reticulum (Prossnitz et al., 2008). An intracellular localization of the receptor is more consistent with estrogen membrane permeability.

It has been determined that the binding of estrogen to GPR30 can be displaced by 17βEstradiol, but not 17αEstradiol, demonstrating the stereo-specificity of the receptor (Thomas et al., 2005). Furthermore, it has been found that GPR30 binds nearly the same amount of estrogen as ERα, suggesting that this receptor does not merely enable estrogen binding, but is involved in estrogen's regulatory fun-

Figure 1

Selected nuclear and nonnuclear actions of estrogen receptors. In classical nuclear pathways, the binding of 17βestradiol to estrogen receptors leads to a translocation of ligand-bound receptor to the nucleus and activation of ERE-dependent transcription. Alternatively, nonnuclear actions may include recruitment of the MAPK pathway, including ERK1/2 through activation of kinases, including Ras. Estrogen receptors can also elicit PI3K and Akt. Ligand binding to GPR30, which is also an estrogen receptor, may lead to activation of several pathways such as PKA and PKC (adapted from Ho & Lia, 2002).

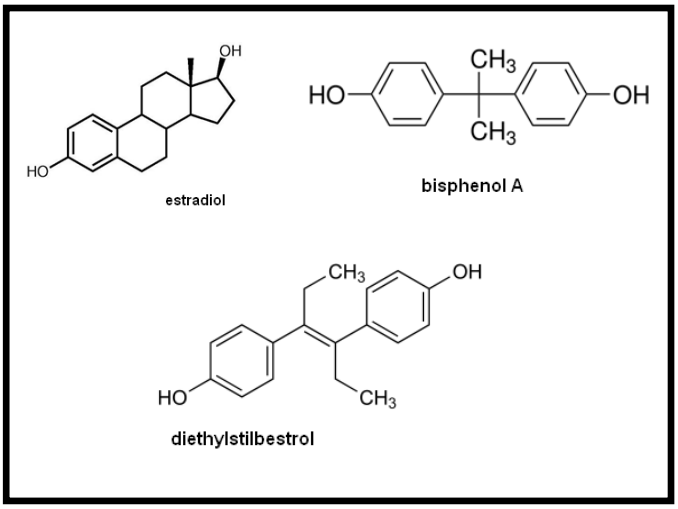


tions within cells (Prossnitz et al., 2008).

The importance of GPR30 in male reproduction is still debatable, and it has even been suggested that GPR30 may be dispensable for the normal development of reproductive organs and reproductive function in mice (Otto et al., 2009). However, there is growing evidence of the involvement of GPR30 with male reproduction. Immunohistochemistry in adult mouse testes was used to demonstrate GPR30 expression in male germ cells, indicating that

Figure 2

Chemical structures of estradiol, Bisphenol A, and diethylstilboestrol.



estrogen effects associated with male reproduction can be induced through this receptor. This study also found, for the first time, that estrogens interacting with GPR30 activate a rapid EGFR/ERK/fos pathway which stimulates mouse spermatogonial cell line proliferation (Sirianni, et al., 2007).

Exogenous Estrogen Ligands

Bisphenol A

Bisphenol A (BPA), is an organic compound with two phenol functional groups and is used as a monomer in polycarbonate plastics. Its structural similarity to estrogen allows BPA to compete with estradiol at binding sites, acting as an exogenous estrogen and endocrine disruptor. One of the highest volume chemicals produced worldwide, BPA can be found in the linings of most food and beverage cans, dental sealants, as well as additives in a wide variety of consumer products (Burridge, 2003). BPA leaches into human food supply through heating of cans, the presence of acidic or basic products in the cans, as well as through repeated washing of polycarbonate products (vom Saal & Hughes, 2005). In the Unites States, BPA has been shown to account for the most estrogenic activity leaching from landfills (Coors et al., 2003).

Suspected of being hazardous to humans since the 1930's, there have been a number of studies of the disruptive effects of BPA to humans. In vitro studies show that BPA's disruptive effects on cells are mediated by genomic and non-genomic estrogen-response mechanisms, with disruptions occurring at doses as low as 1 pM (Wozniak et al., 2005).

A complete background on BPA, although vital, is out of the scope of this review and is available through other sources. A comprehensive document containing references for numerous BPA review articles, mechanisms of action, pharmacokinetics, sources of exposure, and exposure levels in humans, is available online (see review Endocrine Disruptors Group, 2009).

BPA produces estrogenic effects by interacting with ERs. BPA has been shown to be a Selective Estrogen Receptor Modulator (SERM), showing different affinity and regulation of ERα and ERβ and responding differently in various tissues (Routhledge et al., 2000). Furthermore, BPA has recently been shown to act as an androgen antagonist in

the presence of the androgen receptor (AR), blocking testosterone synthesis. Data from this one study, which exposed rats to low doses of BPA, shows that BPA has an inhibitory effect on testicular steroidogenesis. The suppression of pituitary Luteinizing Hormone (LH) along with increased pituitary ERβ mRNA levels in this experiment suggest that BPA's effects are ER mediated (Akingbemi et al., 2004).

With respect to male reproduction, BPA has been shown to decrease daily sperm production and fertility with either developmental or adult exposure in rats. In one study, oral doses of BPA as low as 20 g/kg decreased testicular weight and significantly reduced daily sperm production as well as spermatogenesis (Sakaue et al., 2001).

Diethylstilbestrol

Diethylstilbestrol (DES) is a synthetic, non-steroidal estrogen that has been used traditionally for treatment of multiple pregnancy related problems, including miscarriages, premature birth, and abnormal bleeding (Rubin, 2007).

