Published in final edited form as:
J Neuroendocrinol. 2008 June ; 20(6): 873–879. doi:10.1111/j.1365-2826.2008.01738.x.

Roles of Oestrogen Receptors *α* and *β* in Behavioural Neuroendocrinology: Beyond Yin/Yang

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Abstract

Oestrogen receptor *β* (ER*β*) was discovered more than 10 years ago. It is widely distributed in the brain. In some areas, such as the entorhinal cortex, it is present as the only ER, whereas in other regions, such as the bed nucleus of the stria terminalis and preoptic area, it can be found co-expressed with ER*α*, often within the same neurones. These ERs share ligands, and there are several complex relationships between the two receptors. Initially, the relationship between them was labelled as 'yin/yang', meaning that the actions of each complemented those of the other, but now, years later, other relationships have been described. Based on evidence from neuroendocrine and behavioural studies, three types of interactions between the two oestrogen receptors are described in this review. The first relationship is antagonistic; this is evident from studies on the role of oestrogen in spatial learning. When oestradiol is given in a high, chronic dose, spatial learning is impaired. This action of oestradiol requires ER*α*, and when ER*β* is not functional, lower doses of oestradiol have this negative effect on behaviour. The second relationship between the two receptors is one that is synergistic, and this is illustrated in the combined effects of the two receptors on the production of the neuropeptide oxytocin and its receptor. The third relationship is sequential; separate actions of the two receptors are postulated in activation and organisation of sexually dimorphic reproductive behaviours. Future studies on all of these topics will inform us about how ER selective ligands might affect oestrogen functions at the organismal level.

Keywords

anxiety; lordosis; sexual behaviour; cognition; oxytocin

The first genetically engineered gene knockout (KO) mouse with direct applications to the study of neuroendocrinology was the oestrogen receptor (ER) knockout mouse (1). At the time that Dr Dennis Lubahn and his colleagues produced this mouse, only one ER had been sequenced (2,3), and the idea of a second receptor was heretical. The ERKO mouse was phenotyped by a number of laboratories; their data indicated that mice with mutations for this receptor showed severe deficits in reproductive behaviours, and their hypothalamic–pituitary–gonadal feedback loop was disrupted (4–8). These data were in agreement with the prevailing view that the ER was essential for reproduction at the level of the gonads, pituitary and brain. In 1996, the second ER (ER*β*) was identified from a rat prostate cDNA library (9). Characterisation of ER*β* revealed a 97% similarity between the two receptors in the DNA-binding domain, and the ligand-binding domains were 55% identical (10,11). Initial hopes were that the new receptor would open up novel avenues of investigation that would have far-reaching applications, particularly for women's health, where selective ligands might be used

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to individually treat reproductive disorders, cardiovascular disease, cancer, bone health, hot flushes, obesity and cognitive disorders.

The ERβ mouse knockout was created shortly thereafter, and the initial reproductive phenotype was not nearly as dramatic as that described in the ERαKO mouse (12). Females were subfertile, with ovarian defects accounting for fewer and smaller litters, while males were unremarkable; both displayed normal reproductive behaviour (13). The speculation from the results of these studies, along with brain localisation studies which described more ERβ than ERα outside the hypothalamus, particularly in the cerebellum, cortex and hippocampus (14–16), was that ERβ may mediate the effects of oestradiol (E2) on non-reproductive behaviours (10,17,18).

Antagonistic actions and cognitive behaviours

The role of oestrogens in cognitive behaviours was an exciting research topic in the 1990s, and the arrival of ERβ was heralded as a potential tool to advance women's health research. This was before the release of the first data from the National Institutes of Health's Women's Health Initiative (WHI) which all but shut down exploration of new oestrogen ligands for hormonal replacement therapy (HRT). The WHI results indicated that the treatment regimes under test (oestrogen and progestins) placed post-menopausal women at increased risk of several diseases, with no benefit for cognition (19–21). Closer consideration of the statistical analysis and design of these studies has since placed such a simplistic conclusion in doubt (22–25).

Soon after the ERβKO mice were made by Dr Jan-Ake Gustaffson and his group (12), we started a colony at the University of Virginia. Our ERαKO colony (provided by Dr Dennis Lubahn) was already established, and we were anxious to compare behaviour in the two mice side by side. Our simplistic hypothesis, based on the phenotype of the ERαKO and the location of ERβ in the brain, was that ERβ mediated the beneficial actions of oestrogens on cognition. Because most of the initial research was conducted in rats, we needed to design a comparable behavioural task for mice. We shrank the Morris water maze down to a negotiable size for mice and increased the time needed to learn the task. Before the ERβKO and their wild-type (WT) littermates were available, we tested the effects of different E2 doses in WT C57BL/6J mice on their performance on the Morris water maze. Because one of our other major interests was in sex differences, we used gonadectomised adult males and females treated with either vehicle or E2. Previous work in rodents had shown that males were superior to females in this spatial task (26,27). However, the studies were conducted in gonad-intact animals, and thus the sex differences reported might have been caused by neural organisational differences or by differences in hormone levels at the time of testing. We wanted to pinpoint which one of these mechanisms was involved before switching to animals with gene mutations. One of the drawbacks of working with adult knockout mice is that, if a behavioural problem is found, it is impossible to know if the loss of oestrogen receptors during development or in adulthood was critical.

In our pilot studies, we were surprised that gonadectomised males and female mice performed equally well in the water maze; in fact there was a trend for ovariectomised females to escape more rapidly than castrated males (28). Moreover, ERαKO mice of both sexes did well on this task, and counter to the prevalent idea that oestrogens have positive effects on cognition, learning was impaired in E2-injected WT females. Others showed later that the dosage of E2 treatment is a critical factor for the direction of the effects of oestrogens on learning behaviour, and high doses, like the one we used, may impair various types of learning (29–32). While the fact that ERαKO mice behaved as well as, if not better than, their WT littermates was surprising, it allowed us to test our hypothesis that ERβ was the important oestrogen receptor for spatial learning.

Because the effect of E2 was only noted in females, we restricted our next study to female ERβKO and WT littermates (33). We changed the route of E2 administration from injections to Silastic implants filled with either a high or a low dose of E2. In this study, all of the WT ovariectomised females that were treated or not with E2 exhibited equivalent spatial learning. Only the ERβKO females treated with E2 showed impaired learning. At the low dose, the effect was on the first few testing days and ERβKO females 'caught up' with WT females by the last day. Females with the ERβ mutation that were treated with the high E2 dose took significantly longer to escape than WT females. Taken together with the earlier data showing that ERαKO females were less vulnerable to the negative effects of E2, we hypothesised that, in WT females, the detrimental effect of large doses of E2 is mediate by the ERα; when ERβ is present, more E2 is needed to affect behaviour; in contrast, when ERβ is absent, lower doses of E2 can disrupt cognition (Fig. 1A).

A recent set of behavioural and electrophysiological studies reported specific facilitatory roles for ERβ in learning spatial tasks using both KO mice and rats (34; Fig. 1B). When provided with E2, or an ERβ-specific agonist (Way-200070), ovariectomised female rats performed well in a radial arm maze task, better than females treated with an ERα-specific agonist (PPT) or vehicle. In a water Y maze task, WT female mice treated with E2 outperformed ERβKO mice regardless of their treatment. These behavioural data complement studies carried out *in vitro* showing opposite and/or antagonistic actions of ERα and β. Observations from ERα/ERβ heterodimers, which act in the opposite direction to ERα-only heterodimers, support the yin/yang concept (35). When HeLa cells, transfected with ERs, are exposed to the classic oestrogen receptor antagonist tamoxifen, mixed antagonistic and agonistic effects are apparent on ERα transcription; however, tamoxifen has an exclusively antagonistic action on ERβ (36). In addition, when E2 is present, ERα induces transcription of the cyclin D1 gene via the activator protein 1 (AP1) in HeLa cells (37). In contrast, anti-oestrogens activate ERβ to induce cyclin D1. In summary, both *in vitro* and *in vivo* data suggest that E2 effects are often caused by antagonistic actions between the two ERs (38).

