Responsiveness of endometrial genes Connexin26, Connexin43, C3 and clusterin to primary estrogen, selective estrogen receptor modulators, phyto- and xenoestrogens

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Abstract

Phytohormones and chemical compounds revealing estrogenic effects are of increasing interest for their possible influence on the physiology of the reproductive tract. The gap junction connexin (Cx) genes Cx26 and Cx43, the plasma glycoprotein clusterin gene and the complement C3 gene are highly regulated by estrogen in rat endometrium. To test the value of these genes as markers for estrogenic responsiveness we analyzed the effects of estradiol, diethylstilbestrol, the selective estrogen receptor modulators (SERMs) raloxifene and tamoxifen, the phytoestrogens genistein and daidzein, and the industrial compounds DDT (1,1,1-trichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl) ethane) and polychlorinated biphenyl (PCB) on the transcription of these genes in rat endometrium *in vivo*. Enhancement of Cx26 and decrease of clusterin transcripts expression by estradiol was observed at 0.03 µg/250 g body weight (BW), and induction of C3 expression was observed at 0.05 µg/250 g BW. A comparable effect was obtained by a tenfold higher concentration of diethylstilbestrol. Tamoxifen had a regulatory effect on this set of genes at about a 300-fold higher concentration, while raloxifen revealed much weaker estrogenic activity. No effect on Cx43 transcripts was observed with any of the compounds at the concentrations used. An effect of genistein was observed only on Cx26 expression, while PCB decreased clusterin transcripts. These results show that Cx26, C3 and clusterin reveal a comparable sensitivity to estrogens and SERMs. With respect to the phytoestrogen genistein, however, Cx26 seems to be the most sensitive gene. The analysis of clusters of estrogen-sensitive endometrial genes could help to identify estrogenic substances, assess their potency, and elucidate their mechanism of action.

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Introduction

Pharmaceutical compounds as well as environmental and dietary substances revealing estrogenic effects are of increasing interest because of their potential biological impact on human health. Exposure to such substances has been associated with an increased incidence of hormone-dependent tumorigenesis like testicular, endometrial and breast cancer (Davis *et al.* 1993, Cotton 1994, Safe 1995, Thierfelder *et al.* 1995), disorders of the male reproductive tract (Sharpe & Skakkebaek 1993, Cooper & Kavlock 1997), and interference with reproductive physiology (Auger 1995, Safe 1995,

Strauss *et al.* 1998a). A remarkable diversity of naturally occurring and synthetic compounds has been shown to mimic the biological effects of 17β-estradiol. This includes synthetic estrogens such as tamoxifen and raloxifene, which have been developed as antiestrogens for the therapy of breast cancer (Nass *et al.* 1998, Overmoyer 1999), phytoestrogens from plant origin (Markiewicz *et al.* 1993) as well as pesticides and industrial chemicals (Schaefer *et al.* 1996).

Although many of these substances are known to bind to the estrogen receptor (Kuiper *et al.* 1997), there is little information about the molecular mechanisms and the biological potency of these

compounds. *In vitro* estrogen receptor binding assays do not provide information about the physiological activity of the substances tested (Hopert *et al.* 1998). Receptor binding studies neither discriminate between estrogenic and anti-estrogenic effects nor do they allow for the fact that some non-estrogenic chemicals are metabolized *in vivo* to compounds that are estrogenic (Jorgensen *et al.* 1998). Thus there is a need for biological models to characterize the hormonal activity of substances which reveal estrogenic effects or act as endocrine disruptors to elucidate the systemic mechanisms of their action. Several *in vitro* assays have been developed to assess the estrogen-like activity of individual compounds or natural mixtures (Zacharewski 1997), but they all show limitations as only isolated aspects can be investigated.

