Estrogen in the Fetus

19

Charles E. Wood

Abstract

Estradiol and other estrogens are important modulators of fetal and maternal physiology in pregnancy. Much is known about the biosynthesis of estrogens in fetus and mother, and much is known about the role that estrogen plays in labor and delivery. However, much less is known about the regulation of estrogen biosynthesis throughout the latter half of gestation, and the role that estrogen plays in homeostatic and neuroendocrine control in the fetus. This review focuses on the biosynthesis and actions of estrogen in the fetal circulation, the role that it plays in the development of the fetus in the latter half of gestation, and the role that is played by the estrogen milieu in the control of the timing of birth. Estrogen circulates in fetal blood in both unconjugated and conjugated molecular forms, with the conjugated steroids far more abundant than the unconjugated steroids. This review therefore also addresses the biological significance of the variety of molecular forms of estrogen circulating in fetal and maternal blood.

Keywords

Estradiol • Pregnancy • Estrogens • Fetal blood • Steroids

Estradiol and other estrogens are important modulators of fetal and maternal physiology in pregnancy. Actions of estrogen in the maternal circulation include the modulation of uterine and systemic vascular tone [1–4], uterine growth and differentiation of the uterine glands [5], growth and terminal differentiation of mammary ducts and lactation [6, 7]. Much is known about the ontogeny of estrogen biosynthesis and secretion into the maternal circulation, and much is known about the biochemical mechanism underlying the function of the feto-placental unit in the human being and nonhuman primate. The feto-placental unit, which typifies primate placental estrogen biosynthesis, represents a de facto collaboration between mother and baby [8]. While control of estrogen production

C.E. Wood (✉)
Department of Physiology and Functional Genomics, University of Florida College of Medicine, 32610 Gainesville, FL, USA
e-mail: woodc@ufl.edu

L. Zhang and C.A. Ducsay (eds.), *Advances in Fetal and Neonatal Physiology*, Advances in Experimental Medicine and Biology 814, DOI 10.1007/978-1-4939-1031-1_19,

in the human is dependent upon fetal hypothalamus-pituitary-adrenal axis integrity and activity, more attention has been given to the role of estrogens in the maternal, rather than the fetal, circulation. In this review, I will focus on the biosynthesis and actions of estrogen in the fetal circulation, the role that it plays in the development of the fetus in the latter half of gestation, and the role that is played by the estrogen milieu in the control of the timing of birth. This review also focuses on the biological significance of the variety of molecular forms of estrogen circulating in fetal and maternal blood.

Much of our understanding of the role of estrogens with regard to fetal physiology has derived from work in the chronically catheterized fetal sheep model. Biosynthesis of estrogen in the sheep differs from that in the human or nonhuman primate because of the ability of the sheep placenta to induce 17α hydroxylase activity, encoded by the CYP17 gene, in late gestation [9, 10]. In the human and nonhuman primate (Fig. 19.1), an increase in the secretion of the estrogen precursor, dehydroepiandrosterone sulfate (DHAS), by the fetal adrenal increases placental synthesis of estradiol [8, 11]. In the sheep (Fig. 19.1), the placenta can synthesize estradiol directly from maternally-derived cholesterol after induction of CYP17 but may also be in part dependent on supply of precursors from the fetal adrenal cortex [12–15]. In both cases, the increase in estrogen secreted into the maternal circulation is an important step in the chain of events that culminates in parturition. Also in both cases, the ultimate stimulation of estrogen production is the adrenocorticotropin (ACTH) secretion by the fetal pituitary [16]. Prior to the preparturient induction of CYP17, however, the sheep expresses little or no CYP17 [17, 18]; nevertheless, the ovine fetus—like the human fetus—has estrogen circulating in its blood. The mystery of the origin of these fetal estrogens is perhaps at least partly explained by secretion of estrogen precursors from the adrenal cortex [15].

Viewed through the lens of late gestation and parturition, the role of estrogen in the fetus is at least in part characterized by the action of estradiol to stimulate the fetal HPA axis. We have previously hypothesized [19] that increases in plasma concentrations of estrogens [20] or increases in the abundance or balance of estrogen receptors in target tissues [21] participate in a progressive stimulation of the fetal HPA axis by estradiol and other estrogens. Chronic estradiol treatment of fetal sheep increases basal and stress-induced fetal HPA axis activity [22–25]. Partial blockade of aromatase by infusion of inhibitor into the fetal circulation decreased circulating fetal ACTH, although with little effect on cortisol [26]. In late gestation baboons, blockade of aromatase decreased plasma cortisol concentration in umbilical cord blood [27].

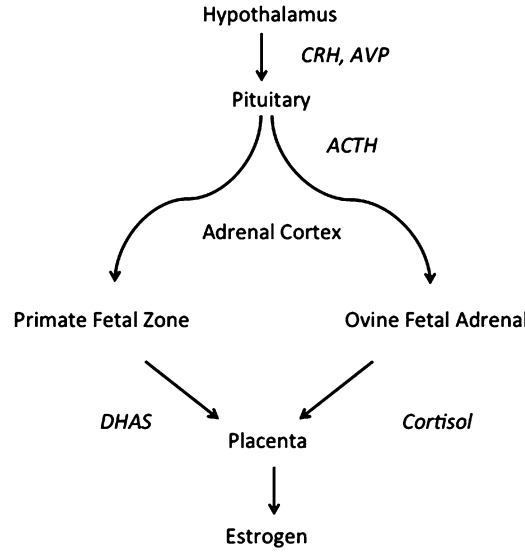

Fig. 19.1 Estrogen production in primate and sheep with respect to control by the fetal hypothalamus-pituitary-adrenal axis. In the human and nonhuman primate, there is a functional feto-placental unit for biosynthesis of estrogen. Estrogen precursors, mainly dehydroepiandrosterone sulfate (DHAS), are supplied to the placenta as substrate for synthesis of estrogens. The placenta of the primate requires DHAS as substrate because of a lack of CYP17 (which is responsible for 17α-hydroxylase activity). The placenta of the sheep lacks CYP17 before it is induced by the preparturient increase in fetal plasma cortisol concentration that heralds the onset of labor in this species. Prior to the induction of CYP17, the sheep placenta synthesizes estrogens from precursors that are secreted by the fetal adrenal cortex

Estradiol appears to have a stimulatory effect on the fetal HPA axis via an action on the fetal central nervous system. Evidence of this is the increasing abundance of arginine vasopressin in the ovine fetal hypothalamus with estradiol treatment [28]. Evidence of estradiol action in the fetal brain can also be seen as increased Fos immunostaining in paraventricular nucleus of the hypothalamus and other regions important for control of ACTH secretion [23]. The mechanism of neuronal stimulation by estradiol is not known, but it is likely that at least one component of the mechanism involves brain prostaglandin biosynthesis.