Synthetic estrogens, such as DES, have been shown to induce bladder, ovarian, testicular, lymphatic, uterine, mammary, and prostatic tumors in mammals (Roy et al., 1997). There is also epidemiological evidence of a slightly increased risk of breast cancer in populations of women exposed to DES (Malone, 1993).

DES has a high affinity for ERs and modifies the expression pattern of androgen and estrogen receptors. Furthermore, neonatal exposure of DES is associated with various reproductive tract anomalies in the male (Goyal et al., 2003; Williams et al., 2001). These changes include reduced growth of the prostate, seminal vesicles, vas deferens, epididymis, and efferent ducts, as well as reduced numbers of Sertoli, Leydig, and germ cells (Williams et al., 2001).

According to another study, DES-exposed rats generally had: (1) altered sperm morphology, (2) decreased sperm production, (3) decreased weight of the testis, epididymis, and seminal vesicles, and (4) decreased sperm fertility as evident from a reduced number of offspring (Goyal et al., 2003).

One study found that prenatal exposure of DES reduces the offspring's Sertoli cell population during adulthood (Sharpe et al., 1998). Here, DES was administered neonatally to rats, and developmental changes in Sertoli cell function were evaluated over time. In adulthood, DES-treated rats had over 60% reduction in testicular weight and very low daily sperm production. Because Sertoli cell population is representative of spermatogenic potential, this finding suggests that neonatal exposure to estrogens may lead to impaired spermatogenesis in adulthood (Sharpe et al., 2003.)

A National Toxicology Program Peer Review Panel stated that DES is an appropriate positive control estrogenic drug that may be used in studies of estrogenic chemicals, such as BPA (Welshons, et al., 2006).

Dose-Response Relationship of Bisphenol A

A review looking at low dose effects of BPA verified the presence of a non-monotonic response function, in which the chemical does not induce change in a classical dose-dependent manner (vom Saal & Hughes, 2005). Instead, it has been shown that low doses of BPA may actually cause a greater response in target cells, while higher doses may inhibit the same response. Several mechanisms could be involved, including changes in tissue expression of affected receptors (Wetherill et al., 2007). Testing the effects of various doses is significant in light of the emerging data concerning the inverted U shape dose-response function of BPA.

Endocrine Disruptors and Estrogen Receptors

The pattern of ER expression is a target for exogenous estrogen action, which can produce either stimulatory or inhibitory effects (Akingbemi, 2005). Although one would assume that an increase in estrogen, whether endogenous or exogenous, would induce increased expression of ERs, the evidence so far suggests a more complex relationship.

A group of mice treated with 50 μg/ml of BPA displayed a significant reduction in mRNA expression of ERβ. In contrast, ERα mRNA expression was increased significantly in the same mice (Takao et al., 2003). Interestingly, a single injection of estradiol benzoate at a 500μg dosage induced the opposite effects in rats, reducing ERα mRNA expression and increasing ERβ mRNA expression (Tena-Sempere et al., 2000). These results suggest that the effects of exogenous estrogen may be differential modulation of ERα and ERβ in the testes. Furthermore, additional research is required to determine whether each endocrine disruptor produces consistent changes in estrogen receptor gene expression, or if the effects may vary with different doses and/or conditions.

The complex effects and disparities of exogenous estrogens on ER expression may be attributed to an inverted U-shaped dosage response, in which low doses of a chemical may be stimulatory but high doses may be inhibitory. Another proposition is that inappropriate estrogen exposure, such as through ingestion of exogenous estrogens, may lead to the down-regulation of ERs. This would result in an estrogen deficiency syndrome, in which mice have deficiencies of ERα similar to ERα knockout mice.

Studies in the testes support this proposal, suggesting that neonatal DES exposure leads to downregulation of ERα and androgen receptor, but an increase in ERβ expression (Tena-Sempere et al., 2000). DES exposure may result in a permanent change in estrogen responsiveness or in estrogen-dependent gene expression (O'Donnell et al., 2001).

Because GPR30 has only recently been recognized as an estrogen receptor in the testes, there is little research studying the effects of endocrine disruptors on gene expression in of this receptor.

Conclusion

Endogenous estrogen plays a significant regulatory role in male reproduction. At the same time, exogenous estrogens, such as endocrine disruptors Bisphenol A and Diethylstilbestrol, have been shown to disrupt development of the male reproductive system and to affect fertility. One mechanism through which these endocrine disruptors can affect reproduction is by changing the expression of estrogen receptor proteins at a cellular level, thus disturbing cellular regulation by endogenous estrogen.

The exact mechanisms through which BPA and DES affect the male reproductive system are complex and may be in part due to changes in the expression of estrogen receptors, which would lead to altered function at a cellular level. Currently, studies show that gene expression of estrogen receptors changes with exposure to BPA or DES, but there is inconclusive evidence as to whether receptor expression is stimulated or inhibited. In fact, gene expression may be influenced differently based on the chemical, dosage, specific estrogen receptor subtype, and tissue (Akingbemi, 2005).

Additional research is required to understand the complex relationship that exists between exogenous estrogens and gene expression of estrogen receptors ERα, ERβ, and GPR30. Future studies should focus on clarifying whether this relationship is stimulatory and/or inhibitory. Furthermore, by studying the effects of different dosages of exogenous estrogens, it may be possible to determine if the changes in gene expression induced are dose-dependent, or if they follow the proposed inverted U-shaped dose-response.

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