The uterus is a classical target organ for estrogenic action. The widely used uterotrophic assay determines the increase in uterine wet weight (Lan & Katzenellenbogen 1976); however, in this assay different effects leading to this increase in weight cannot be discriminated. Meanwhile, it has been shown that compounds revealing estrogenic effects may lead to only a partial estrogenic transformation. In the past years a large number of genes has been identified which are regulated in the endometrium and can be used as marker genes for estrogenicity. It has been shown that estrogen enhances transcription of the complement C3 gene (Sundstrom *et al.* 1989, Hopert *et al.* 1998, Diel *et al.* 2000) but inhibits expression of the clusterin gene (Wunsche *et al.* 1998, Diel *et al.* 2000) and thus reveals selective regulatory effects on endometrial genes.

Recently, we demonstrated that the gap junction protein connexin (Cx) 26 is sensitively regulated by estrogen in rat endometrium (Grümmer *et al.* 1994, 1999) and may serve as a marker gene to identify estrogenic effects in the uterus. Since the differences in signaling pathways of estrogens, selective estrogen modulators (SERMs) and substances known as endocrine disruptors are still unknown, a screening system for a set of marker genes is needed to evaluate the estrogenic effects in detail. In order to evaluate genes as sensitive indicators for estrogenic effects we have compared the regulation properties of a set of endometrial genes — Cx26, Cx43, complement C3, and clusterin. These estrogen-sensitive genes have been chosen to evaluate their functional significance as marker

genes for screening the estrogenic potential of the estrogen agonist diethylstilbestrol (DES), the SERMs tamoxifen and raloxifen, as well as the phytoestrogens genistein and daidzein, and the xenoestrogens DDT (1,1,1-trichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl) ethane) and polychlorinated biphenyl (PCB). We demonstrate that Cx26, clusterin and C3 show a similar responsiveness to estrogen and to SERMs, whereas with regard to the phytoestrogen genistein Cx26 seems to be the most sensitive. Thus this endometrial gene cluster is highly suited for the investigation of the biological potential of natural and synthetic estrogens and for the evaluation of the signal cascades involved.

Materials and methods

Animal care

Adult female Sprague-Dawley rats were housed—under defined conditions with a temperature of 22 ± 1 °C, an atmospheric humidity of $55 \pm 10\%$, and a 12-h darkness:12-h light cycle. They were fed standard pellet food and water was provided *ad libitum*. All animal experiments were approved by the institutional animal care committee.

Treatment and compounds

Rats of 250 g body weight were ovariectomized and kept for 14 days before treatment to allow the endogenous ovarian hormone levels to decline. A single injection of the following compounds was administered subcutaneously in 200 µl sesame oil: 17β-estradiol (E_2 ; 0.01, 0.03, 0.05, 0.08 µg/rat), DES (0.05, 0.1, 0.3, 0.5, 1, 10 µg/rat), tamoxifen (1, 10, 100 µg/rat), genistein and daidzein (2.5 mg/rat) were obtained from Sigma (Deisenhofen, Germany), 1,1,1-trichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl) ethane (o,p'-DDT; 2.5 mg/rat) was obtained from the Institute of Organic Industrial Chemistry (Warsaw, Poland), polychlorinated biphenyl (PCB; 2.5 mg/rat) was obtained from Environmental Solutions Inc. (Longwood, USA), Raloxifen (1, 10 µg/rat) was kindly provided by Schering AG (Berlin, Germany), and ICI 182,780 (ICI; 0.5 mg/rat) by A Wakeling (Zeneca Pharmaceuticals, UK). E_2 , DES, tamoxifen, ICI, genistein, and PCB were dissolved in a 1:1 mixture of ethanol and benzylbenzoate, raloxifen and DDT were

dissolved in ethanol, and daidzein was dissolved in DMSO. Rats were killed 24 h after injection of compounds. Controls were given an equal volume of vehicle only. Three animals were used for each experiment.

Tissue collection

Animals were killed by ether and uteri were removed. Small pieces of the uteri were frozen in liquid nitrogen for subsequent histochemical analysis, the remaining uteri were opened longitudinally on an ice-cold glass plate and the endometrium was carefully scraped off. Histological examination of the removed endometrium revealed no contamination with myometrial tissue (data not shown). The endometrial samples of the three rats per experimental approach were pooled, frozen in liquid nitrogen, and stored at -80°C .