There is a longstanding recognition of the effect of prostaglandin E2 (PGE2) on fetal ACTH and cortisol secretion [29, 30]. Parturition is triggered after prolonged administration of PGE2 [31]. There is a dramatic increase in circulating concentrations of PGE2 in the plasma of fetal sheep that originate in the placenta and peaks at the time of spontaneous parturition [30, 32]. Intravenous infusion of high doses of PGE2 into the fetus increase fetal HPA axis activity [33]. Whole body blockade of prostaglandin biosynthesis with nimesulide (a cyclooxygenase-2, COX2, inhibitor) inhibits HPA axis activity in fetuses of laboring sheep [34]. In a similar experiment, McKeown and colleagues demonstrated that, after initiation of labor with RU-486, treatment of fetal sheep with meloxicam (a COX2 inhibitor) decreased plasma PGE2 and fetal ACTH concentrations [35]. Inhibition of prostaglandin synthesis has been shown to prolong gestation in several species, although this is the direct result of reduced prostaglandin stimulation of the myometrium. The link between COX2, ACTH, and parturition in sheep is clear, although the critical site of COX2-mediated prostaglandin biosynthesis is the fetal brain. Infusion of PGE2 into the carotid arterial blood of fetal sheep at rates that modestly exaggerate the spontaneous increase in plasma PGE2 from the placenta are ineffective in stimulating fetal pituitary ACTH secretion [36]. Intravenous infusion of PGE2 is more effective at releasing immunoreactive ACTH from lung than from pituitary [36]. Blockade of COX2 specifically in the fetal brain

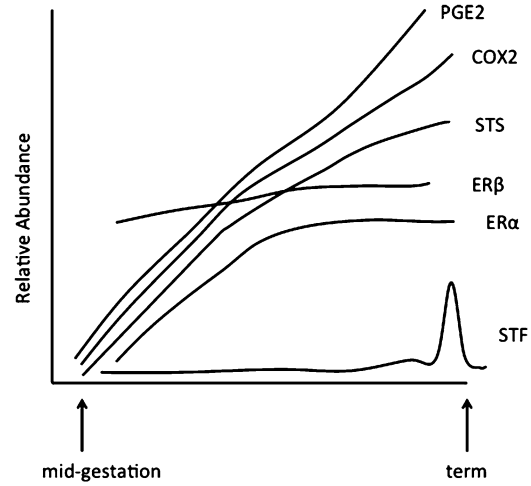

Fig. 19.2 Schematic representation of tissue content of prostaglandin E2 (PGE2), mRNA abundance of cyclooxygenase 2 (COX2), steroid sulfatase (STS), estrogen sulfotransferase (STF), and estrogen receptors alpha (ERα) and beta (ERβ) in the cerebral cortex of fetal sheep throughout the latter half of gestation [21, 41, 92]. The relative abundance of these enzymes is consistent with the net deconjugation of sulfoconjugated estrogen, increasing transcriptional activity of the estrogen receptor, increasing transcription of COX2, and increasing local biosynthesis of PGE2

(by infusion of small amounts of nimesulide into the lateral cerebral ventricle of the fetal sheep) decreases fetal ACTH responses to stress (cerebral hypoperfusion) and prolongs gestation [37, 38]. NMDA glutamatergic neurotransmission, which mediates the fetal ACTH response to cerebral hypoperfusion [39], stimulates the HPA axis in part by increasing brain (COX2-dependent) prostaglandin biosynthesis [40]. The concentration of PGE2 in cerebral cortex and hippocampus greatly exceeds that in plasma, suggesting local biosynthesis, and the concentrations increase in these brain regions prior to parturition [41]. The fetal brain and pituitary express the enzymatic machinery needed for prostaglandin biosynthesis (Fig. 19.2) [41–43]. Activation of neuronal pathways subserving fetal responsiveness to stress increase the expression of these enzymes [44].

It is possible that there exists a positive feedback relationship between placenta and the fetal HPA axis. This idea was first proposed as a link between placental PGE2 production and

fetal ACTH secretion [32]. However, even with the understanding that the site of prostaglandin production relevant to fetal ACTH secretion is in the fetal brain and/or pituitary, it is still logical to posit a functional communication between placenta and fetal brain. There is evidence to support the notion that estrogen secreted by the placenta augments fetal HPA activity, and that at least a part of that mechanism of the estrogen effect on ACTH is via stimulation of COX2 expression in the fetal brain. Estradiol treatment increases COX2 abundance in fetal brain and increases the magnitude of the increase in COX2 abudance after cerebral hypoperfusion [24]. Infusion of ICI 182,780, an estrogen receptor antagonist intracerebroventricularly into fetal sheep decreases COX2 mRNA abundance [45]. The mechanism of the estrogen effect on COX2 expression in the brain is unknown.

There is evidence that estrogen alters COX2 expression in endothelial cells via an effect on membrane-bound ER, transduced by the activity of phosphatidylinositol 3-phosphate/Akt [46]. Similarly, upregulation of COX2 expression in breast cancer cells by the xenoestrogen o′p′-DDT is dependent on CRE activation, and PKA and Akt/PI3 kinase activities [47]. As might be expected, several endocrine stimuli to COX2 generation utilize phosphorylation of CREB as the mechanism. For example, in adult fibroblasts, cortisol upregulates COX2 via phosphorylation of CREB [48].

In cardiac myocytes, corticosterone increases COX2 transcription via binding of the GR and C/EBP to the COX2 promoter. In the placenta of sheep, the mechanism of upregulation of COX2 at the end of gestation might also be glucocorticoid, not estrogen, dependent. Whittle and colleagues reported that cortisol increases COX2 mRNA in placenta, and the effect is not blocked by concomitant blockade of aromatase [49]. It is possible that, in differing cell types within placenta, both glucocorticoid and estrogen receptor upregulated the PTGS2 (COX2) gene expression using the same molecular mechanism, CREB phosphorylation. However, in many tissues, the predominant effect of glucocorticoid is inhibition of the PTGS2 gene, secondary to a GR-NFkB interaction that blocks the upregulation of gene expression by NFkB [50].