Synergistic actions of ERα and ERβ in neuroendocrinology

Oestrogen receptors act as transcription factors to regulate many downstream genes, including those encoding neurotransmitters and peptides. Another way to distinguish the actions of ERα and β is to ask which genes are regulated by each ER. Oestrogens can enhance the actions of the neuropeptide oxytocin, by increasing the production of both the peptide and its receptor (39–42). The supraoptic area and the paraventricular nucleus (PVN) contain the vast majority of the oxytocin-producing cells. In rats, ERβ is more highly expressed in the PVN than is ERα, whereas the rest of the hypothalamus has both ERs, with more ERα than ERβ in some locations (i.e. the ventromedial nucleus) (43). Moreover, after ERβ was discovered, several studies reported co-localisation of ERβ within oxytocin-producing neurones in the rat PVN (44–46) and later in the mouse (47). In collaboration with Drs Heather Patisaul and Larry Young, we asked which ER induced oxytocin and its receptor. Using gonadectomised WT and ERαKO mice of both sexes, we treated some animals with E2 and others with vehicle. Regardless of sex, E2 induced oxytocin binding (a reflection of receptor abundance) throughout the hypothalamus. This response was blocked in ERαKO mice (48). In another study we used ERβKO and WT female mice to examine oxytocin mRNA in the PVN. In this study, hormone priming was conducted, followed by *in situ* hybridisation. Oxytocin mRNA was elevated in the WT PVN after hormone treatment, but not in the ERβKO brain (47). As confirmation of the early data on the oxytocin receptor, in both the WT and ERβKO female mice, receptor message in the hypothalamus and amygdala was induced with hormone priming. This is an example of both an autonomous and a synergistic relationship, as ERβ increases the amount of oxytocin peptide produced, and ERα increases expression of its receptor (Fig. 2).

A different type of ER synergy has been noted in the anteroventral periventricular nucleus (AVPV), a subregion of the medial preoptic area. The cells in this area are sexually dimorphic, with females having more than males (49). Often the population of cells is revealed with immunocytochemistry for tyrosine hydroxylase (TH), and the TH-positive labelled neurones in the AVPV are known to produce dopamine. Not only is this a structural sex difference, but it is also functional as the dopamine-containing neurones project to nearby gonadotrophin-releasing hormone (GnRH)-containing cells, and are thought to be involved in the ovulatory release of GnRH from these cells (50). Studies with the ER*α*KO mouse showed that WT females had more cells positive for TH in the AVPV than WT males, ER*α*KO males or ER*α*KO females (51). At the time this observation was made, ER*β* had just been discovered, and thus little was made of the fact that ER*α*KO males and females had AVPV cell numbers that were not completely 'female-like' and instead were intermediate between WT males and females. A few years ago we decided to revisit this question of ER regulation and labelled neurones in the AVPV with TH in brains of mice from both ERKO strains and double-KO individuals (52). Only in WT individuals was the sex difference in the AVPV present. All the KO mice, both males and females, had 'female-like' high numbers of dopaminergic neurones in the AVPV. These data showed that the two ERs work in concert in this region to elaborate the sex difference in cell numbers. We presumed that the actions occurred during development as this sex difference is organised prior to postnatal day 7 in rats (49,53). Interestingly, ER*α*KO females lack positive and negative feedback to E2 (8,54), and ER*β*KO mice have normal negative feedback but ovulate a reduced number of ova as compared with WT females (12,55). Afferents from GnRH neurones are specifically associated with ER*α*-containing neurones in the AVPV, suggesting that the ER*α* in the AVPV is directly involved in GnRH regulation (54).

Sequential actions of ER*α* and ER*β*

One of the most important and unifying concepts in the field of behavioural neuroendocrinology is that steroid hormones act at two distinct and sequential times in the lifespan to affect behaviours (56,57). The first occurs during development, in which steroids act (via their receptors) to shape neural cell clusters and connections, and the second occurs in adulthood, in which steroids activate gene transcription which ultimately results in a behavioural change. In many of the animals used for these studies, the major steroid involved at both time-points is E2 (58). When the ER*α*KO mouse first became available, and tests for sexually differentiated behaviours were conducted by our group and Drs Don Pfaff and Sonoko Ogawa, we found severe deficits in male sexual behaviour, female sexual behaviour, and partner preferences (4,6,7,17,59–61). Because the ER*α* gene mutation is present at all times of the lifespan, these data revealed nothing about when ER*α* is critical.

By contrast, ER*β*KO males display normal male sexual behaviour and partner preferences (13,62) while the female ER*β*KO mice have normal, if not enhanced, lordosis (the female receptivity posture) (13,63). Taken together, these findings suggest that ER*β* is not essential for development and for the expression of sex-typical behaviours; lordosis in females, and partner preferences in males. However, a true test of the organisational effects of a specific receptor cannot be performed unless you examine opposite sex-typical behaviours. To do this, you need to determine whether females can display partner preferences or males can perform lordosis. Thus we started by testing ER*β*KO males for lordosis. When treated with activational hormones, identical to the treatments given to ovariectomised females to hormonally prime them to become sexually receptive, we found that ER*β*KO males had higher levels of lordosis than WT males (62), but the ER*β*KO males displayed male partner preferences similar to those of WT males. To confirm our hypothesis that ER*β* is required in development for 'defeminisation' in males, we asked if treatment with a selective ER*β* agonist during the perinatal period would also 'defeminise' females. We injected pups from the day of birth for

three consecutive days with E2, the ER*α* agonist PPT, an ER*β* agonist (DPN) or vehicle. E2 and DPN animals had reduced lordosis expression in adulthood as compared with females that received PPT or vehicle (64). These data show that the activation of the ER*β* during the early postnatal period blocks development of female-typical lordosis (Fig. 3). Our hypothetical model suggests that ER*β* is activated normally in the perinatal male, and this activation selectively defeminises adult behavioural potential without affecting masculinization of male-typical behaviours.

Data on neural sexual dimorphisms support this hypothesis. We treated adult WT and ER*β*KO gonadectomised mice with either vehicle or E2, and then processed their brains for ER*α* and progestin receptor (PR) immunoreactivity (ir) (65). E2 treatment promoted a reduction in ER*α*-ir cells in the preoptic area, arcuate nucleus and the ventromedial nucleus (VMN) of WT males when compared with their vehicle-treated male counterparts, but E2 had no effect in these areas in WT females. In ER*β*KO males, E2 treatment also had no effect. The difference between the WT and ER*β*KO males and the similarity between WT females and ER*β*KO males suggests that ER*β* activation during development makes ER*α* in brains of WT males more sensitive to the down-regulatory effects of E2 in adulthood. When we examined PR-ir cell numbers in the same regions, we noted that, in areas and animals were ER*α*-ir was not decreased by E2, PR-ir was enhanced by this same treatment. Sex differences between WT males and females were noted in the preoptic area and VMN, and ER*β*KO males again had the same pattern as did WT females. Thus ER*β* may act during development in the normal male brain to ‘defeminise’ adult responses to E2.

What does the future hold?

Over the past 10 years much has been uncovered about the relationship between ER*α* and ER*β*, but we have a long way to go to unravel the complexity of relationships between the two receptors. New technologies are being used to enhance the ER ‘tool box’. Examination of the roles of ER*α* in hypothalamic–pituitary–gonadal feedback has been elegantly accomplished with tissue-specific ER*α*KO mice. In these transgenics the ER*α* gene is knocked out only in the brain (54). Moreover, development of conditional and tissue-specific KO mice would greatly enhance the possibilities for brain research. Mice with ER*β* tagged with green fluorescent protein (GFP) make identification of these cells *in vitro* or *in vivo* easier (http://www.gensat.org). Agonists and specific antagonists for the ERs are very useful as they can be used to ask questions about ER actions in any animal, and at any time during development, whereas the KO work has been limited to mice and the classic models remove the receptors at all times in the lifespan. The technologies for the use of siRNA to block specific gene transcription are becoming more specific and simpler all the time (http://www.ambion.com/techlib/tn/131/5.html). We can anticipate new discoveries based on these improved technologies.

Several new developments have broadened our view of ER*β*. For example, a metabolite of dihydrotestosterone, 5*α*-androstane-3*β*,17*β*-diol (3*β*-diol), acts on ER*β*, but not ER*α* (66). ER*β* is present in the prostate [this is one of the tissues in which ER*β* was discovered (9)] and is activated there not only by oestrogens, but importantly by 3*β*-diol (66,67). Moreover, this certainly occurs in the brain. For example, ER*β* appears to mediate oestrogen action on anxiety (68,69). However, ER*β* may also mediate anxiolytic actions of testosterone on anxiety (70). The discovery of this endogenous specific ligand for ER*β* opens a new frontier for examining independent actions of this ER.

In our laboratory, sex differences are a major research focus and thus we are currently asking how ER*β* ‘defeminises’ the male brain during development. Others are also studying this, and one very applicable aspect of this work stems from the fact that environmental oestrogens and

phytoestrogens preferential activate the ERβ (71,72). Documentation of actions of environmental oestrogens and phytoestrogens during development in animal models is underway and the relevance to human development, particularly in infants on soy-based formula, is potentially enormous (72–74). While the scientific community initially hoped that the discovery of ERβ would produce new HRT treatments, through the power and imagination of basic science research we now have several other, equally exciting, ideas about ERβ and the translational value to human medicine is vast.

Acknowledgements

I thank Dr Jin Ho Park for constructive comments on this manuscript. My laboratory is indebted to Aileen Wills and Savera Shetty for expert technical assistance. The work referenced here from my laboratory was funded by NIH (MH057759).

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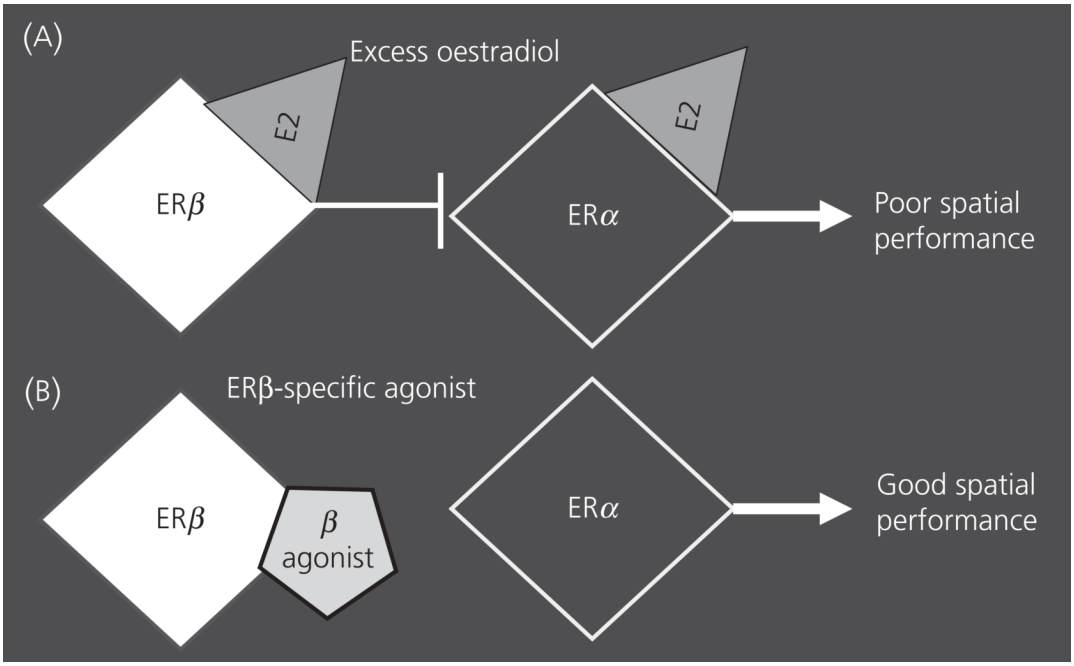

Fig. 1.

This cartoon illustrates the antagonistic relationship between the two oestrogen receptors (ERs). The white diamond represents ERβ, the black diamond represents ERα, and the grey triangle is oestradiol activating the receptors. An ERβ-specific agonist is represented by the off-grey pentagon. (A) The example shows the impact of excess oestradiol on the two ERs and the resulting behaviour. (B) The relationship is shown when an ERβ agonist is used.

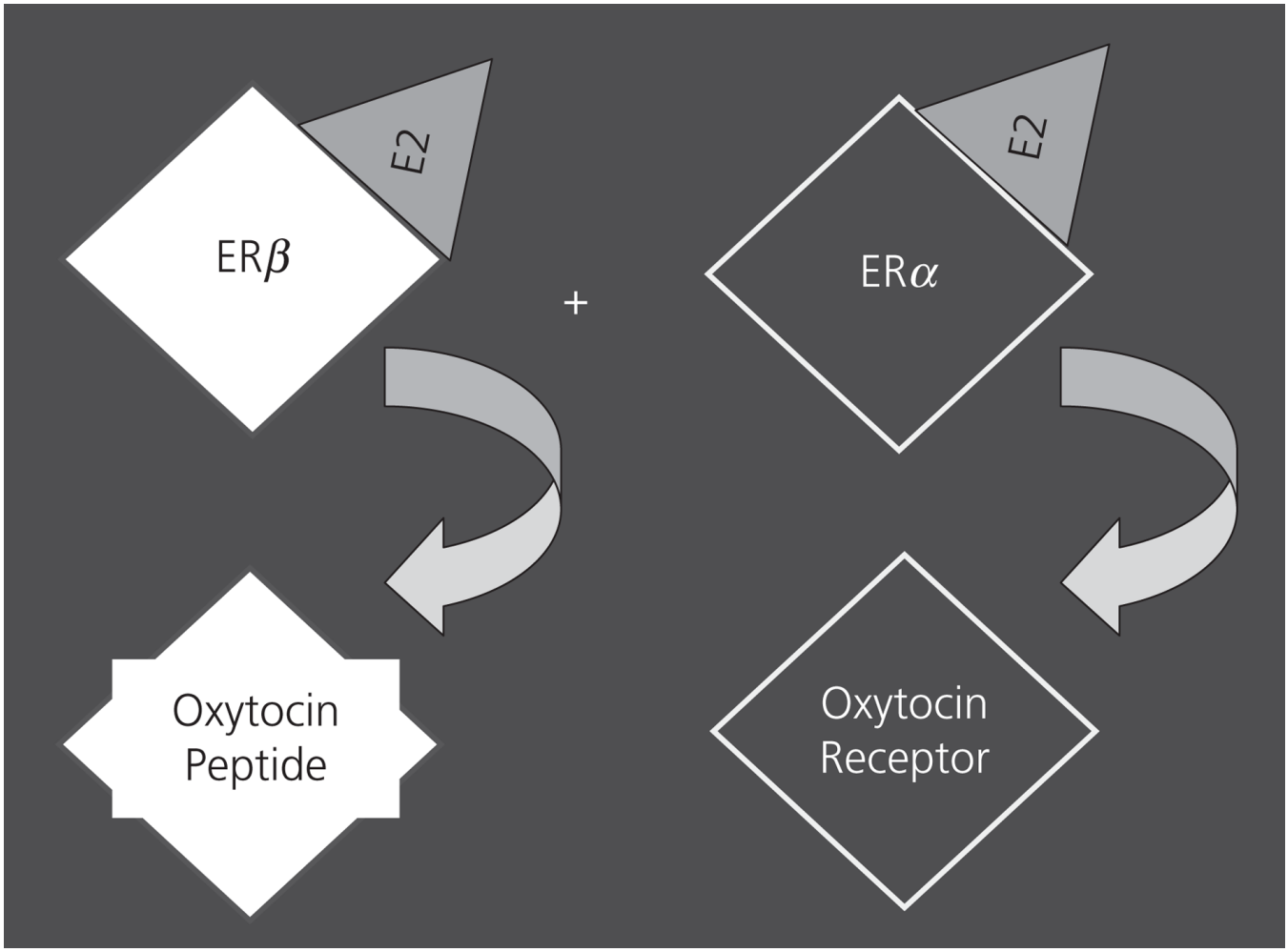

Fig. 2.

This cartoon figure illustrates the synergistic relationship between the two oestrogen receptors (ERs). The white diamond represents ERβ, the black diamond represents ERα, and the grey triangle is oestradiol activating the receptors. Below each receptor is the oxytocin gene that it affects.

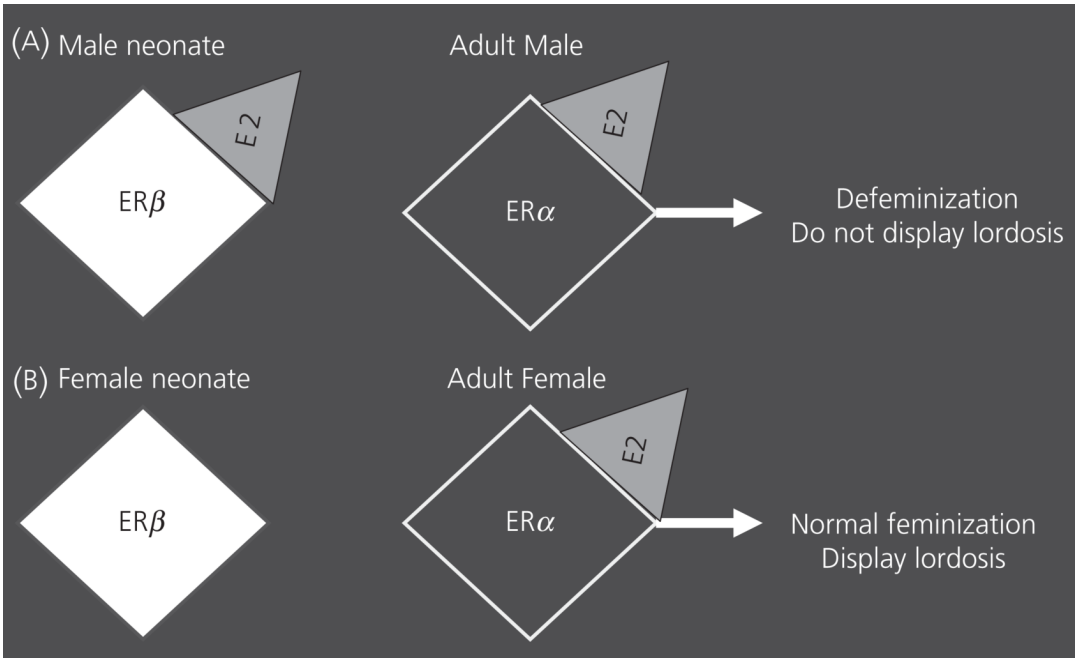

Fig. 3.

This cartoon illustrates a sequential relationship between the two oestrogen receptors (ERs) during development. The white diamond represents ER*β*, the black diamond represents ER*α*, and the grey triangle is oestradiol activating the receptors. (A) An illustration of the roles of ER*β* activation in neonatal males followed by activation of the ER*α* in adult males. The end result of the two actions is defeminisation of adult male behaviour. (B) An illustration of the situation in a normal female. Here, the lack of ER*β* activation in the neonatal period is responsible for the adult activational effect of oestradiol on lordosis.