Northern blot analysis

Total RNA was extracted from endometrial tissue using the RNeasy midi kit (Qiagen, Hilden, Germany). Five micrograms (as estimated from optical absorbance measurements at 260 nm) were electrophoresed on a denaturing agarose-formaldehyde gel and blotted onto nylon membranes (Hybond-M, Amersham-Bucher GmbH). cDNA probes were random-prime labeled with [^{32}P]dCTP and hybridized with the RNA blots overnight at 42°C in a solution containing 55% deionized formamide, 1 M NaCl, 1% sodium dodecyl sulfate (SDS), 10% dextran sulfate, and 100 $\mu\text{g}/\text{ml}$ salmon sperm DNA. cDNAs of the following genes were used for hybridization: rat Cx26 (Zhang & Nicholson 1989), rat Cx43 (Beyer *et al.* 1987), rat complement C3 (Sundstrom *et al.* 1989), and rat clusterin (Guenette *et al.* 1994). Blots were washed at 60°C in $1 \times \text{SSC}$, 0.1% SDS for 1 h, in $0.5 \times \text{SSC}$, 0.1% SDS for 30 min, and in $0.2 \times \text{SSC}$, 0.1% SDS for 30 min. Exposure to Kodak XAR-5 films took place at -80°C with intensifying screens. After exposure, each blot was re-hybridized with a rat actin-specific cDNA probe (Reuss *et al.* 1996) using the same conditions of hybridization.

In each experiment the endometrium of 3 animals was pooled. Signals detected by autoradiography were quantified by densitometry. Densitometric values for gene expression were calculated relative to the β -actin level of the corresponding lane for possible differences in RNA loading.

Immunohistochemistry

Immunohistochemical staining was performed on cryostat sections (4–6 μm) as described previously (Winterhager *et al.* 1991) using affinity purified rabbit antibodies (1 $\mu\text{g}/\text{ml}$) to Cx26 from mouse liver gap junctions (Traub *et al.* 1989) and to a synthetic peptide representing the C-terminal 22 amino acids of rat Cx43 (Traub *et al.* 1992). For positive controls, rat heart (Cx43) and liver (Cx26) were tested. For controls, rabbit preimmune serum was used instead of the primary antibody. Photographs were taken with an Axiophot microscope (Zeiss) equipped for epifluorescence.

Results

Regulation of Cx26, Cx43, C3 and clusterin gene expression by estrogen and DES

E_2 was applied to ovariectomized rats as a single injection at concentrations ranging from 0.01 to 0.08 $\mu\text{g}/\text{rat}$. mRNA levels of Cx26, Cx43, C3 and clusterin genes were analyzed in the endometrium 24 h after treatment. Cx26 gene expression was already induced at an E_2 concentration as low as 0.03 $\mu\text{g}/\text{rat}$, and complement C3 gene expression was induced at 0.05 $\mu\text{g}/\text{rat}$ (Fig. 1). The amount of transcripts of both genes increased in a gene-dose-dependent manner. Using the same concentrations, however, no increase in Cx43 mRNA could be observed. In former studies, an increase in Cx43 gene expression was induced by E_2 levels from 0.1 $\mu\text{g}/\text{rat}$ upwards (Grümmer *et al.* 1999). Clusterin gene expression which is known to be suppressed by E_2 was decreased from a concentration of 0.03 $\mu\text{g}/\text{rat}$ upwards (Fig. 1), indicating a similar sensitivity to E_2 compared with C3 and Cx26.

The strong estrogen agonist DES revealed a similar effect on endometrial gene expression; however, compared with E_2 , DES had to be applied in about a tenfold higher concentration (0.3 $\mu\text{g}/\text{rat}$) to induce comparable levels of Cx26 and C3 transcripts or to inhibit clusterin mRNA. No effect on Cx43 gene expression was observed after application of DES at concentrations of 1 and 10 $\mu\text{g}/\text{rat}$ respectively (Fig. 1). It has already been shown that induction of connexin genes by E_2 is mediated via the estrogen receptor (Grümmer *et al.* 1999). Also, the action of DES on Cx26 and C3 gene induction and the suppression of clusterin is mediated by the estrogen receptor since the effect of DES on all endometrial genes tested is abolished

Figure 1 Northern blot (left) and corresponding densitometric evaluation (right) of endometrial RNA 24 h after injection of E₂ or DES. Induction of Cx26 and C3 gene expression was observed from a concentration of 0.03 μg/rat and 0.5 μg/rat, respectively, and upwards, while there was no increase in Cx43 transcripts. Clusterin gene expression was decreased at an E₂ concentration as low as 0.03 μg/rat. DES had to be applied in a tenfold higher concentration compared with E₂ to achieve a similar effect on endometrial gene expression, which could be inhibited by simultaneous injection of 0.5 mg ICI 182,780 (ICI). Controls (C) were treated with vehicle only.

by simultaneous application of 0.5 mg of the antiestrogen ICI 182,780 (Fig. 1).

Expression and localization of connexin proteins after estrogen and DES treatment was investigated by immunohistochemistry. The corresponding Cx26

protein was detected 24 h after application of 0.08 μg E₂ (Fig. 2b) or 1 μg DES (Fig. 2d). Application of lower amounts of E₂ and DES respectively, which increased mRNA levels, did not lead to detectable levels of Cx26 protein (Fig. 2a,c). Cx43 protein was

Figure 2 Immunohistochemical localization of Cx26 and Cx43 in endometrium of ovariectomized rats treated with estrogen agonists and SERMs, respectively. While no Cx26 protein is detected 24 h after application of 0.03 μg E₂/rat (a) and 0.03 μg DES/rat (c), a clear punctate staining for Cx26 can be seen in the epithelium (E) of rats treated with 0.08 μg E₂ (b) or 1 μg DES (d) respectively. Cx43 protein is expressed in the stromal compartment (S) of untreated ovariectomized rats (e) and shows no change in strength of reaction when rats were treated with up to 0.1 μg E₂ (f). While application of 100 μg tamoxifen leads to a distinct punctate reaction for Cx26 antigen in the epithelial cells (g), no staining can be observed 24 h after injection of 100 μg raloxifen (h). Bar in a and c–h=50 μm, in b=30 μm.

Figure 3 Northern blot (left) and corresponding densitometric evaluation (right) of endometrial RNA 24 h after application of tamoxifen (Tam) or raloxifen (Ral) to ovariectomized rats. At a concentration of 100 $\mu\text{g}/\text{rat}$ both compounds reveal a clear inductive effect on Cx26 and C3 gene expression but this treatment leads to a decrease in clusterin transcripts, raloxifen showing a lower estrogenic effect than tamoxifen. These effects are inhibited by simultaneous application of 0.5 mg ICI 182,780 (ICI), which even causes an enhancement of clusterin transcript expression. Controls (C) were treated with vehicle only.

expressed in the endometrial stromal compartment of untreated ovariectomized rats independent of hormonal treatment (Fig. 2c); however, no obvious difference in immunolabeling was seen compared with rats treated with 0.1 μg E_2 (Fig. 2f).

Regulation by tamoxifen and raloxifen

The effect of the selective estrogen receptor modulators (SERMs) tamoxifen and raloxifen on endometrial gene expression has also been analyzed 24 h after a single subcutaneous injection of these SERMs at different concentrations. Both compounds were able to induce Cx26 and C3 gene expression and to decrease the amount of clusterin transcripts; however, much higher concentrations

were needed compared with estrogen and DES (Fig. 3). An inductive effect on Cx26 and C3 gene expression and a decrease in clusterin transcripts could be achieved by application of 100 μg tamoxifen/rat. In comparison with tamoxifen, the same concentration of raloxifen acts only as a weak agonist on endometrial gene expression. This difference in agonistic action of the two SERMs could be confirmed by immunohistochemistry for Cx26 protein expression. While there was a distinct punctate reaction for Cx26 antigen in the endometrial epithelium 24 h after injection of 100 μg tamoxifen (Fig. 2g), no protein expression was observed after application of the same amount of raloxifen (Fig. 2h). No regulatory effect of either SERM could be seen on Cx43 gene transcription.

Figure 4 Northern blot (left) and corresponding densitometric evaluation (right) of rat endometrial RNA. Application of the phytoestrogen genistein leads to induction only of Cx26 gene expression, which can be inhibited by simultaneous application of 0.5 mg ICI 182,780 (ICI). The amount of clusterin transcript expression is reduced 24 h after injection of PCB. Daidzein and DDT have no clear effect on endometrial gene expression 24 h after application. Controls (C) were treated with vehicle only.

The estrogenic effect of tamoxifen and raloxifen also acts via the estrogen receptor, as it could be inhibited by application of the antiestrogen ICI 182,780 (Fig. 3). Interestingly, the amount of clusterin transcripts was clearly enhanced by simultaneous application of 0.5 mg ICI 182,780 compared with untreated ovariectomized rats. This phenomenon was also observed after application of DES and ICI 182,780 (compare Fig. 1 with Fig. 3).

Regulation of gene expression by phytoestrogens and industrial compounds

A single subcutaneous injection of 2.5 mg of the phytoestrogen genistein led to an increase in Cx26

gene expression within 24 h. This induction could be suppressed by simultaneous application of the antiestrogen ICI 182,780. However, at this concentration genistein caused no effect with regard to the amounts of Cx43, C3 and clusterin gene transcripts (Fig. 4). In contrast to genistein, a single application of 2.5 mg of the phytohormone daidzein revealed no visible effect on either of the endometrial genes tested 24 h after subcutaneous injection (Fig. 4).

The industrial compound DDT at a concentration of 2.5 mg/rat had no effect on the endometrial genes tested, whereas 24 h after the application of the xenoestrogen PCB, a slight reduction of clusterin mRNA could be observed (Fig. 4).

Discussion

To obtain more comprehensive information about the estrogenic activity of natural and synthetic estrogens we tested short-term regulation of the estrogen responsive genes Cx26, Cx43, C3 and clusterin in rat endometrium. Estrogen agonists, as well as the SERMs tamoxifen and raloxifen, demonstrated a similar effect on regulation of Cx26, C3 and clusterin gene transcription, and the three genes showed comparable sensitivity to these compounds. Previous studies revealed that Cx26 is regulated sensitively by estrogen (Grümmer & Winterhager 1998, Grümmer *et al.* 1999). Here, we confirm this responsiveness to even lower estrogen concentrations as well as to SERMs and phytoestrogens. Cx43, which is known to be regulated by estrogen at higher concentrations (Grümmer *et al.* 1999), is obviously less sensitive to estrogens, and its expression was not modulated by the conditions used in the present study. Differences with regard to transcriptional regulation of endometrial genes, however, were observed after application of phyto- and xenoestrogens. Thus the gene cluster analysis in response to different estrogenic stimuli revealed that different signaling pathways may be involved.

In general, the most sensitive regulation of gene expression was obtained by application of 17 β -estradiol. The strong E₂ agonist DES, which reveals an even higher binding capacity to the estrogen receptor (ER) than E₂ (Kuiper *et al.* 1997), had less effect on Cx26, C3 and clusterin gene transcription than comparable concentrations of estradiol. Thus the physiological effect of DES seems to be less than could have been expected from ER binding assays. This confirms the observation of other groups that estrogen receptor binding assays do not allow conclusions to be drawn regarding the biological activity of estrogenic substances (Hopert *et al.* 1998, Strunck *et al.* 2000), and that hormonal activity can only be evaluated in a biological model.

Selective estrogen receptor modulators (SERMs) act either as ER agonists or antagonists, dependent on the target tissue. The synthetic estrogen, tamoxifen, which is widely used as an antiestrogen in the treatment of breast cancer (Nass *et al.* 1998, Overmoyer 1999) reveals agonistic effects in the uterus which lead to an increased risk of development of endometrial cancer after long-term tamoxifen therapy (Assikis & Jordan 1995, Sato

et al. 1996, Carmichael 1998). We confirmed this agonistic effect on the endometrium by its influence on transcriptional regulation of Cx26, C3 and clusterin gene expression.

Considerable effort has been made to identify compounds which lack unwanted agonistic effects. The related substance raloxifen, which also acts as an estrogen antagonist in breast tissue (Jordan 1998) does not stimulate endometrial proliferation. Corresponding to the studies of Sato and coworkers (1996) who showed a slight agonistic effect in the uterus, raloxifen also reveals weak estrogenic effects in rat endometrium with regard to regulation of the reference genes Cx26, C3 and clusterin. The effects of these antiestrogens are mediated by the estrogen receptor as shown in this and other publications (Grese *et al.* 1997, Paech *et al.* 1997). However, the molecular mechanisms for gene- and tissue-specific effects of selective estrogen receptor modulators are not known in detail.

Recently, a novel ER subtype (ERbeta) was discovered by Kuiper and coworkers (1996). The two ER subtypes, alpha and beta, are expressed differentially in estrogen target tissues (Kuiper *et al.* 1997). It is supposed that the tissue-specific effects of SERMs correspond to the tissue distribution of the different estrogen receptor subtypes. It could be shown that tamoxifen is a potent transcriptional activator with ERbeta via an AP1 site, whereas estradiol inhibits transcription after binding to ERbeta, but activates transcription with ERalpha (Paech *et al.* 1997). In addition, different expression of transcriptional coactivators and corepressors which modulate receptor activity may result in tissue-specific activation of certain genes (Horwitz *et al.* 1996, McKenna & O'Malley 2000).

Much less is known about the regulatory effects of phytoestrogens on gene expression patterns *in vivo*. Phytoestrogens constitute a diverse group of plant-derived compounds possessing estrogenic activity (Strauss *et al.* 1998a) and revealing multiple biological effects, including beneficial effects on osteoporosis, breast cancer, prostate cancer and menopausal symptoms (Adlerkreutz & Mazur 1997, Barnes 1998, Clarkson *et al.* 1998), and on the cardiovascular system (St Clair 1998). On the other hand, epidemiological and experimental studies suggest that the consumption of a phytoestrogen-rich diet may decrease the risk of estrogen-related cancers such as breast and prostate

cancer (Adlerkreutz 1995, Strauss *et al.* 1998a). However, the causal relationship as well as the mechanisms of action still remain to be demonstrated, and there is only sparse evidence of an antiestrogenic effect of phytoestrogens (Mäkelä *et al.* 1994, Ruh *et al.* 1995). Phytoestrogens bind to both types of ER, induce estrogen-responsive genes and their action can be blocked by antiestrogens (Mäkelä *et al.* 1994, Kuiper *et al.* 1997). However, most of the phytoestrogens bind to the estrogen receptor with a lower affinity than 17 β -estradiol (Kuiper *et al.* 1997) and are weakly estrogenic *in vitro* (Markiewicz *et al.* 1993) and *in vivo* (Diel *et al.* 2000). The single largest source of phytoestrogens in the human diet is from soy or soy products. Consumption of soy has been shown to lengthen the menstrual cycle (Phipps *et al.* 1993, Cassidy *et al.* 1994), and animal studies suggest that soy protein is able to prevent cardiovascular diseases (Anthony *et al.* 1996) but may also cause severe reproductive tract disorders including impaired fertility (Burroughs *et al.* 1990, Strauss *et al.* 1998b) which could be explained by the estrogenic activity of phytoestrogens. Here, we have shown that the soy isoflavonoid genistein exclusively increases Cx26 gene expression in rat endometrium via the ER, but at the concentration used in this study it had no effect on transcription of the C3 and clusterin genes which, however, revealed a similar sensitivity to estrogen as Cx26. This indicates a specific signaling pathway for genistein action. It is known that genistein is a specific inhibitor of protein tyrosine kinases (Akiyama *et al.* 1987) and thus may interfere with the regulation of certain endometrial genes. It has to be taken into consideration that sesame oil, a natural plant oil which has been used as a vehicle in this study, might itself reveal estrogenic effects (Markaverich *et al.* 2002). However, controls which have included injecting sesame oil as vehicle revealed no induction of the target gene products, showing that the influence of sesame oil is negligible in this study.

Involvement of different estrogen receptor cofactors as well as estrogen receptor subtypes has to be considered when investigating the estrogenic activity of natural and synthetic estrogens (Kuiper *et al.* 1998). Although the biological role of ERbeta is poorly understood, the relative binding affinity of specific estrogens to ERbeta and ERalpha varies widely. This could, in addition to the

recruitment of different subsets of coactivator or corepressor complexes, provide a mechanism by which SERMs as well as environmental estrogens reveal tissue selective actions. To evaluate the role of the different ER subtypes, experiments with ERalpha and ERbeta knockout mice have to be performed.

In contrast to our results, Hopert and coworkers (1998) showed in an *in vitro* assay using established rat endometrial adenocarcinoma cells that genistein and daidzein increased formation of complement C3. The uterotrophic activity of daidzein has previously been described as low (Farmakalidis *et al.* 1985). Our results indicate that genistein reveals a higher short-term estrogenic potency *in vivo* than its inactive analog daidzein. However, daidzein has been shown to be capable of regulating clusterin and C3 mRNA expression in the rat uterus after 3 days application via a gastric tube (Diel *et al.* 2000).

The industrial compound DDT had no visible effect on endometrial gene transcription within 24 h of application. In previous studies, it had proved to be a potent estrogen as regards the uterotrophic response and the regulation of progesterone receptor and clusterin gene expression (Diel *et al.* 2000). In contrast to the present study, however, in those experiments DDT had been applied orally and for a longer time period. Polychlorinated biphenyls (PCBs) belong to the most widespread industrial products in the ecosystem, giving rise to serious environmental contamination, and are a potential hazard to health. It was shown in previous studies that they possess the ability to mimic estrogen action mediated through estrogen receptors (Nesaretnam *et al.* 1996); this was confirmed in the present study as they revealed an estrogenic effect by suppressing clusterin gene expression.

In agreement with the results of Diel and coworkers (2000) we have demonstrated that alteration of the expression of specific uterine genes is a more estrogen-sensitive parameter than, for example, increase in uterine weight. In addition, we have shown that the endometrial genes Cx26 and clusterin are at least as sensitive parameters as C3 and thus are highly suited to investigate the biological potential of natural and synthetic estrogens. An estrogen responsive element which promotes a direct effect of the activated ER on the promotor has been identified only for the C3

gene (Vik *et al.* 1991). The signal cascades involved are still unknown and may vary for different genes with similar sensitivity to estrogenic compounds.

Investigating gene expression of tissues from exposed animals offers the possibility to assay for induction of genes that are regulated by still unknown signaling pathways and it ensures that effects derived both from the tested compound and from its metabolites are detected.

The endometrium is one of the main target tissues for estrogenic effects. The present study reveals that analysis of clusters of estrogen-sensitive endometrial genes could help to identify compounds revealing estrogenic potency, assess their biological activity, and elucidate the involvement of different signal cascades. In addition, endocrine disruptors which could interfere with the physiological differentiation state of the endometrium could be identified by cDNA arrays composed of these genes as a simple approach for screening conspicuous substances.

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