1 Cellular Responses to Estrogen in the Fetus

Numerous studies have demonstrated that estrogen receptors are expressed in various tissues in the late-gestation fetus [51, 52]. ERα functions as a ligand-gated transcription factor (Fig. 19.3) [53, 54], or as an estrogen binding site tethered to the plasma membrane [55–58]. The role of ERβ is somewhat less clear. ERβ could act as a functional inhibitor of ERα action [59, 60]. ERβ is also known to play an important role in mitochondrial function [61–63]. Estrogen receptors are found in the fetal brain

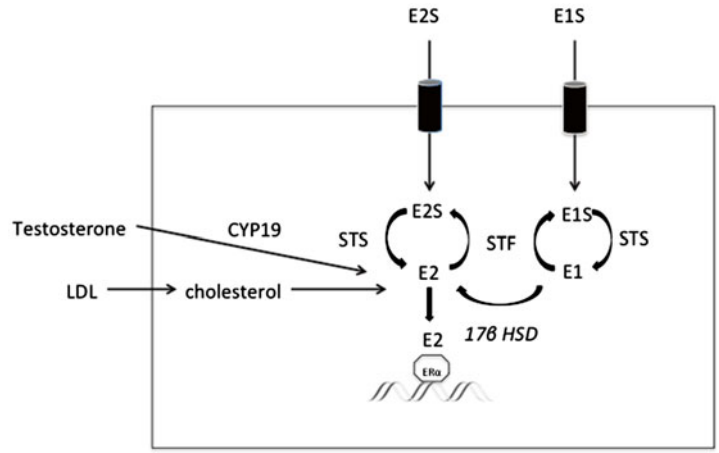

Fig. 19.3 Schematic representing the local synthesis of estradiol (E2) from testosterone and de novo from cholesterol, the interconversion of E2 and estrone (E1), the sulfonation/desulfonation cycle catalyzed by steroid sulfatase (STS) and sulfotransferase (STF), and entry of E2S and E1S into the cell by carrier-mediated transport

of sheep [64–66], and mouse [67, 68]. In the cerebral cortex of the bovine fetus, ERα, ERβ, and aromatase are expressed throughout gestation, with increases in both ERα and ERβ at the end of gestation [69].

Changes in the abundance of estrogen receptors might predict developmental changes in sensitivity to estrogen. In a recent study, we reported that there is an increase in ERα and a decrease in ERβ in the ovine fetal pituitary in late gestation [21], suggesting a possible increase in pituitary sensitivity to estradiol in the peripartum period. Interestingly, the ratio of ERα/ERβ (at the level of mRNA abundance) favors ERα in the pituitary, but ERβ throughout the fetal brain. In hypothalamus, ERα and ERβ abundances are highest at 120 days gestation (~80 % gestation), with decreases at term. The decreases might be caused by receptor downregulation secondary to rising plasma concentrations of estradiol. In hippocampus, the brain region in which there is increasing biosynthesis of PGE2 in late gestation, there is an increase in both ERα and ERβ from 120 days through the end of gestation, with further increases postnatally. These changes in ER abundance in pituitary and in various brain regions suggests an increasing pattern of estrogen signaling in the brain as the fetus matures and approaches the normal time of spontaneous parturition. The molecular development of the brain could therefore account for changes in estrogen responsiveness prior to the ontogenetic increase in plasma concentrations of estradiol at term.

2 17β-Hydroxysteroid Dehydrogenase

In addition to developmental changes in estrogen receptor abundance, local synthesis and enzymatic biotransformation of steroids within target tissues can alter estrogen signaling independent of changes in plasma concentrations of estradiol (Figs. 19.3 and 19.4). Estradiol and estrone are interconverted by the action of 17β-hydroxysteroid dehydrogenase (17β-HSD) activity. Resko and Stadelman reported that this activity, converting E2 to E1, was higher in the fetal pituitary of the rhesus monkey at 80 (~48 % gestation) days gestation compared to 120 (~73 % gestation) and 150 (~90 % gestation) days gestation [70]. In that study, the investigators reported lower levels of

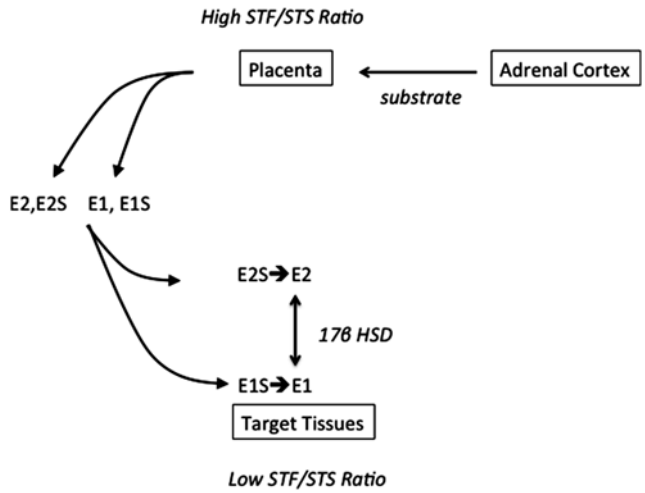

Fig. 19.4 Schematic representation of the targeting of estrogen from its source (placenta) to tissues that deconjugate E2S and E1S and therefore enhance local estrogen signaling. The placenta expresses sulfotransferase but very little sulfatase, resulting in predominant secretion of sulfoconjugated estrogen. Target tissues that express sulfatase but little sulfotransferase would be expected to have higher cellular content of unconjugated estrogen than tissues that cannot locally deconjugate the high concentrations of E2S and E1S that are in fetal blood

17β-HSD activity in the brain of the fetus than in pituitary and other peripheral tissues. In first and second trimester human fetuses, Millewich, MacDonald, and Carr reported higher oxidative activity (E2 to E1) in liver, intestine, stomach, kidney, brain, and heart, and relatively higher reductive activity (E1 to E2) in placenta and in the fetal zone of the fetal adrenal cortex [71]. In a later study, Takeyama and colleagues reported that in human fetuses 11–20 weeks gestation, 17β-HSD activity could be measured in various tissues, but that both the oxidative and reductive activities were far lower in fetal brain than in placenta and liver, with intermediate activity in regions of the gastrointestinal tract and in the kidney [72]. Using Northern blot, these investigators reported that type 1 enzyme (HSD17B1) was found in placenta, while the type 2 enzyme (HSD17B2) was found in liver, kidney, and gastrointestinal tract [72]. Recent molecular studies have revealed that there are multiple enzymes with 17β-HSD activity. Currently, there are 14 known enzymes that have been identified as members of the family of enzymes that have similarities in enzyme activity and gene structure. These are named HSD17Bx (HSD17B1, HSD17B2, etc.) [73]. Our understanding of the distribution of these enzymes in the developing fetus is incomplete, and an improvement in this regard can improve our understanding of molecular targets that can be exploited for the purpose of altering the course of fetal development or timing of parturition.

