MINIREVIEW

Aging-Related Changes in Ovarian Hormones, Their Receptors, and Neuroendocrine Function

TANDRA R. CHAKRABORTY* AND ANDREA C. GORE†,[1]

*Fishberg Research Center for Neurobiology, Mount Sinai School of Medicine, New York, New York 10029; and †Division of Pharmacology/Toxicology, College of Pharmacy, Institute for Neuroscience, and Institute for Cellular & Molecular Biology, University of Texas at Austin, Austin, Texas 78712

Ovarian steroid hormones exert a broad range of effects on the body and brain. In the nervous system, estrogen and progesterone have crucial feedback actions on the hypothalamic neurons that drive the reproductive axis. In addition, hormones exert a variety of actions on other traditionally nonreproductive functions such as cognition, learning and memory, neuroprotection, mood and affective behavior, and locomotor activity. The actions of hormones on the hypothalamus are largely mediated by their nuclear hormone receptors, the two estrogen receptors, ERα and ERβ, and the two progesterone receptor isoforms, PR-A and PR-B. Thus, changes in the circulating concentrations of estrogens and progestins during the life cycle can result in differential activation of their receptors. Furthermore, changes in the numbers, activity, and distribution of hypothalamic ERs and PRs can occur as a function of developmental age. The purpose of this article is to review the literature on the causes and consequences of alterations in steroid hormones, their neural receptors, and their interactions on reproductive senescence. We have also discussed several important experimental design considerations, focusing on rodent models in current use for understanding the mechanisms of menopause in women. Exp Biol Med 229:977–987, 2004

Key words: estrogen receptor; progesterone receptor; aging; menopause; neuroendocrine; hypothalamus

This work was supported by the National Institutes of Health Grant 1 PO1 AG16765 to A.C.G.

[1] To whom correspondence should be addressed at Division of Pharmacology/Toxicology, University of Texas at Austin, Austin, TX 78712. E-mail: andrea.gore@mail.utexas.edu

1535-3702/04/22910-0977$15.00

Reproductive aging in women is characterized by dramatic changes in ovarian function, the result of which is a precipitous loss of estrogen and alterations in progesterone secretion. These menopausal changes in circulating hormones have broad consequences on the body and brain. Although most research on menopause in women has focused on ovarian hormonal changes, the hypothalamic and pituitary levels of the reproductive axis also change during reproductive aging (1–3). The close interrelationships among the hypothalamus, pituitary, and gonad make it difficult to differentiate the causes and consequences of reproductive hormonal changes, and several important questions remain unanswered. First, whether the hypothalamus plays any causal role in reproductive aging is unclear. Reports showing that gonadotropin levels change in women before ovarian failure (1–3) and that hypothalamic and pituitary hormone concentrations change in rodents before there is any appreciable loss in ovarian follicles (4–11) support a role of the hypothalamus in reproductive senescence. A recent study demonstrated an age-related increase in pulsatile gonadotropin-releasing hormone (GnRH) release from the hypothalamus of perimenopausal rhesus monkeys (12), although again, whether this precedes ovarian hormonal changes is yet to be determined. Second, the hypothalamic and pituitary responses to the positive and negative feedback effects of hormones are likely to be strongly affected by age-related changes in concentrations of circulating hormones (13). For example, women in their later postmenopause years have diminished gonadotropin release relative to women in the early postmenopause years (2). This finding has direct consequences for the timing at which hormone replacement ought to be initiated relative to

the menopause. Third, there may be age-related changes in expression or responsiveness of the neural estrogen and progestin receptors; these can occur as a consequence of steroid changes, but it is also possible that hormone receptor levels in the brain change with chronological age, independently of circulating hormone levels. Thus, the three levels of the hypothalamic-pituitary-gonadal axis undergo age-related changes, some interrelated, others unique to each level, that together have consequences on the physiology of reproductive senescence. The purpose of this minireview is to provide an overview of the causes and outcomes of age-related changes in circulating hormones and their effects on the central nervous system.

Experimental Models to Study the Role of Estrogen in Neuroendocrine Aging

In women, estrogen levels decline at menopause as a result of the loss of ovarian follicles (14–17). However, the menopausal process appears to be fairly unique to humans and some higher primates, as even nonhuman primates such as rhesus monkeys may menstruate until the end of their maximal lifespan (12, 18). Reproductive failure also occurs in rodents, but at an age when there are few if any primary changes in the ovary or decreases in the ovarian follicular stores. Nevertheless, rats and other laboratory rodents undergo a transition in midlife that our laboratory and others have referred to as "estropause," characterized initially by irregular, usually prolonged, estrous cycles and eventually by acyclicity (11, 19–23). When rats first become acyclic, they typically enter a stage referred to as persistent estrus, characterized by chronically high estrogen levels and cornified vaginal cells. As rats continue to age, they usually enter a stage called persistent diestrus, in which estrogen levels are lower (albeit still detectable), and they exhibit a leukocytic vaginal cytology (11, 20–23). In addition, rats exhibit decreased fertility and reduced litter sizes and increased fetal resorptions during pregnancies that occur later in life (24). Although there is less information available on mice, females of this species also undergo a transitional period during which estrous cycles increase in length and eventually cease (reviewed in Ref. 25). In mice, as in menopausal primates, pituitary and plasma gonadotropin levels increase with age (26). Therefore, laboratory rodents exhibit some characteristics of reproductive aging in women, including a gradual cessation of spontaneous reproductive cycles and a decrease in fertility. Rats differ from primates in that their estrogen levels continue to be maintained at relatively high levels even at advanced ages, whereas in primates, estrogen levels are substantially decreased (12). As a result, ovariectomized (OVX) rats are often used as a model for the menopause in women. Despite some obvious limitations, the OVX rats are a strong model for a precipitous loss of estrogen and are even more relevant to menopause if the OVX is performed at the rat's chronological equivalent of middle age.

Many laboratories have capitalized on the OVX plus estrogen replacement model in female rats. Nevertheless, experimental differences exist among these studies regarding the age at OVX, the post-OVX duration before experimentation, and the mode and duration of hormone replacement. The effects of OVX with hormone replacement on neuroendocrine outcomes in young and aged rats are summarized in Table 1 for a representative group of studies. Although the outcomes shown in this table vary, several general and important conclusions about effects of OVX, with or without hormone replacement, can be drawn. First, the responsiveness of rats to OVX is typically more robust and rapid in younger rodents with regular estrous cycles (typically aged 2–6 months). Thus, by 1 week post-OVX, young rats have very low or undetectable concentrations of circulating ovarian steroid hormones and demonstrate maximal concentrations of circulating gonadotropins in response to the loss of steroid hormone negative feedback (Table 1; Refs. 27–29). By contrast, middle-aged and old rats (typically aged 9–14 months for middle-aged and 18–30 months for old) still have detectable circulating estrogens and progestins for weeks or even months post-OVX and require more time to exhibit the increased gonadotropin (and presumably GnRH) levels that result from the loss of ovarian steroid feedback (27, 28, 30). Second, the negative feedback response to hormone replacement in OVX rats is greater and more rapid in young rats compared to older rats. For example, estrogen-induced negative feedback on luteinizing hormone (LH) concentrations occurs after fewer days of estrogen replacement in young than older rats (31, 32). Third, the positive feedback effects of estrogen (with or without progesterone) on the GnRH/LH surge differ with aging. In young rats, fewer days of estrogen replacement are required to induce a surge, compared with middle-aged rats, and the amplitude of the LH surge is lower and delayed in middle-aged compared with young rats (Table 1; Refs. 28, 29, 33).

These results underscore the importance of the following considerations for designing and interpreting neuroendocrine aging studies in rodents: (i) If the clearance of circulating steroid hormones is desirable, an adequate post-OVX interval is necessary to enable rats of different ages to attain the maximal gonadotropin increase in response to the removal of negative feedback. In middle-aged and old rats, the post-OVX decline in steroid hormones does not occur for several weeks post-OVX, necessitating a post-OVX period of at least 3–4 weeks. (ii) An appropriate period is required for hormone replacement because of the slower neuroendocrine responses of older animals. (iii) There is a need for comparisons among rats of different ages, and it is not adequate to simply rely on the young OVX rat model. Differences in neuroendocrine responses among rats at different life stages, presented in Table 1, to some extent limit the utility of young OVX rats as a model for neuroendocrine aging. (iv) The age at OVX must be taken into consideration. For example, if animals are to be studied at 2 years, should OVX be done during the mid-life

transition to acyclicity (e.g., at 12 months) and animals used at 24 months, or is it adequate and/or equivalent to ovariectomize them at 23 months and use them at 24 months? (v) The reproductive status of the animals must also be known before OVX. This is particularly important for middle-aged rats that may have either regular estrous cycles or irregular cycles, or be acyclic, the latter either in persistent estrus (PE) or persistent diestrus (PD). This consideration is exemplified by a study showing that intact middle-aged rats that are used in early PE can exhibit an LH surge in response to estrogen plus progesterone replacement, whereas when they are in long-term PE, neither estrogen nor estrogen plus progesterone can induce an LH surge (34). In addition to the considerations discussed above, there are alternative models that provide important insights into mechanisms of aging. For example, Tsai and Legan (35–37) have published three elegant studies using young rats that are immediately implanted with estradiol at the time of OVX, the result of which is to mimic the changes in GnRH neurons and LH surges that occur in intact rats at middle age. This is a powerful approach to modeling reproductive aging in young rats given chronic estrogen replacement.

Estrogen Actions in the Brain: Mechanisms and Targets

The changes in ovarian steroids that occur in aging females have broad implications for the body and brain. In mammals, effects of estrogen in the brain are mediated by the two known nuclear estrogen receptors, estrogen receptor (ER) α and β. Estrogen receptors are members of the steroid hormone superfamily. In ER-expressing cells, the binding of estrogen to its intracellular receptor causes receptor-ligand dimerization and complexing with other co-factors in the cell. This complex can bind directly to an estrogen-response element located on the promoter of specific genes, with the effect of altering gene transcription (38). This genomic effect of estrogen, acting through the nuclear ER, can be contrasted with the nongenomic and more rapid mechanism of estrogen action that probably involves a membrane ER that is not a transcription factor (39, 40). Because the nongenomic ER has not yet been well investigated in the context of the hypothalamic-pituitary-gonadal axis, this review will focus on age-related changes in the genomic ERs in the hypothalamus.

The ERα is widely expressed in those brain regions controlling reproduction as well as in other brain regions that are not typically associated with reproductive function. In regions involved in the control of physiology and behavior of reproduction in rodents (mostly hypothalamic, preoptic, and limbic structures), nuclear ERα mRNA or protein is detected in high levels. Examples of regions with high ERα expression in rats include anteroventral periventricular nucleus (AVPV), medial preoptic nucleus (MPN), median preoptic area, bed nucleus of the stria terminalis (BST), lateral septum, medial amygdala, arcuate nucleus (ARC), periventricular nucleus of the hypothalamus, and ventromedial nucleus (VMN) of the hypothalamus (41–46). In brain areas not traditionally associated with reproduction, ERα mRNA or protein is detectable in relatively high levels in olfactory regions, cerebellum, area postrema, and substantia gelatinosa of the spinal cord (41, 46, 47). The murine nervous system in general has similar distributions of ERα and ERβ, although there are several region-specific differences; excellent coverage of this subject is provided by Mitra *et al.* (48). Estrogen can act on the brain at diverse targets and exert a broad range of actions, and this finding is at least in part explained by this broad distribution of ERs in the brain. For example, along with its feedback actions on the hypothalamus and preoptic area, estrogen also plays roles in neuroprotection, locomotor activity, mood, memory, and cognition, and these latter effects are largely mediated by nonhypothalamic/preoptic regions (38, 49, 50).

The ERβ is also abundantly expressed in limbic-hypothalamic regions involved in reproduction of rodents, including those in which ERα is expressed. Examples of regions in rat and mouse expressing ERβ, that also express ERα, include the BST, medial preoptic area, MPN, medial amygdala, and AVPV (Fig. 1; Refs. 46, 48, 51, 52). However, ERβ is also expressed in moderate to high levels in other hypothalamic and preoptic regions in which ERα is absent or in very low abundance, such as the diagonal band of Broca, supraoptic area, and paraventricular nucleus (46, 48, 52, 53). In brain regions that are not associated with reproduction, ERβ is expressed most abundantly in hippocampus, olfactory regions, spinal cord, cerebellum, substantia nigra, ventral tegmental area, dorsal Raphe, and locus coeruleus (46–48, 52, 54, 55). Therefore, in the rodent brain, there are regions where effects of estrogen are mediated by only one of the ERs because of lack of expression of the other, whereas in other brain regions, either or both ERs can mediate these effects because both receptors are expressed. The regions in which both ERα and ERβ are expressed, particularly AVPV, BST, medial amygdala, medial preoptic area, and MPN, are critically involved in reproductive physiology and behavior. In fact, ERα and ERβ have been shown to be co-expressed within a high proportion of the same cells in these latter regions, ranging from 25% of ERα-containing cells also expressing ERβ in preoptic nuclei to 95% in the BST (56, 57).

Although estrogens can bind to both ERs, they do so with different affinities, with the ERα having a higher affinity for estradiol than does the ERβ (58). Thus, although circulating estrogen can enter the entire brain, its effects are determined by which of the two ERs is expressed in a particular brain region, the cellular co-factors that can interact with these ERs, and the binding to the estrogen response element that enables gene transcription. All of these actions enable estrogen's signals to be differentially transduced in the brain.

Table 1. Effects of Aging and Hormone Replacement on Neuroendocrine Outcomes in Ovariectomized Female Rats[a]

Strain[b]	Age at OVX[c]	Post-OVX duration	Estrogen replacement[d]	Serum E concentrations (pg/ml)[e]
L-E	4–6 months (Y) 23–30 months (O)	Immediately, 18 hrs, 7 days or 12 days	EB (0, 0.1, 0.5, 1, 5 mg/100 g) sc daily beginning Day 13 through Day 24 post-OVX	Not assayed
L-E	4–5 months (Y) 22–24 months (O)	Up to 10 weeks	In the 7th week post-OVX, give EB (0.5 mg/100 g) sc daily for 8 days	Not assayed
L-E	3–4 months (Y) 10–12 months (MA) 18–30 months (O)	Up to 27 days	EB (10 mg/100 g) twice, 3 days apart (Day 1 and Day 3)	After 2nd EB at 1400 hrs: Y: 473 MA: ~480 O: 422
L-E	4 months (Y) 18 months (O)	Immediately (Experiment 1)	EB 8 mg/100 g sc	O OVX: 4.5 O OVX + EB: 24–38 Y OVX + EB: 60
	Same as above	5 weeks later (Experiment 2)	17β-E_2 (50% in 3 mm Silastic) every 2 weeks	
Wistar	3–4 months (Y) 18–20 months (O)	3 weeks	EB (1 mg) sc	Not assayed
S-D	3–4 months (Y) 9–12 months (MA)	1 week	17β-E_2 capsule (150 mg/ml) for 4 days	Y: 14–18 MA: 19–23
S-D	2–4 months (Y) 8–11 months (MA)	3–4 weeks	EB (4 mg/100 g) sc	Not assayed
S-D	3–4 months (Y) 21–24 months (O)	3–4 weeks	EB (25 mg/kg) sc daily for 3 days	Not assayed
S-D	2–4 months (Y) 8–11 months (MA)	3–4 weeks	EB (4 mg/100 g) sc 2 days later	Y OVX + E: 34 MA OVX + E: 39
S-D	4–5 months (Y) 12–14 months (MA) 24–26 months (O)	1 month 1 or 6 months 1 or 6 months	17β-E_2 capsule (10%; 1, 1.5, or 2 cm) 2 days or 2 weeks	Y OVX: 10 Y OVX + E: 83 MA OVX: 11 MA OVX + E: 127 O OVX: 9 O OVX + E: 131

[a] Studies are presented chronologically by publication date.
[b] Abbreviations of rat strains: S-D, Sprague-Dawley; L-E, Long-Evans.
[c] Y, young; MA, middle-aged; O, old.
[d] EB, estradiol benzoate; sc, subcutaneous; OVX, ovariectomy/ovariectomized; 17β-E_2, 17β-estradiol.
[e] E, estrogen.
[f] LH, luteinizing hormone; FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone.

Effects of Aging on Nuclear ER Expression in the Brain

ERα. The expression and properties of hormone receptors in the brain can change during reproductive aging. Evidence for such age-related changes in the functions of nuclear ERs were first provided by reports using ligand-binding assays to compare effects of estrogen binding in young and aging rats. One group reported that aged female rats have decreased levels of estrogen binding in nuclear extracts of the preoptic area compared to young rats, but they found no differences in the amygdala, medial basal hypothalamus, or pituitary gland (59). By contrast, another laboratory found decreased estrogen binding in nuclear extracts from hypothalamus, preoptic area, and pituitary (60). This age difference is region-specific: Decreased binding between middle-aged and old female rats was detected in the medial preoptic area, VMN, ARC, and pituitary gland (61). These three studies are in agreement on

Table 1. (Extended)

Strain[b]	Progesterone replacement	Serum P concentrations (ng/ml)	Neuroendocrine outcome[f]
L-E	None	Not assayed	The post-OVX increase in LH occurs more rapidly in young than old rats. The percent decrease in LH is similar, but the absolute decrease is greater in young than old rats. After cessation of daily EB, the increase in LH is much greater in young than old rats (27).
L-E	None	Not assayed	The post-OVX increase in LH is much greater in young than old rats. The percent decrease in LH post-EB injections is also greater in young rats, as is the rate of recovery (28).
L-E	0.5 mg/100 g on Day 4	After 2nd EB at 1400 hrs: Y: 24 MA: ~28 O: 24	Post-OVX increase in LH is far slower in MA and old than young rats, even at 27 days post-OVX. The EB-induced LH surge on Day 4 after EB occurs in young but not MA or old rats; by Day 5 the surge occurs in young and MA rats, but not in old rats. The EB + P surge occurs on Day 4 in young and MA, not in old rats (29).
L-E	0.5 mg/100 g sc on 3 days later	Not assayed	Progesterone potentiates the EB-induced LH release more in young than old rats.
	0.5 mg/100 g sc on 3 days later		Similar results as Experiment 1, although the age difference is less pronounced (32).
Wistar	None	Not assayed	Plasma concentrations of LH are greater in young than old rats. 3 days of EB decreases LH in rats of both ages. There are no differences in concentrations of FSH (30).
S-D	None	Not assayed	Daily LH surges are greater in young than MA rats (33).
S-D	0.8 mg/100 g sc	Not assayed	GnRH is higher in young than middle-aged rats, as measured by push-pull perfusion. 2 days after EB, the LH surge is much greater in young than MA rats (81).
S-D	None	Not assayed	Plasma LH concentrations are greater in young than old rats. EB decreases LH in young but not old rats (31).
S-D	0.8 mg/100 g sc	Not assayed	The percentage of GnRH neurons that co-express Fos, and the magnitude of the LH surge, are much greater in young than MA rats (5).
S-D	None	Not assayed	2 weeks of E decreases LH concentrations more in young and MA than old rats. The post-OVX increase in LH is greater in young and MA than old rats. There is no effect of the post-OVX interval on GnRH mRNA levels, and no effect of E on GnRH mRNA levels (9).

a loss of estrogen binding in preoptic nuclei with aging, but they disagree on results in basal hypothalamus, making interpretation difficult and highlighting the need for an additional approach to the question of hormone receptor changes during aging.

Several research groups have addressed the question of changes in ERs in the brain during aging using *in situ* hybridization histochemistry of ER mRNA levels (Table 2). The results of these studies have suggested little or no age-related change in ERα mRNA levels in the preoptic area and hypothalamus (62–65). Nevertheless, it is also important to measure the ER protein because protein levels may not parallel mRNA levels if there is a posttranscriptional or translational regulation, as has been demonstrated for other neuroendocrine molecules (66). To our knowledge, there have been only two quantitative stereologic analyses on effects of aging on ERα protein (Table 2). One study by Madeira *et al.* showed that ERα cell numbers do not change from 6 to 24 months in intact female Wistar rats in the MPN (67). A second study from our laboratory quantified ERα cell number in young, middle-aged, or old (3–4 months, 10–12 months, or 24–26 months, respectively) Sprague-Dawley rats that were OVX for 2–3 weeks and given 2 days of estrogen or vehicle replacement. A stereologic methodology was used in four neuroendocrine brain regions: the AVPV, MPN, ARC, and VMN (44). A representative example of ERα nuclear labeling in the AVPV is shown in Figure 2. In MPN and ARC, no age-related differences were detected,

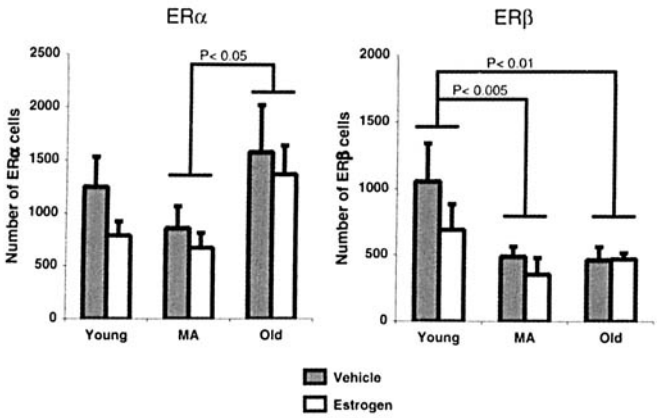

Figure 1. Age-related changes in estrogen receptor α (ERα, left) and estrogen receptor β (ERβ, right) in the anteroventral periventricular nucleus (AVPV) of female rats. ER-positive cells were counted by unbiased stereologic methodology in young (~4 months), middle-aged (MA; ~12 months), and old (~24 months) Sprague-Dawley rats (n = 4–8 rats per group). Animals were ovariectomized for 2–3 weeks and given vehicle (gray bars) or estradiol (white bars) replacement for 2 days. ERα cell number was significantly higher in old than middle-aged rats ($P < 0.05$). ERβ cell number was significantly lower in middle-aged and old than young rats ($P < 0.005$ and 0.01, respectively). No significant effects of estrogen were detected on either ERα or ERβ cell number. (Modified from Refs. 44 and 51).

and the result in MPN is consistent with the report by Madeira *et al.* in intact rats (67). In AVPV and VMN, we found age-related differences in ERα cell number: In AVPV, a significant increase in ERα cell numbers occurred from the middle-aged to the old group, whereas in VMN, a significant decrease from young to middle-aged, and then an increase from middle-aged to old, was detected (Fig.1; Ref. 44). These latter results differ from the mRNA studies that suggest little or no change in ERα mRNA in hypothalamus and POA. Our results indicate that not only does the ERα protein continue to be expressed with aging, but it can even increase the number of cells in which it is expressed. Moreover, ERα protein expression does not necessarily parallel gene expression, suggesting a posttranscriptional or translational mechanism of ERα biosynthesis.

ERβ. Using *in situ* hybridization histochemistry, Wise's laboratory showed no effect of age on ERβ mRNA in the periventricular preoptic nucleus, MPN, or paraventricular nuclei (Table 2; Ref. 65). They also reported a significant decrease in ERβ mRNA levels in the supraoptic nucleus between middle-aged and old OVX, estrogen-treated rats. In order to address this question at the level of the ERβ protein, our laboratory has utilized an unbiased stereological approach to study the effects of age on the number of ERβ-immunoreactive cells in the AVPV and principal nucleus of the bed nucleus of the stria terminalis (pBST; Table 2; Ref. 51). In that study, rats (young, 3–4 months; middle-aged, 10–12 months; or old, 24–26 months) were OVX for 2–3 weeks and given estrogen or vehicle for 2 days. A representative photomicrograph of ERβ protein expression in the AVPV is shown in Figure 2. In the pBST, no effect of age was observed on the number of ERβ-immunoreactive cells. However, in the AVPV, a significant decrease in the number of ERβ-immunoreactive cells occurred with aging (Figure 1). Specifically, ERβ cell numbers were significantly greater in young than middle-aged or old animals. This result is in contrast to our finding for ERα in the AVPV, where an age-related *increase* in ERα cell numbers was detected (Fig. 1; Ref. 44). Therefore, the two ERs undergo differential changes in expression during aging, with the likely net outcome of a differential response to estrogen in the aging brain.

Effects of Estrogen Replacement on Nuclear ER Expression in the Brain of Aging Rats

ERα. Effects of estrogen on the hypothalamic and preoptic ERα expression have been widely studied. These experiments have differed considerably in methodology, including postovariectomy interval, duration, dose, and mode of estrogen replacement, species, strain, age, and/or brain region. For the most part ERs have been quantified at the level of their mRNA using *in situ* hybridization, and the results have supported a down-regulation of ERα mRNA levels by estrogen treatment (e.g., 62–64, 68, 69). There are also several descriptive studies on regulation of the ERα protein by estrogen, showing that the ERα protein is down-regulated by estrogen in hypothalamic and preoptic nuclei of female rats (57, 70, 71). These studies have often relied on semiquantitative measurements of ERα staining intensity. The decrease in ERα is regionally specific and depends on the dose and duration of estrogen treatment.

Our group used an unbiased stereologic approach to quantify the regulation of ERα cell numbers by estrogen. We reported that the ERα cell number in Sprague-Dawley rats (young, middle-aged, or old) that were OVX for 3–4 weeks is not significantly altered following 2 days of estrogen compared to vehicle treatment in AVPV, MPN, arcuate nucleus, and VMN, regardless of age (Fig. 1; Table 2; Refs. 43, 44). Although this result challenges the dogma of the down-regulation of the ERα by estrogen, we believe that the finding is compelling and important. Because our study entailed a quantitative analysis at the level of the ERα nuclear protein, as opposed to its mRNA, we believe that the down-regulation of ERα mRNA by estrogen that has previously been reported (62–64, 68, 69) must be compensated by a posttranscriptional or translational change that allows ERα protein levels to be maintained. This result provides new insight into the regulation of ERα expression in the brain.

The results of our study on ERα cell number in the context of estrogen and aging together reveal that in general the effects of aging are greater than those of estrogen on the numbers of ERα-immunoreactive neurons. In AVPV, ERα cell numbers are higher in old than middle-aged rats, and in VMN, cell numbers are lowest in middle-aged rats (44). However, we did not find any significant effects of estrogen on ERα cell numbers in any brain region that we examined.

Table 2. Effects of Aging, Ovariectomy, and Estrogen Replacement on Neural Estrogen Receptors in Female Rats

Strain	Age at OVX	Post-OVX duration	Estrogen replacement	Serum E concentrations (pg/ml)	Effect on estrogen receptor
F344[a]	3 months (Y) 11 months (MA) 20 months (O)	Immediately	17β-E_2 capsule (50%; 7 mm), kill 2 weeks later	Y: 34 MA: 33 O: 36	ER mRNA in the POA is decreased by E, but not age (63).
F344	2 months (Y) 9 months (MA) 14 months (O)	10 days	17β-E_2 capsule (5%), kill 4 days later	Not assayed	ERα mRNA in VMN and ARC is decreased by E only in young rats; in POA in MA rats only. Induction of PR is similar in rats of all 3 ages (64).
Wistar	6 months (Y) 24 months (O)	None (Intact)	None	Not assayed	There is no effect of age on ERα cell number in the MPN (67).
S-D	3–4 months (Y) 11–12 months (MA) 19–24 months (O)	1 week	17β-E_2 capsule (180mg/ml), 20 (Y) or 30 (MA, O) cm for 2 days	Not assayed	ERβ is not altered by age in MPN,PVN, but is higher in young than MA or old rats in cortex and SON. ERα does not differ in MPN, ARC, VMN but is higher in young and MA than old rats in the PePOA (65).
S-D	3–4 months (Y) 10–12 months (MA) 24–26 months (O)	2–3 weeks	17β-E_2 capsule (10%; 1, 1.5, or 2 cm) for 2 days	Not assayed	E does not affect the number of ERβ cells in the AVPV; ERβ cell number decreases with aging (51).
S-D	3–4 months (Y) 10–12 months (MA) 24–26 months (O)	2–3 weeks	17β-E_2 capsule (10%; 1, 1.5, or 2 cm) for 2 days	Not assayed	ERα cell number increases with age in AVPV and VMN; there is no effect of E on ERα cell number (44).

[a] Studies are presented chronologically by publication date. Rat strains: S-D, Sprague-Dawley; F344, Fischer 344.
Other abbreviations: E, estrogen; MA, middle-aged; Y, young; O, old; OVX, ovariectomy/ovariectomized; LH, luteinizing hormone; GnRH, gonadotropin-releasing hormone; 17β-E_2, 17β-estradiol; ER, estrogen receptor; ARC, arcuate nucleus; AVPV, anteroventral periventricular nucleus; MPN, medial preoptic nucleus; POA, preoptic area; PePOA, periventricular preoptic area; PVN, paraventricular nucleus; SON, supraoptic nucleus; VMN, ventromedial nucleus.

Nevertheless, there were trends for a decrease in ERα cell number in response to estrogen in AVPV, MPN, and VMN, and this trend was greater for the young than the older animals. We feel that this point is important because the trend for estrogen to cause a small decrease in ERα cell number differs with aging, with older animals being less responsive to estrogen than younger animals. This result emphasizes the significance of studying effects of estrogen on the brain in age-appropriate models, as the young OVX rat is not identical to the old OVX rat in its response to estrogen replacement.

ERβ. We have also examined effects of estrogen replacement on ERβ cell number in OVX rats at different life stages (Table 2; Ref. 51). In the AVPV, as was the case for ERα, estrogen did not alter ERβ cell number in rats at any of the three ages examined (Fig. 1). We performed the same analysis in the pBST and also did not detect any

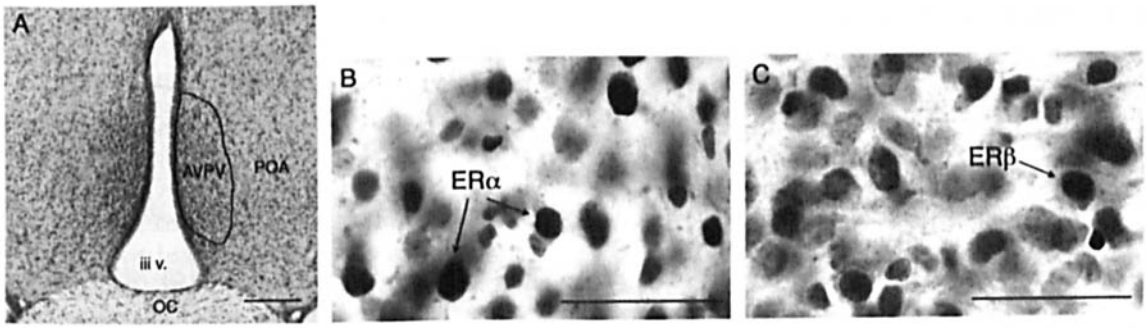

Figure 2. Photomicrographs of ERα (A and B) and ERβ (C) in AVPV of representative OVX rats. Panel A indicates the region of the AVPV at ×2.5. Panel B shows ERα labeling in the AVPV at ×63. ERα-immunopositive nuclei are seen in dark brown, as detected by DAB, and representative nuclei are indicated by arrows. Panel C shows ERβ-immunoreactive nuclei, labeled by DAB-peroxidase reaction, in the AVPV at ×63. A representative ERβ-positive nucleus is indicated by the arrow. All tissues were counterstained with cresyl violet. Abbreviations (panel A): AVPV, anteroventral periventricular nucleus; iii v., third ventricle; OC, optic chiasm; POA, preoptic area. Scale bars: A, 250 μm; B and C, 25 μm.

Table 3. Effects of Aging, Ovarian Status, and Estrogen Replacement on Neural Progesterone Receptors in Female Rats[a]

Strain	Age at OVX	Post-OVX duration	Estrogen replacement	Serum E concentrations (pg/ml)
S-D[b]	3–4 months (Y) 10–12 months (MA)	1 week	17β-E_2 capsule for 2 or 4 days	Y OVX: 3.0 Y 2 days E: 16.2 Y 4 days E: 15.5 MA OVX: 3.1 MA 2 days E: 18.8 MA 4 days E: 17.1
S-D	2.5 months (Y) 8–10 months (MA) 19 months (O)	17 days	17β-E_2 capsule for 3 days	Y: ~90 MA: ~120 O: ~125
F344	3 months (Y) 10 months (MA) 15 months (O)	10 days	17β-E_2 capsule for 4 days	Not assayed
L-E	2–3 months (Y) 10–12 months (MA, early PE) 13–15 months (MA, long-term PE)	None (Intact)	None	Not assayed

[a] Studies are presented chronologically by publication year.
[b] Abbreviations of rat strains: S-D, Sprague-Dawley; L-E, Long-Evans; F344, Fischer 344. Other abbreviations: E, estrogen; P, progesterone; sc, subcutaneously; MA, middle-aged; Y, young; O, old; OVX, ovariectomized; PE, persistent estrus; PR, progesterone receptor; BST, bed nucleus of the stria terminalis; POA, preoptic area; MBH, medial basal hypothalamus; VMN, ventromedial nucleus.

estrogen regulation of ERβ cell number (51). Because we found an age-related decrease in ERβ cell number in the AVPV but no effect of estrogen, we conclude that in this brain region, aging has greater effects on ERβ cell number than does estrogen replacement. This result is similar to our finding for ERα, for which we reported age-related changes but no significant effects of estrogen replacement (44). Again, this finding has consequences for models of hormone replacement in female rats and demonstrate the need to use experimental rodents that are at an older age.

The potential physiological outcomes of age-related changes in ERs and their differential regulation by estrogen are currently unknown. At the level of the ERα protein, the increase in ERα immunoreactive cell numbers that we see in AVPV and VMN with aging in OVX rats (44) may reflect a compensatory up-regulation in response to decreased positive and negative responsiveness to estrogen (27–29, 31–33). At the level of the ERβ protein, for which we have observed an age-related decrease in cell numbers in AVPV (51), this may serve to counteract the age-related change in ERα cell numbers or represent a differential sensitivity to both aging and estrogen between the two nuclear ERs. Clearly, future research is necessary to provide a better understanding of both the causes and consequences of changes in estrogen receptor numbers in the aging brain and their regulation by ovarian hormones.

Progesterone, the Progesterone Receptor, and Aging

The ovarian hormone progesterone, acting through the PR, plays a central role in female reproductive physiology. In rodents, progesterone levels fluctuate during the estrous cycle and are highest on proestrus and lowest on estrus (20, 72, 73). In rats with regular estrous cycles, the increase in progesterone on proestrus is delayed and attenuated in middle-aged compared to young rats (74). When rats make the transition from regular estrous cycles to acyclicity at middle-age, progesterone levels decrease compared to young cycling rats (20). It is noteworthy that persistent diestrus rats, which can be quite aged, have significantly elevated progesterone levels compared to much younger animals, indicating the continued capacity for progesterone biosynthesis even at advanced ages in female rats (20, 73).

The physiological and behavioral changes associated with progesterone are mediated by its nuclear receptor, which is expressed as two isoforms, PR-A and PR-B (75). In rats and guinea pigs, PR mRNA and immunoreactivity are most abundant in the arcuate nucleus, periventricular preoptic regions, MPN, medial preoptic area, and VMN (76, 77). In most cases, expression of the PR is regulated by estrogen preexposure (76, 78, 79).

Few studies have assessed changes in hypothalamic PR binding and gene expression during aging (Table 3). Wise and Parsons (80) showed that cytosolic PR binding is decreased in middle-aged compared to young OVX rats in the preoptic area and medial basal hypothalamus but not amygdala or pituitary, following 2 days of estradiol treatment (80). By contrast, another group reported no age difference in estradiol-induced PR induction, using binding assays (61). A study by Funabashi *et al.* using *in situ* hybridization to measure PR mRNA expression also reported no differences in the induction of PR mRNA by

Table 3. (Extended)

Strain	Progesterone replacement	Serum P concentrations (ng/ml)	Effect on progesterone receptor
S-D[b]	0.2 mg/kg sc on day 2 or 4	Y OVX: 0.6 Y 2 days E: 0.7 Y 4 days E: 0.9 MA OVX: 0.9 MA 2 days E: 0.9 MA 4 days E: 1.2	Estradiol (2 days) increases PR binding sites in POA and MBH more in young than middle-aged rats. There is no difference with 4 days of estradiol treatment (80).
S-D	None	Not assayed	There is no age difference in induction of PR binding by estradiol in VMN, arcuate nucleus, BST, periventricular preoptic area (61).
F344	None	Not assayed	Induction of PR mRNA (measured by *in situ* hybridization) does not differ among the three age groups in POA, VMN, arcuate nucleus (64).
L-E	None	Not assayed	PR mRNA levels are lower in long-term PE rats compared to early PE and young proestrous rats, in VMN and arcuate nucleus. PR mRNA levels are lower in both early and long-term PE rats compared to young rats. There is no age difference in medial preoptic nucleus (34).

estradiol in the VMN, arcuate nucleus, and preoptic area of OVX, estrogen-treated rats at any age (young, middle-aged, or old; Ref. 64). Differences among these results could be attributable to the measurement of PR binding as opposed to PR mRNA levels, and other technical differences in protocols. It is clear that further research on age-related changes in PRs and their regulation by estrogen will be a valuable addition to the literature.

A recent study by Mills *et al.* (34) tested whether changes in the ability of endogenous estradiol to induce the PR in the hypothalamus may be related to the transition to persistent estrus in middle-aged rats (Table 3). Using intact animals, that group found that PR mRNA levels were significantly lower only in long-term persistent-estrous middle-aged rats but not early persistent-estrous middle-aged rats compared to young proestrous rats in the VMN and ARC (34). In the AVPV, PR mRNA levels were significantly lower in both middle-aged groups compared to young rats. No effects of aging on PR mRNA were seen in the MPN. This latter study suggests region-specific changes in PR mRNA levels during the transition to reproductive senescence in female rats. Although these findings differ somewhat from those of Funabashi *et al.* (64), who did not observe an age-related change in PR mRNA, the two studies differ considerably in the ovarian status of the animals (intact versus OVX with estrogen priming).

Summary and Conclusions

Reproductive function in female mammals requires the precise regulation and coordination of the hypothalamus, pituitary, and ovary. There is increasing evidence that all three of these levels undergo age-related changes, some of which are occur independently, others of which occur in response to altered output from other levels. For example, changes in hypothalamic GnRH release with age will impact pituitary gonadotropin release and, subsequently, gonadal function. Similarly, a loss of ovarian follicles with age, and its consequent decline in estradiol, results in a decrease in the feedback actions of gonadal steroid hormones on the hypothalamus. Studies in rodents suggest that the aging brain may not respond in the same way as a young brain to effects of steroid hormones because of changes in the binding properties of the receptors, receptor numbers, or other functional aspects of these receptors.

The present review has discussed the concepts that (i) circulating hormone levels change during aging in female mammals; (ii) the expression of neural receptors that bind to these hormones is also subject to regulation with aging; and (iii) the consequences of these changes include the consideration that even the same levels of hormones may act differently on the brain of a younger individual compared with the brain of an older individual. These concepts have implications for the treatment of menopausal symptoms related to declining steroid hormone levels in women.

We thank Dr. Weiling Yin for helpful comments on the manuscript.

1. Gill S, Sharpless JL, Rado K, Hall JE. Evidence that GnRH decreases with gonadal steroid feedback but increases with age in postmenopausal women. J Clin Endocrinol Metab 87:2290–2296, 2002.
2. Hall JE, Lavoie HB, Marsh EE, Martin KA. Decrease in gonadotropin-releasing hormone (GnRH) pulse frequency with aging in postmenopausal women. J Clin Endocrinol Metab 85:1794–1800, 2000.
3. Reame NE, Kelch RP, Beitins IZ, Yu M-Y, Zawacki CM, Padmanabhan V. Age effects on follicle-stimulating hormone and pulsatile luteinizing hormone secretion across the menstrual cycle of premenopausal women. J Clin Endocrinol Metab 81:1512–1518, 1996.

4. Lloyd JM, Hoffman GE, Wise PM. Decline in immediate early gene expression in gonadotropin-releasing hormone neurons during proestrus in regularly cycling, middle-aged rats. Endocrinology 134:1800–1805, 1994.
5. Rubin BS, Lee CE, King JC. A reduced proportion of luteinizing hormone (LH)-releasing hormone neurons express Fos protein during the preovulatory or steroid-induced LH surge in middle-aged rats. Biol Reprod 51:1264–1272, 1994.
6. Sahu A, Kalra SP. Absence of increased neuropeptide Y neuronal activity before and during the luteinizing hormone (LH) surge may underlie the attenuated preovulatory LH surge in middle-aged rats. Endocrinology 139:696–702, 1998.
7. Miller BH, Gore AC. Alterations in hypothalamic IGF-I and its associations with GnRH neurons during reproductive development and aging. J Neuroendocrinol 13:728–736, 2001.
8. Miller BH, Gore AC. NMDA receptor subunit expression in gonadotropin-releasing hormone neurons changes during reproductive senescence in the female rat. Endocrinology 143:3568–3574, 2002.
9. Gore AC, Oung T, Woller MJ. Age-related changes in hypothalamic gonadotropin-releasing hormone (GnRH) and NMDA receptor gene expression and their regulation by estrogen in the female rat. J Neuroendocrinol 14:300–309, 2002.
10. Gore AC, Yeung G, Morrison JH, Oung T. Neuroendocrine aging in the female rat: The changing relationship of hypothalamic gonadotropin-releasing hormone neurons and N-methyl-D-aspartate receptors. Endocrinology 141:4757–4767, 2000.
11. Gore AC. Gonadotropin-releasing hormone neurons, NMDA receptors, and their regulation by steroid hormones across the reproductive life cycle. Brain Res Rev 37:235–248, 2001.
12. Gore AC, Windsor-Engnell BM, Terasawa E. Menopausal increases in pulsatile gonadotropin-releasing hormone (GnRH) release in a non-human primate (*Macaca mulatta*). Endocrinology 145:4653–4659, 2004.
13. Rossmanith WG, Handke-Vesel A, Wirth U, Scherbaum WA. Does the gonadotropin pulsatility of postmenopausal women represent the unrestrained hypothalamic-pituitary activity? Eur J Endocrinol 130:485–493, 1994.
14. Burger HG. The endocrinology of the menopause. Maturitas 23:129–136, 1996.
15. Gosden RG, Faddy MJ. Ovarian aging, follicular depletion, and steroidogenesis. Exp Gerontol 29:265–274, 1994.
16. Judd HL, Fournet N. Changes of ovarian hormonal function with aging. Exp Gerontol 29:285–298, 1994.
17. Wich BK, Carnes M. Menopause and the aging female reproductive system. Endocrinol Metab Clin North Am 24:273–295, 1995.
18. Woller MJ, Everson-Binotto G, Nichols E, Acheson A, Keen KL, Bowers CY, Terasawa E. Aging-related changes in release of growth hormone and luteinizing hormone in female rhesus monkeys. J Clin Endocrinol Metab 87:5160–5167, 2002.
19. Wise PM, Scarbrough K, Larson GH, Lloyd JM, Weiland NG, Chiu S. Neuroendocrine influences on aging of the female reproductive system. Front Neuroendocrinol 12:323–356, 1991.
20. Lu KH, Hopper BR, Vargo TM, Yen SSC. Chronological changes in sex steroid, gonadotropin and prolactin secretion in aging female rats displaying different reproductive states. Biol Reprod 21:193–203, 1979.
21. Huang HH, Meites J. Reproductive capacity of aging female rats. Neuroendocrinology 17:289–295, 1975.
22. LeFevre J, McClintock MK. Reproductive senescence in female rats: A longitudinal study of individual differences in estrous cycles and behavior. Biol Reprod 38:780–789, 1988.
23. Gore AC. Gonadotropin-releasing hormone (GnRH) neurons: gene expression and neuroanatomical studies. In: Parhar I, Ed. Progress in Brain Research. Amsterdam: Elsevier, pp 193–208, 2002.
24. Matt DW, Gilson MP, Sales TE, Krieg RJ, Kerbeshian MC, Veldhuis JD, Evans WS. Characterization of attenuated proestrous luteinizing hormone surges in middle-aged rats by deconvolution analysis. Biol Reprod 59:1477–1482, 1998.
25. Nelson JF. Puberty, gonadal steroids and fertility: potential reproductive markers of aging. Exp Gerontol 23:359–367, 1988.
26. Belisle S, Bellabarba D, Lehoux J-G. Hypothalamic-pituitary axis during reproductive aging in mice. Mech Ageing Dev 52:207–217, 1990.
27. Shaar CJ, Euker JS, Riegle GD, Meites J. Effects of castration and gonadal steroids on serum luteinizing hormone and prolactin in old and young rats. J Endocrinol 66:45–51, 1975.
28. Huang H-H, Marshall S, Meites J. Capacity of old versus young female rats to secrete LH, FSH and prolactin. Biol Reprod 14:538–543, 1976.
29. Steger RW, Huang HH, Chamberlain DS, Meites J. Changes in control of gonadotropin secretion in the transition period between regular cycles and constant estrus in aging female rats. Biol Reprod 22:595–603, 1980.
30. Steger RW, Sonntag WE, Peluso JJ, Meites J. Effects of ovariectomy and steroid replacement on hypothalamic LHRH content in aging female rats. Neurobiol Aging 4:53–57, 1983.
31. Hwang C, Pu H-F, Hwang C-Y, Liu J-Y, Yao H-C, Tung Y-F, Wang PS. Age-related differences in the release of luteinizing hormone and gonadotropin-releasing hormone in ovariectomized rats. Neuroendocrinology 52:127–132, 1990.
32. Lu JKH, Gilman DP, Meldrum DR, Judd HL, Sawyer CH. Relationship between circulating estrogens and the central mechanisms by which ovarian steroids stimulate luteinizing hormone secretion in aged and young female rats. Endocrinology 108:836–841, 1981.
33. Wise PM. Estradiol-induced daily luteinizing hormone and prolactin surges in young and middle-aged rats: Correlations with age-related changes in pituitary responsiveness and catecholamine turnover rates in microdissected brain areas. Endocrinology 115:801–809, 1984.
34. Mills RH, Romeo HE, Lu JKH, Micevych PE. Site-specific decrease of progesterone receptor mRNA expression in the hypothalamus of middle-aged persistently estrus rats. Brain Res 955:200–206, 2002.
35. Tsai H-W, Legan SJ. Chronic elevation of estradiol in young ovariectomized rats causes aging-like loss of steroid-induced luteinizing hormone surges. Biol Reprod 64:684–688, 2001.
36. Tsai H-W, Legan SJ. Loss of luteinizing hormone surges induced by chronic estradiol is associated with decreased activation of gonadotropin-releasing hormone neurons. Biol Reprod 66:1104–1110, 2002.
37. Legan SJ, Tsai H-W. Oestrogen receptor-α and -β immunoreactivity in gonadotropin-releasing hormone neurones after ovariectomy and chronic exposure to oestradiol. J Neuroendocrinol 15:1164–1170, 2003.
38. McEwen BS, Alves SE. Estrogen actions in the central nervous system. Endocrine Rev 20:279–307, 1999.
39. Falkenstein E, Tillmann H-C, Christ M, Feuring M, Wehling M. Multiple actions of steroid hormones—a focus on rapid, nongenomic effects. Pharmacol Rev 52:513–555, 2000.
40. Toran-Allerand CD, Guan X, MacLusky NJ, Horvath TL, Diano S, Singh M, Connolly ES Jr, Nethrapalli IS, Tinnikov AA. ER-X: A novel, plasma membrane-associated, putative estrogen receptor that is regulated during development and after ischemic brain injury. J Neurosci 22:8391–8401, 2002.
41. Simerly RB, Chang C, Muramatsu M, Swanson LW. Distribution of androgen and estrogen receptor mRNA-containing cells in the rat brain: An in situ hybridization study. J Comp Neurol 294:76–95, 1990.
42. Simonian SX, Spratt D, Herbison A. Identification and characterization of estrogen receptor α-containing neurons projecting to the vicinity of the gonadotropin-releasing hormone perikarya in the rostral preoptic area of the rat. J Comp Neurol 411:346–358, 1999.
43. Chakraborty TR, Ng L, Gore AC. Colocalization and hormone regulation of estrogen receptor alpha and NMDA receptor in the hypothalamus of female rats. Endocrinology 144:299–305, 2003.
44. Chakraborty TR, Hof PR, Ng L, Gore AC. Stereologic analysis of estrogen receptor alpha (ERα) expression in rat hypothalamus and its regulation by aging and estrogen. J Comp Neurol 466:409–421, 2003.

45. Yokosuka M, Okamura H, Hayashi S. Postnatal development and sex difference in neurons containing estrogen receptor-alpha immunoreactivity in the preoptic brain, the diencephalon, and the amygdala in the rat. J Comp Neurol 389:81–93, 1997.
46. Laflamme N, Nappi RE, Drolet G, Labrie C, Rivest S. Expression and neuropeptidergic characterization of estrogen receptors (ER-alpha and ER-beta) throughout the rat brain: anatomical evidence of distinct roles of each subtype. J Neurobiol 36:357–378, 1998.
47. Belcher SM. Regulated expression of estrogen receptor α and β mRNA in granule cells during development of the rat cerebellum. Dev Brain Res 115:57–69, 1999.
48. Mitra SW, Hoskin E, Yudkovitz J, