Estrogen Signaling in the Regulation of Female Reproductive Functions

J.K. Findlay,
Prince Henry's Institute of Medical Research, PO BOX 5152, Clayton, VIC 3168, Australia

S.H. Liew,
Prince Henry's Institute of Medical Research, PO BOX 5152, Clayton, VIC 3168, Australia

E.R. Simpson, and
Prince Henry's Institute of Medical Research, PO BOX 5152, Clayton, VIC 3168, Australia

K.S. Korach
Laboratory of Reproductive and Developmental Toxicology, NIEHS/NIH, Room 12233, Research Triangle Park, NC 27709, USA

J.K. Findlay: jock.findlay@princehenrys.org; K.S. Korach: korach@niehs.nih.gov

Abstract

Estrogens influence fertility and infertility in animals. This chapter reviews the use of estrogen as a contraceptive through the regulation of its production and action. It is concluded that the use of specific agonists and antagonists of estrogen action that avoid the global and unwanted side effects of estrogen offers new potential methods of contraception.

Keywords

Aromatase; Contraception; Estrogen receptors; Estrogens

1 Introduction

Estrogens have an important role to influence fertility and infertility in mammals. They are members of the steroid hormone family produced principally by the gonads and placenta, but in numerous other tissues also such as breast, bone, skin, vasculature, adipose mesenchymal cells, and numerous sites in the brain. They were shown to have negative and positive feedback effects on the hypothalamic–pituitary axis (Diczfalusy and Fraser 1998). It was established also that estrogens acted on target organs such as the uterus, hypothalamus, pituitary, bone, mammary tissue, and liver, as well as having local actions within the gonads (Hisaw 1947; Hall et al. 2001). These properties of estrogen were exploited by Pincus et al. (1958) in the development of the contraceptive pill for women. This extensive list of target tissues is important when considering targeting estrogens as contraceptives as will be discussed below. Estrogens were shown to act on target cells via nuclear transcription factors, or estrogen receptors (ER), of which two have been identified, ERa and ERb (Jensen and DeSombre 1973; Kuiper et al. 1996). More recently, there is evidence of a membrane form of ER that might transmit the estrogen signal (Levin 2009).

However, our knowledge of the regulation of the biological actions of estrogens is incomplete. Extrinsic estrogens were shown to have actions other than infertility as observed with the contraceptive pill. Compounds with estrogenic activity developed for the agricultural and plastics industries were shown to cause infertility and tumors in mammals (Sharara et al. 1998). Children born of pregnant women treated with diethylstilbestrol had malformation of the reproductive tracts and development of vaginal cancer (Swan 2000). The known mechanisms of estrogen action could not explain the benefits of phytoestrogens used for hormone replacement therapy (Adlercreutz 1995).

The aim of this chapter is to review the use of estrogen as a contraceptive through the regulation of its production and action. After reviewing the mechanisms for estrogen production and signaling, we shall examine the actions of estrogen that allow us to evaluate their novel contraceptive potential.

2 Production of Estrogens

Estrogens are produced from androgens by the enzyme known as aromatase, which is a member of the cytochrome P450 superfamily and its gene designation is CYP19A1. The gene encoding the aromatase enzyme is some 120 kb long of which 91 kb comprise an extensive 5′ untranslated region. This contains a number of 5′ untranslated first exons which are spliced into the coding region in a tissue-specific fashion. The splicing in each case occurs at a common 3′ junction upstream of the start of translation (Simpson et al. 2002). Thus, the coding region is always the same regardless of the tissue site of expression. This occurs because of the presence upstream of each of these 5′ untranslated exons of a tissue-specific promoter. These promoters have different *trans-* and *cis*-acting elements regulating their activity. Hence, the expression of aromatase in each of the tissue-specific sites of expression is different. The distribution of aromatase activity in the body includes the gonads, hypothalamus, adipose tissue, bone, and placenta, as well as in some tumors and endometriotic tissues (Simpson et al. 2002). Thus, expression in ovarian granulosa cells and Sertoli cells of the testes is driven by a proximal promoter PII which contains a couple of CREs and hence binds CREB and is regulated by factors which stimulate adenylyl cyclase, leading to increased cyclic AMP and PKA activation. In granulosa cells and Sertoli cells, FSH appears to be the primary trophic hormone responsible for this activation. In the case of adipose tissue, the primary promoter being utilized is the distal promoter I.4, which is regulated by Class I cytokines such as interleukin 6 and TNFα in the presence of glucocorticoids. This response is mediated by a JAK1/STAT3 regulatory pathway. In the case of the placenta, the most distal promoter, promoter I.1, is employed which is some 91 kb upstream from the start of translation. Aromatase expression in adipose tissue is normally quite low; however, in breast adipose tissue in the presence of a tumor, aromatase expression increases three- or fourfold. This increase is due to the use of promoter PII, which is driven by inflammatory factors such as prostaglandin E_2 produced by the tumorous epithelium, which also activates adenylyl cyclase and hence cyclic AMP. Other transcription factors and response elements are also employed by each of these promoters, for example in the case of promoter PII, a monomeric orphan member of the nuclear receptor family is also absolutely required for expression and binds to a nuclear receptor half site downstream of the CREs. In the case of the ovary, this nuclear receptor appears to be SF1, whereas in the

tumorous mesenchymal cells of the breast it is LRH1, a closely related member of the nuclear receptor superfamily (Clyne et al. 2002).

3 Cellular Mechanisms of Action

ER is a protein that functions as a major component in the mechanisms of estrogen action, where it binds estrogens to initiate the tissue responses. There are two separate ER proteins, ERα and ERβ, which have distinct tissue expression patterns (Mueller and Korach 2001) in both humans and rodents. ERα and ERβ are encoded from separate genes and chromosomal locations, *ESR1* and *ESR2*, and likely arose due to gene duplication. Development of the homologous recombination technology has allowed scientists to develop unique experimental animal models (Hewett et al. 2005). Gene-targeted knock-out mouse models lacking these receptors exhibit distinct phenotypes (Couse and Korach 1999).

Entry of the hormone into the target cell is thought to be by diffusion where it becomes bound by the ER, which is located primarily in the nucleus, but can also be associated with other cellular organelles such as the plasma membrane. The nuclear ER–estrogen complex can regulate genes, positively or negatively, by binding directly to specific unique DNA sequences, referred to as estrogen response elements (ERE) contained in the promoter region of regulated genes. Once the hormone receptor complex is formed there is believed to be recruitment of co-regulatory proteins (coactivators or corepressors) which associate directly with the receptor protein at the promoter, in addition to the general transcription complex, producing increased or decreased mRNA levels and associated protein production, and resulting physiological responses (Couse et al. 2006).

An alternatively described mode of action involves an indirect mechanism where the ER does not bind directly to the DNA but interacts with existing transcription factors (e.g., f*os/ jun*), which is referred to as the tethered mechanism of nuclear receptor gene transcription. To elicit the many actions of the hormone this "genomic" mechanism typically occurs over the course of hours in most tissues and has been shown to involve unique gene groups at different times (Hewitt et al. 2003).

Another mode of action of estrogen is thought to involve a nongenomic mechanism. Such a mechanism has been shown to occur very rapidly within minutes of hormone exposure. Components of this cellular mechanism are shown to be the ER protein itself located in or adjacent to the plasma membrane involving interactions with adaptor proteins such as striatin, caveolin-1 or Shc, or through other, recently described nonER plasma membrane-associated estrogen-binding proteins, such as GPR30 (Otto et al. 2009; Levin 2009), resulting in cellular responses such as activation of MAP kinases which can then act to prime the genomic actions.

Besides the two previously mentioned cellular mechanisms, a third ER activity can also occur which involves the ligand independent activation of the receptor protein. Such an action has been shown experimentally in cells and experimental animal models, involving the activation of kinase cascades (e.g., MAPK or IP3K) by growth factors or other membrane signaling agents (Curtis et al. 1996). The extent to which any of these specific mechanisms are involved in mediating the physiological actions of estrogen is still requiring

considerable study to develop effective biomedical understanding and therapeutic application.

4 Estrogens and Contraception

Estrogens, estradiol-17β in particular, are essential for fertility in mammals. They are known to act at key points in the reproductive process in females, such as:

- Development of the ovulatory follicle(s)

- Triggering the midcycle preovulatory surge of gonadotropins

- Altering the consistency of cervical mucus to facilitate sperm transport

- Preparing the endometrial lining of the uterus for implantation

Alterations to the production and or actions of estrogen can disrupt these processes leading to infertility.

4.1 Regulation of Estrogen Production

Targeting the intrinsic production of estrogen as a means of contraception is complex and can lead to untoward side effects. This is exemplified by the use of aromatase inhibitors in breast cancer therapy. In recent years, inhibitors such as Arimidex, Letrozole, and Exemestane have supplanted Tamoxifen in first and second line therapy as well as adjuvant and neoadjuvant therapy for breast cancer. However, use of these compounds results in significant contraindications such as bone loss, joint arthralgia, and possibly cognitive defects. This is because these compounds inhibit the catalytic activity of the aromatase enzyme and thus inhibit its activity globally not only in the breast but in other sites where estrogens have important roles such as the reproductive tissues, bone, brain, and cardiovascular system. Thus, there is no tissue specificity in their action. The only way to achieve this would be to take advantage of the specificity presented by the use of tissue-specific promoters to design inhibitors which block expression of aroma-tase specifically in a given tissue. This is theoretically feasible. For example, in the postmenopausal woman LRH1 uniquely regulates aromatase expression in the breast but not, as far as we understand, in other tissue sites of expression. Thus LRH1 is a potential target for breast-specific ablation of aromatase expression (Simpson et al. 2002).

In the case of premenopausal women, the use of aromatase inhibitors appears to be less effective since they do not reduce circulating estrogen levels to the extent that they do in the postmenopausal situation and are therefore less likely to be useful as contraceptives. Furthermore, their use could lead to collateral problems of estrogen deficiency.

4.2 Regulation of Estrogen Action

Estrogens can be produced and act locally or they can be secreted and act distally. In both cases, they act on the target cells via specific receptors. The actions of estrogen will be governed by the rate at which they are secreted and metabolized, the presence if any of binding proteins, and the concentrations of receptors. Alterations in metabolism of estrogen

or the properties of their binding proteins have not offered any potential as a contraceptive. However, there are agonists and antagonists of the receptors that may offer a new approach.

Contraception has been a successful medical treatment for a number of years. Early studies of Pincus, Greep, Hertz, Greenblatt, and others showed the use of estrogen or estrogen receptor agonists could effectively inhibit gonadotropin secretion and subsequent stimulation of the ovary and ovulation (Pincus et al. 1958). From those early years, using estradiol itself, estrogen derivatives or synthetic estrogens included high doses resulting in concerns over the potential side effects of increasing cancer susceptibility in endocrine responsive tissues such as the endometrium and breast. Years of investigation have now resulted in a therapeutic approach involving much lower dosing schemes and development of neuroendocrine selective agonists.

Advancement from both clinical cases (e.g., aromatase or ER mutant patients) and experimental animal models, e.g., aromatase knockout (ArKO) (Fisher et al. 1998, Britt and Findlay 2002, 2003) and ER knockout (ERKO) (Couse and Korach 1999) mice, has provided new insights into both the mode of action and the specific estrogen receptor (i.e., ERα or ERβ) signaling molecule involved. Such knowledge allows us to know which type of selective estrogen receptor modulator (SERM) or tissue selective estrogen complex (TSEC) will be effective in contraceptive development and regulation of the hypothalamic pituitary gonadal axis. It appears that the primary mediator of negative feedback is ERα, although studies have implemented ERβ as possibly involved in the ovulatory (proestrus) LH surge required for ovulation. Additionally, ERβ has been shown to be required in the ovary for effective follicle ovulation. Therefore, blocking the LH surge with an ERβ selective antagonist maybe a highly effective approach at both the neuroendocrine and target tissue (e.g., ovarian) level. Since ERβ has minimal, if any mitogenic activity in the uterus or breast, the most common side effect should not be a concern. Another option would be a tissue selective delivery of estrogen or a derivative to neuroendocrine sites to act as an agonist and contraceptive. Such an approach does not concern itself with the specificity of the compound and receptor involved, but rather to the tissue selective delivery and action. Finally, use of an ERα selective agonist to induce negative feedback has the age-old problem of also stimulating ERα activities potentially associated with carcinogenesis. So, the side effects may outweigh the usefulness of that earlier approach, based on our current knowledge of LH regulation. It is obvious that understanding the precise mechanism for ER regulation of LH expression will also provide alternative modes of contraceptive development such as through other signaling pathways or the target tissue selective action at the ovary.

5 Conclusions

While the contraceptive pill containing very low doses of estrogen remains a very effective method, the use of receptor-specific agonists and antagonists that avoid the global and unwanted side effects of estrogen offers new potential methods of contraception. Receptor agonists and antagonists rather than aromatase inhibitors would appear to be a preferable option to explore at this stage.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Acknowledgments

References

Adlercreutz H. Phytoestrogens:epidemiology and a possible role in cancer protection. Environ Health Perspect. 1995; 103(Suppl 7):103–112. [PubMed: 8593855]

Britt KL, Findlay JK. Estrogen actions in the ovary revisited. J Endocrinol. 2002; 175:269–276. [PubMed: 12429025]

Britt KL, Findlay JK. Regulation of the phenotype of ovarian somatic cells by estrogen. Mol Cell Endocrinol. 2003; 202:11–17. [PubMed 12770724]

Clyne CD, Speed CJ, Zhou J, Simpson ER. Liver receptor homologue-1 (LRH-1) regulates expression of aromatase in preadipocytes. J Biol Chem. 2002; 277:20591–20597. [PubMed: 11927588]

Couse JF, Korach KS. Estrogen receptor null mice: what have we learned and where will they lead us? Endocr Rev. 1999; 20:358–417. [PubMed: 10368776]

Couse, JF.; Hewitt, SC.; Korach, KS. Steroid receptors in the ovary and uterus. In: Neill, JD., editor. Knobil and Neill: physiology of reproduction. 3rd. Academic Press; New York: 2006. p. 593-678.

Curtis SW, Washburn TF, Sewall C, DiAugustine R, Lindzey J, Couse JF, Korach KS. Physiological coupling of growth factor and steroid signaling pathways: estrogen receptor knockout mice lack estrogen-like response to EGF. Proc Natl Acad Sci USA. 1996; 93:12626–12630. [PubMed: 8901633]

Diczfalusy, E.; Fraser, IS. The discovery of reproductive steroid hormones and recognition of their physiological roles. In: Fraser, IS.; Jansen, RPS.; Lobo, RA.; Whitehead, MI., editors. Estrogens and progesterones in clinical practice. Churchill Livingstone; London: 1998. p. 3-18.

Fisher CR, Graves KH, Parlow AF, Simpson ER. Characterization of mice deficient in aromatase (ArKO) because of targeted disruption of the cyp19 gene. Proc Natl Acad Sci USA. 1998; 95:6965–6970. [PubMed: 9618522]

Hall JM, Couse JF, Korach KS. The multifaceted mechanisms of estradiol and estrogen receptor signaling. J Biol Chem. 2001; 276:36869–36872. [PubMed: 11459850]

Hewitt SC, Deroo B, Hansen K, Collins J, Grissom S, Afshari CA, Korach KS. Estrogen receptor-dependent genomic responses in the mouse uterus mirror the biphasic physiological response to estrogen. Mol Endocrinol. 2003; 17:2070–2083. [PubMed: 12893882]

Hewitt SC, Harrell JC, Korach KS. Lessons in estrogen biology from knockout and transgenic animals. Ann Rev Physiol. 2005; 67:285–308. [PubMed: 15709960]

Hisaw FL. Development of the Graafian follicle and ovulation. Physiol Rev. 1947; 27:95–119. [PubMed: 20282155]

Jensen EV, DeSombre ER. Estrogen-receptor interaction. Science. 1973; 182:126–134. [PubMed: 4354173]

Kuiper GGJM, Enmark E, Pelto-Huikko M, Nilsson S, Gustafsson JA. Cloning of a novel estrogen receptor expressed in the rat prostate and ovary. Proc Natl Acad Sci USA. 1996; 93:5925–5930. [PubMed: 8650195]

Levin ER. G protein-coupled receptor 30: estrogen receptor or collaborator? Endocrinology. 2009; 150:1563–1565. [PubMed: 19307418]

Mueller SO, Korach KS. Estrogen receptors and endocrine diseases: lessons from estrogen receptor knockout mice. Curr Opin Pharmacol. 2001; 1:613–619. [PubMed: 11757817]

Otto C, Fuchs I, Kauselmann G, Kern H, Zevnik B, Andreasen P, Schwarz G, Altmann H, Klewer M, Schoor M, Vonk R, Fritzemeier KH. GPR30 does not mediate estrogenic responses in reproductive organs in mice. Biol Reprod. 2009; 80:34–41. [PubMed: 18799753]

Pincus G, Rock J, Garcia C-R, Rice-Way E, Paniagua M, Rodriguez B, Pedras R. Fertility control with oral contraception. Am J Obstet Gynecol. 1958; 75:1333–1346. [PubMed: 13545267]

Sharara FI, Seifer DB, Flaws JA. Environmental toxins and female reproduction. Fert Steril. 1998; 70:613–622.

Simpson ER, Clyne CD, Rubin GL, Boon WC, Robertson KM, Britt KL, Speed CJ, Jones MEE. Aromatase – a brief overview. Ann Rev Physiol. 2002; 64:93–127. [PubMed: 11826265]

Swan SH. Intrauterine exposure to diethylstilbestrol: long term effects on humans. APMIS. 2000; 108:793–804. [PubMed: 11252812]