3 Aromatase

Aromatase (CYP19), is present in brain [74, 75] and is usually understood as a marker of local estrogen synthesis (Fig. 19.3) [76]. In the developing rat brain, aromatase activity increases between day 16 of gestation (~75 % gestation) and day 20 of gestation [77]. Highest activities were found in the preoptic area. However, because the altricial nature of the in utero development of the rat brain, these results cannot easily be extrapolated to species that are more mature at birth. Aromatase activity has been found in the term and newborn rhesus monkey brain, with highest levels in the POA and hypothalamus [78, 79], and has been found in the fetal sheep brain, again with high levels of activity in the fetal hypothalamus [80, 81]. Similarly, CYP19 mRNA is found in human fetal (15–19 weeks gestation) brain [82]. Tissue-specific expression of the CYP19 gene involves multiple alternative splicing schema, although the alternative splicing is not in the coding region [83].

The origin of steroid in the fetal brain is generally thought to be placental or maternal in origin. In the case of glucocorticoids and mineralocorticoids, the fetal and maternal adrenal cortices are the most likely source. However, there is increasing evidence of the possibility that steroids are synthesized from cholesterol in the brain. Adult human brain expresses low levels of steroidogenic enzymes that could subserve local synthesis of mineralocorticoid and glucocorticoid [84]. Brain steroid concentrations in adrenalectomized rats reveals functional local steroidogenesis in varous brain regions [85]. Pezzi and coworkers, using PCR, have demonstrated the presence in the fetal brain of StAR protein and all the enzymes needed for synthesis of estradiol from cholesterol [82].

4 Sulfoconjugation of Estrogens

The most abundant molecular forms of estrogen in fetal and maternal blood are the conjugated estrogens. In the sheep, E1 sulfate (E1S) is more abundant than E1, and E2 sulfate (E2S) is more abundant than E2 [86, 87]. The placenta of the sheep has abundant activity of estrogen sulfotransferase [88, 89] but very little estrogen sulfatase activity [18]. In the maternal circulation, E1 and E1S increase progressively through the latter half of gestation from approximately 10 to approximately 50 pg/mL and from approximately 50–100 to approximately 2,000–5,000 pg/mL, respectively [20]. The pattern of E1 and E1S in the fetus is similar, although plasma concentrations of E1S are higher than on the maternal side of the placenta [20, 86]. Maternal plasma E2, like

E1, increases throughout the latter half of gestation, increasing from <10 pg/mL at mid-gestation to approximately 50 pg/mL at term [86]. Maternal E2S displays the same pattern but at higher levels, increasing from <100 pg/mL to approximately 500 pg/mL [86]. Arteriovenous differences across the uterine vascular bed support the conclusion that estrogen synthesis is dominated by the placenta, but it is clear that the pattern of change in the plasma concentrations for E1, E1S, E2, and E2S are not identical in fetal and maternal blood, suggesting either directional secretion of steroid or an independent source of estrogen secretion in the fetus. In any event, the high concentration of E2S in uterine venous blood compared to E2S in jugular venous blood strongly suggests that E2S is secreted from the placenta.

What is the endocrine function of the sulfoconjugated E1 and E2 in fetal and maternal blood? Is it simply a metabolite or is it an active hormone? We have proposed that required steps involved in cellular action of sulfoconjugated estrogens would include carrier-mediated transport [90] and deconjugation [91, 92]. Sulfoconjugation blocks binding of the steroid to the estrogen receptor [93, 94]. Removal of the sulfate group, liberating E1 or E2, would be required to convert the sulfoconjugated steroid to a steroid that can act at the ER. It is possible that secretion of sulfoconjugated steroid allows targeting to tissues that express the deconjugating enzyme [19]. Local deconjugation has been proposed as one cellular mechanism by which breast cancer increases local estrogen action [95]. However, a similar mechanism could work in both mother and fetus. Fetal brain takes up sulfoconjugated estrogen from fetal plasma [96], and fetal brain contains both conjugating and deconjugating enzymes [91, 97]. The ratios of mRNA abundance for the two enzymes [92] suggest that the predominant reaction is deconjugation (i.e., liberation of E2 from E2S and E1 from E1S). Quantification of the ratio of mRNA for the deconjugating to the conjugating enzymes reveals that, at term, this ratio ranges from approximately 10 to 100, depending on brain region [92]. The general pattern of expression in most brain regions (brainstem, cerebellum, and cerebral cortex) is an increasing expression for steroid sulfatase (the deconjugating enzyme) and in several of the brain regions (cerebellum, hippocampus, and hypothalamus) an increasing expression of the sulfotransferase (conjugating) enzyme [92]. It is possible that, as the fetus matures and the HPA axis is activated, the increasing plasma cortisol concentration induces SULT1E1, the gene that encodes the sulfotransferase enzyme [98]. If so, estrogen action in the fetal brain could be modified by cortisol. Along with the dynamics of deconjugation and conjugation, the dynamics of E1 and E2 interconversion in the fetal brain, local synthesis of estrogen from cholesterol or from aromatization of testosterone and androstenedione should be considered as a possible factor in the action of estrogen on the fetal brain. Tissue content of E1, E2, E1S, and E2S are higher in the cerebral cortex than in fetal plasma (Wood, Chang, Keller-Wood, submitted manuscript), suggesting either that these estrogens are concentrated in the brain or that they are synthesized locally.

Contrary to the hypothesis that E1S and E2S actions require deconjugation and subsequent binding to the ER, we have found that the physiological and molecular responses of the fetus to E2S appear to have overlapping, yet distinct, actions compared to the responses to E2 [92, 96, 99]. This suggests that E2S acts, at least in part, through mechanisms not subserved by deconjugation and binding to the ER. One possibility is that E2S has a neurosteroid action on $GABA_A$ or on NMDA receptors, analogous to the action of pregnenolone sulfate [100–103]. E2S stimulates genomic responses in the fetal hypothalamus that suggest that it is orexigenic, perhaps encouraging feeding behavior in early neonatal life. E2S also stimulates a subset of genes that are hypoxia-sensitive, including genes important for neovascularization [99].

It is likely that estradiol sulfate acts, in part, via cellular mechanisms that do not involve classical estrogen receptor binding. However, little is known about the binding of E2S or E1S to cell surface receptors. To date, there have been no reports of studies reporting the binding affinity of E2S or E1S with the palmitoylated and membrane bound ERα or the putative GPCR estrogen receptor GP30 (GPER). However, there is

reported evidence of sulfoconjugated estrogen interacting with ion channels [104], analogous to the neurosteroid action of pregnenolone sulfate [102]. For example, microinjection of estradiol sulfate into the striatum of rats impaired response learning [105]. In the striatum, membrane-bound ER activates metabotropic mGluR3 and mGluR5 glutamate receptors, which in turn alter MAP kinase-dependent CREB phosphorylation [106]. Perhaps using the same mechanism, DHEAS is known to be neuroprotective, working by activation of CREB and NFkB [107].

5 A Model of Fetal Estrogens in Late Gestation

Estrogens in plasma and target tissues are important components of the endocrine milieu that modulate fetal physiology and—in late gestation—are involved as a component of the mechanism by which parturition is triggered. It is perhaps most likely that estrogen acts via several mechanisms. The large supply of sulfoconjugated estrogen in fetal blood, the ready availability of the deconjugating enzyme (STS) in the fetal brain, the abundant uptake of sulfoconjugated estrogen into the fetal brain from blood, and the expression of estrogen receptors in the brain are all consistent with local deconjugation and action of the sulfoconjugated estrogen (Fig. 19.4). In this model, the majority of estrogen secretion in the fetus is in the sulfoconjugated form, affording a large supply of water-soluble precursor hormone that can be locally deconjugated in and targeted to the brain and other tissues that express steroid sulfatase. However, the unique endocrine and molecular responses to E2S as compared to E2 suggests that there are mechanisms of action unique to sulfoconjugated estrogens, perhaps as neurosteroids interacting with glutamate or GABA receptors.

The importance of estrogen supporting the physiological changes associated with a healthy pregnancy is well-known, as is the importance of estrogen in normal, spontaneous parturition. The role of sex steroids in patterning of early brain development is also well-known. However, the view of biologically active fetal estrogen as E2 and/or E1, and the view of the nuclear ERα as the unitary signal transduction mechanism for estrogen are overly simplistic. The secretion of both conjugated and unconjugated forms of E1 and E2, the tissue-specific and gestation-specific expression of sulfatase and sulfotransferase, the known activities of ERα and ERβ as both ligand-gated transcription factors and membrane receptors, and the possibility of estrogen signaling mediated by GPR30, suggests a far more complex endocrine axis. The complexity of estrogen signaling in the fetus strongly argues for a better understanding of the role of the sulfoconjugated estrogens in fetal development. Concentrations of sulfoconjugated steroid, long assumed to be inactive, should be routinely included in the assessment of steroid concentrations in the fetus and neonate.

References

1. Magness RR, Rosenfeld CR. Local and systemic estradiol-17 beta: effects on uterine and systemic vasodilation. Am J Physiol. 1989;256:E536–42.
2. Longo LD. Maternal blood volume and cardiac output during pregnancy: a hypothesis of endocrinologic control. Am J Physiol. 1983;245:R720–9.
3. Ueda S, Fortune V, Bull BS, Valenzuela GJ, Longo LD. Estrogen effects on plasma volume, arterial blood pressure, interstitial space, plasma proteins, and blood viscosity in sheep. Am J Obstet Gynecol. 1986;155:195–201.
4. Greiss Jr FC, Anderson SG. Effect of ovarian hormones on the uterine vascular bed. Am J Obstet Gynecol. 1970;107:829–36.
5. Price D, Harvey H. The relation of estrogen dosage to the precocious development of uterine glands in the rat. Anat Rec. 1947;99:658.
6. Russo J, Russo IH. Breast development, hormones and cancer. Adv Exp Med Biol. 2008;630:52–6.
7. Buhimschi CS. Endocrinology of lactation. Obstet Gynecol Clin North Am. 2004;31:963–79. xii.
8. Diczfalusy E. Endocrine functions of the human fetoplacental unit. Fed Proc. 1964;23:791–8.
9. Anderson ABM, Flint AP, Turnbull AC. Mechanism of activation of glucocorticoids in induction of ovine parturition: effect on placental steroid metabolism. J Endocrinol. 1975;66:61–70.
10. Miller WL. Steroidogenic enzymes. Endocr Dev. 2008;13:1–18.
11. Parker Jr CR. Dehydroepiandrosterone and dehydroepiandrosterone sulfate production in the human

adrenal during development and aging. Steroids. 1999;64:640–7.
12. Su YX, Carey LC, Rose JC. Ontogeny of StAR and ACTH-R genes in ovine placenta. Placenta. 2004;25:658–63.
13. de la Llosa-Hermier MP, Martal J, Ricour A, Hermier C. Evidence for modulation of progesterone secretion by calcium and protein kinase C activators in ovine chorionic cells. Placenta. 1991;12:511–20.
14. de la Llosa-Hermier MP, Zongazo MA, Martal J, Hermier C. Lack of short-term modulation of in vitro placental progesterone secretion in sheep. Placenta. 1988;9:623–31.
15. Mitchell BF, Lye SJ, Lukash L, Challis JRG. Androstenedione metabolism in the late gestation sheep fetus. Endocrinology. 1986;118:63–8.
16. Deayton JM, Young IR, Thorburn GD. Early hypophysectomy of sheep fetuses: effects on growth, placental steroidogenesis and prostaglandin production. J Reprod Fertil. 1993;97:513–20.
17. France JT, Magness RR, Murry BA, Rosenfeld CR, Mason JI. The regulation of ovine placental steroid 17 alpha-hydroxylase and aromatase by glucocorticoid. Mol Endocrinol. 1988;2:193–9.
18. Mason JI, France JT, Magness RR, Murry BA, Rosenfeld CR. Ovine placental steroid 17 alpha-hydroxylase/C-17,20-lyase, aromatase and sulphatase in dexamethasone-induced and natural parturition. J Endocrinol. 1989;122:351–9.
19. Wood CE. Estrogen/hypothalamus-pituitary-adrenal axis interactions in the fetus: the interplay between placenta and fetal brain. J Soc Gynecol Investig. 2005;12:67–76.
20. Nathanielsz PW, Elsner C, Magyar D, Fridshal D, Freeman A, Buster JE. Time trend analysis of plasma unconjugated and sulfoconjugated estrone and 3 beta-delta 5-steroids in fetal and maternal sheep plasma in relation to spontaneous parturition at term. Endocrinology. 1982;110:1402–7.
21. Schaub CE, Gersting JA, Keller-Wood M, Wood CE. Development of ER-alpha and ER-beta expression in the developing ovine brain and pituitary. Gene Expr Patterns. 2008;8:457–63.
22. Wood CE, Saoud CJ. Influence of estradiol and androstenedione on ACTH and cortisol secretion in the ovine fetus. J Soc Gynecol Investig. 1997;4:279–83.
23. Purinton SC, Wood CE. Oestrogen augments the fetal ovine hypothalamus- pituitary-adrenal axis in response to hypotension. J Physiol. 2002;544:919–29.
24. Wood CE, Giroux D. Central nervous system prostaglandin endoperoxide synthase-1 and -2 responses to oestradiol and cerebral hypoperfusion in late-gestation fetal sheep. J Physiol. 2003;549:573–81.
25. Saoud CJ, Wood CE. Modulation of ovine fetal adrenocorticotropin secretion by androstenedione and 17beta-estradiol. Am J Physiol. 1997;272:R1128–34.
26. Holloway AC, Whittle WL, Challis JR. Effects of cortisol and estradiol on pituitary expression of proopiomelanocortin, prohormone convertase-1, prohormone convertase-2, and glucocorticoid receptor mRNA in fetal sheep. Endocrine. 2001; 14:343–8.
27. Pepe GJ, Ballard PL, Albrecht ED. Fetal lung maturation in estrogen-deprived baboons. J Clin Endocrinol Metab. 2003;88:471–7.
28. Wood CE, Saoud CJ, Stoner TA, Keller-Wood M. Estrogen and androgen influence hypothalamic AVP and CRF concentrations in fetal and adult sheep. Regul Pept. 2001;98:63–8.
29. Hollingworth SA, Deayton JM, Young IR, Thorburn GD. Prostaglandin E2 administered to fetal sheep increases the plasma concentration of adrenocorticotropin (ACTH) and the proportion of ACTH in low molecular weight forms. Endocrinology. 1995;136: 1233–40.
30. Challis JR, Hart I, Louis TM, Mitchell MD, Jenkin G, Robinson JS, et al. Prostaglandins in the sheep fetus: implications for fetal function. Adv Prostaglandin Thromboxane Res. 1978;4:115–32.
31. Young IR, Deayton JM, Hollingworth SA, Thorburn GD. Continuous intrafetal infusion of prostaglandin E2 prematurely activates the hypothalamo-pituitary-adrenal axis and induces parturition in sheep. Endocrinology. 1996;137:2424–31.
32. Thorburn GD, Hollingworth SA, Hooper SB. The trigger for parturition in sheep: fetal hypothalamus or placenta? J Dev Physiol. 1991;15:71–9.
33. Ratter S, Rees LH, Landon JR, Hillier K, Jansen CAM, Beck NFG, et al. The effect of prostaglandin E2 infusion in the fetal lamb on fetal plasma ACTH, prolactin and cortisol concentrations. Prostaglandins. 1979;18:101–16.
34. Unno N, Wu WX, Wong CH, Bennett PR, Shinozuka N, Nathanielsz PW. Prostaglandin regulation of fetal plasma adrenocorticotropin and cortisol concentrations in late-gestation sheep. Biol Reprod. 1998; 58:514–9.
35. McKeown KJ, Challis JR, Small C, Adamson L, Bocking AD, Fraser M, et al. Altered fetal pituitary-adrenal function in the ovine fetus treated with RU486 and meloxicam, an inhibitor of prostaglandin synthase-II. Biol Reprod. 2000; 63:1899–904.
36. Cudd TA, Wood CE. Prostaglandin E2 releases ovine fetal ACTH from a site not perfused by the carotid vasculature. Am J Physiol. 1992;263:R136–40.
37. Gersting J, Schaub CE, Keller-Wood M, Wood CE. Inhibition of brain prostaglandin endoperoxide synthase-2 prevents the preparturient increase in fetal adrenocorticotropin secretion in the sheep fetus. Endocrinology. 2008;149:4128–36.
38. Wood CE, Powers MJ, Keller-Wood M. Blockade of PGHS-2 inhibits the hypothalamus-pituitary-adrenal axis response to cerebral hypoperfusion in the sheep fetus. Am J Physiol Regul Integr Comp Physiol. 2009;296:R1813–9.
39. Powers MJ, Wood CE. Ketamine inhibits fetal ACTH responses to cerebral hypoperfusion. Am J Physiol Regul Integr Comp Physiol. 2007;292: 1542–9.

40. Knutson N, Wood CE. Interaction of PGHS-2 and glutamatergic mechanisms controlling the ovine fetal hypothalamus-pituitary-adrenal axis. Am J Physiol Regul Integr Comp Physiol. 2010;299: R365–70.
41. Gersting JA, Schaub CE, Wood CE. Development of prostaglandin endoperoxide synthase expression in the ovine fetal central nervous system and pituitary. Gene Expr Patterns. 2009;9:603–11.
42. Deauseault D, Giroux D, Wood CE. Ontogeny of immunoreactive prostaglandin endoperoxide synthase isoforms in ovine fetal pituitary, hypothalamus, and brainstem. Neuroendocrinology. 2000;71: 287–91.
43. Reimsnider SK, Wood CE. Colocalisation of prostaglandin endoperoxide synthase and immunoreactive adrenocorticotropic hormone in ovine foetal pituitary. J Endocrinol. 2004;180:303–10.
44. Fraites MJ, Wood CE. Chemoreflex activity increases prostaglandin endoperoxide synthase mRNA expression in the late-gestation fetal sheep brain. Reprod Sci. 2011;18:824–31.
45. Schaub CE, Keller-Wood M, Wood CE. Blockade of estrogen receptors decreases CNS and pituitary prostaglandin synthase expression in fetal sheep. Neuroendocrinology. 2008;87:121–8.
46. Pedram A, Razandi M, Aitkenhead M, Hughes CC, Levin ER. Integration of the non-genomic and genomic actions of estrogen. Membrane-initiated signaling by steroid to transcription and cell biology. J Biol Chem. 2002;277:50768–75.
47. Han EH, Kim HG, Hwang YP, Choi JH, Im JH, Park B, et al. The role of cyclooxygenase-2-dependent signaling via cyclic AMP response element activation on aromatase up-regulation by o, p′-DDT in human breast cancer cells. Toxicol Lett. 2010;198:331–41.
48. Zhu XO, Yang Z, Guo CM, Ni XT, Li JN, Ge YC, et al. Paradoxical stimulation of cyclooxygenase-2 expression by glucocorticoids via a cyclic AMP response element in human amnion fibroblasts. Mol Endocrinol. 2009;23:1839–49.
49. Whittle WL, Holloway AC, Lye SJ, Gibb W, Challis JR. Prostaglandin production at the onset of ovine parturition is regulated by both estrogen-independent and estrogen-dependent pathways. Endocrinology. 2000;141:3783–91.
50. Tsatsanis C, Androulidaki A, Venihaki M, Margioris AN. Signalling networks regulating cyclooxygenase-2. Int J Biochem Cell Biol. 2006;38:1654–61.
51. Brandenberger AW, Tee MK, Lee JY, Chao V, Jaffe RB. Tissue distribution of estrogen receptors alpha (ER-alpha) and beta (ER-beta) mRNA in the midgestational human fetus. J Clin Endocrinol Metab. 1997;82:3509–12.
52. Malayer JR, Woods VM. Development of oestrogen receptor expression and hormone response in the uterus of the bovine fetus. J Reprod Fertil. 1998;112: 289–300.
53. Lonard DM, O'Malley BW. Nuclear receptor coregulators: modulators of pathology and therapeutic targets. Nat Rev Endocrinol. 2012;8:598–604.
54. Bjornstrom L, Sjoberg M. Mechanisms of estrogen receptor signaling: convergence of genomic and nongenomic actions on target genes. Mol Endocrinol. 2005;19:833–42.
55. Acconcia F, Ascenzi P, Bocedi A, Spisni E, Tomasi V, Trentalance A, et al. Palmitoylation-dependent estrogen receptor alpha membrane localization: regulation by 17beta-estradiol. Mol Biol Cell. 2005;16: 231–7.
56. Marino M, Ascenzi P. Membrane association of estrogen receptor alpha and beta influences 17beta-estradiol-mediated cancer cell proliferation. Steroids. 2008;73:853–8.
57. Marino M, Ascenzi P. Steroid hormone rapid signaling: the pivotal role of S-palmitoylation. IUBMB Life. 2006;58:716–9.
58. Christensen A, Micevych P. CAV1 siRNA reduces membrane estrogen receptor-alpha levels and attenuates sexual receptivity. Endocrinology. 2012;153: 3872–7.
59. McEwen BS. Invited review: estrogens effects on the brain: multiple sites and molecular mechanisms. J Appl Physiol. 2001;91:2785–801.
60. Roa J, Vigo E, Castellano JM, Gaytan F, Navarro VM, Aguilar E, et al. Opposite roles of estrogen receptor (ER)-alpha and ERbeta in the modulation of luteinizing hormone responses to kisspeptin in the female rat: implications for the generation of the preovulatory surge. Endocrinology. 2008;149:1627–37.
61. Simpkins JW, Yang SH, Sarkar SN, Pearce V. Estrogen actions on mitochondria-physiological and pathological implications. Mol Cell Endocrinol. 2008; 290:51–9.
62. Levin ER. Plasma membrane estrogen receptors. Trends Endocrinol Metab. 2009;20:477–82.
63. Klinge CM. Estrogenic control of mitochondrial function and biogenesis. J Cell Biochem. 2008; 105:1342–51.
64. Lehman MN, Ebling FJP, Moenter SM, Karsch FJ. Distribution of estrogen receptor-immunoreactive cells in the sheep brain. Endocrinology. 1993;133: 876–86.
65. Scott CJ, Rawson JA, Pereira AM, Clarke IJ. The distribution of estrogen receptors in the brainstem of female sheep. Neurosci Lett. 1998;241:29–32.
66. Scott CJ, Tilbrook AJ, Simmons DM, Rawson JA, Chu S, Fuller PJ, et al. The distribution of cells containing estrogen receptor-alpha (ERalpha) and ERbeta messenger ribonucleic acid in the preoptic area and hypothalamus of the sheep: comparison of males and females. Endocrinology. 2000;141: 2951–62.
67. Friedman WJ, McEwen BS, Toran-Allerand CD, Gerlach JL. Perinatal development of hypothalamic and cortical estrogen receptors in mouse brain: methodological aspects. Brain Res. 1983;313:19–27.

68. Gerlach JL, McEwen BS, Toran-Allerand CD, Friedman WJ. Perinatal development of estrogen receptors in mouse brain assessed by radioautography, nuclear isolation and receptor assay. Brain Res. 1983;313:7–18.
69. Peruffo A, Giacomello M, Montelli S, Corain L, Cozzi B. Expression and localization of aromatase P450AROM, estrogen receptor-alpha, and estrogen receptor-beta in the developing fetal bovine frontal cortex. Gen Comp Endocrinol. 2011;172:211–7.
70. Resko JA, Stadelman HL. 17 beta-hydroxysteroid dehydrogenase activity in tissues of fetal rhesus macaques. Proc Soc Exp Biol Med. 1982;171:233–7.
71. Milewich L, MacDonald PC, Carr BR. Activity of 17 beta-hydroxysteroid oxidoreductase in tissues of the human fetus. J Endocrinol. 1989;123:509–18.
72. Takeyama J, Suzuki T, Hirasawa G, Muramatsu Y, Nagura H, Iinuma K, et al. 17beta-hydroxysteroid dehydrogenase type 1 and 2 expression in the human fetus. J Clin Endocrinol Metab. 2000;85:410–6.
73. Adamski J, Jakob FJ. A guide to 17beta-hydroxysteroid dehydrogenases. Mol Cell Endocrinol. 2001;171:1–4.
74. Selmanoff MK, Brodkin LD, Weiner RI, Siiteri PK. Aromatization and 5alpha-reduction of androgens in discrete hypothalamic and limbic regions of the male and female rat. Endocrinology. 1977;101:841–8.
75. McEwen BS, Lieberburg I, Chaptal C, Krey LC. Aromatization: important for sexual differentiation of the neonatal rat brain. Horm Behav. 1977; 9:249–63.
76. Weisz J. In vitro assays of aromatase and their role in studies of estrogen formation in target tissues. Cancer Res. 1982;42 Suppl 8:3295s–8.
77. George FW, Ojeda SR. Changes in aromatase activity in the rat brain during embryonic, neonatal, and infantile development. Endocrinology. 1982;111:522–9.
78. MacLusky NJ, Roy EJ, Shanabrough M, Eisenfeld AJ. Preservation of steroid receptors in frozen brain and pituitary tissue: use of the cryoprotective agent, dimethylsulfoxide. J Neurosci Methods. 1986; 16:131–40.
79. Roselli CE, Resko JA. Effects of gonadectomy and androgen treatment on aromatase activity in the fetal monkey brain. Biol Reprod. 1986;35:106–12.
80. Roselli CE, Resko JA, Stormshak F. Estrogen synthesis in fetal sheep brain: effect of maternal treatment with an aromatase inhibitor. Biol Reprod. 2003;68:370–4.
81. Mura A, Gadau S, Lepore G, Balzano F, Zedda M, Mura E, et al. Expression and distribution of P450-aromatase in the ovine hypothalamus at different stages of fetal development. Neuro Endocrinol Lett. 2010;31:690–9.
82. Pezzi V, Mathis JM, Rainey WE, Carr BR. Profiling transcript levels for steroidogenic enzymes in fetal tissues. J Steroid Biochem Mol Biol. 2003;87:181–9.
83. Toda K, Simpson ER, Mendelson CR, Shizuta Y, Kilgore MW. Expression of the gene encoding aromatase cytochrome P450 (CYP19) in fetal tissues. Mol Endocrinol. 1994;8:210–7.
84. Yu L, Romero DG, Gomez-Sanchez CE, Gomez-Sanchez EP. Steroidogenic enzyme gene expression in the human brain. Mol Cell Endocrinol. 2002; 190:9–17.
85. Gomez-Sanchez EP, Ahmad N, Romero DG, Gomez-Sanchez CE. Is aldosterone synthesized within the rat brain? Am J Physiol Endocrinol Metab. 2005;288:E342–6.
86. Carnegie JA, Robertson HA. Conjugated and unconjugated estrogens in fetal and maternal fluids of the pregnant ewe: a possible role for estrone sulfate during early pregnancy. Biol Reprod. 1978;19:202–11.
87. Tsang CPW. Changes in plasma levels of estrone sulfate and estrone in the pregnant ewe around parturition. Steroids. 1974;23:855–68.
88. James MO, Li W, Summerlot DP, Rowland-Faux L, Wood CE. Triclosan is a potent inhibitor of estradiol and estrone sulfonation in sheep placenta. Environ Int. 2010;36:942–9.
89. Pierrepoint CG, Anderson AB, Harvey G, Turnbull AC, Griffiths K. The conversion in vitro of C19-steroids to oestrogen sulphates by the sheep placenta. J Endocrinol. 1971;50:537–8.
90. Cousins R, Wood CE. Expression of organic anion transporters 1 and 3 in the ovine fetal brain during the latter half of gestation. Neurosci Lett. 2010;484: 22–5.
91. Purinton SC, Newman H, Castro MI, Wood CE. Ontogeny of estrogen sulfatase activity in ovine fetal hypothalamus, hippocampus, and brain stem. Am J Physiol. 1999;276:R1647–52.
92. Winikor J, Schlaerth C, Rabaglino MB, Cousins R, Sutherland M, Wood CE. Complex actions of estradiol-3-sulfate in late gestation fetal brain. Reprod Sci. 2011;18:654–65.
93. Payne AH, Lawrence CC, Foster DL, Jaffe RB. Intranuclear binding of 17-estradiol and estrone in female ovine pituitaries following incubation with estrone sulfate. J Biol Chem. 1973;248:1598–602.
94. Kuiper GG, Carlsson B, Grandien K, Enmark E, Haggblad J, Nilsson S, et al. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. Endocrinology. 1997;138:863–70.
95. Pasqualini JR, Gelly C, Nguyen BL, Vella C. Importance of estrogen sulfates in breast cancer. J Steroid Biochem. 1989;34:155–63.
96. Wood CE, Gridley KE, Keller-Wood M. Biological activity of 17beta-estradiol-3-sulfate in ovine fetal plasma and uptake in fetal brain. Endocrinology. 2003;144:599–604.
97. Purinton SC, Wood CE. Ovine fetal estrogen sulfotransferase in brain regions important for hypothalamus-pituitary-adrenal axis control. Neuroendocrinology. 2000;71:237–42.
98. Gong H, Jarzynka MJ, Cole TJ, Lee JH, Wada T, Zhang B, et al. Glucocorticoids antagonize estrogens

by glucocorticoid receptor-mediated activation of estrogen sulfotransferase. Cancer Res. 2008;68: 7386–93.
99. Rabaglino MB, Richards E, Denslow N, Keller-Wood M, Wood CE. Genomics of estradiol-3-sulfate action in the ovine fetal hypothalamus. Physiol Genomics. 2012;44:669–77.
100. Baulieu EE, Robel P. Neurosteroids: a new brain function? J Steroid Biochem Mol Biol. 1990;37: 395–403.
101. Baulieu EE, Robel P, Schumacher M. Neurosteroids: beginning of the story. Int Rev Neurobiol. 2001;46: 1–32.
102. Schumacher M, Liere P, Akwa Y, Rajkowski K, Griffiths W, Bodin K, et al. Pregnenolone sulfate in the brain: a controversial neurosteroid. Neurochem Int. 2008;52:522–40.
103. Petrovic M, Sedlacek M, Cais O, Horak M, Chodounska H, Vyklicky Jr L. Pregnenolone sulfate modulation of N-methyl-D-aspartate receptors is phosphorylation dependent. Neuroscience. 2009; 160:616–28.
104. Kakusaka S, Asayama M, Kaihara A, Sasano T, Suzuki T, Kurokawa J, et al. A receptor-independent effect of estrone sulfate on the HERG channel. J Pharmacol Sci. 2009;109:152–6.
105. Zurkovsky L, Serio SJ, Korol DL. Intra-striatal estradiol in female rats impairs response learning within two hours of treatment. Horm Behav. 2011;60:470–7.
106. Grove-Strawser D, Boulware MI, Mermelstein PG. Membrane estrogen receptors activate the metabotropic glutamate receptors mGluR5 and mGluR3 to bidirectionally regulate CREB phosphorylation in female rat striatal neurons. Neuroscience. 2010;170: 1045–55.
107. Charalampopoulos I, Alexaki VI, Tsatsanis C, Minas V, Dermitzaki E, Lasaridis I, et al. Neurosteroids as endogenous inhibitors of neuronal cell apoptosis in aging. Ann N Y Acad Sci. 2006;1088:139–52.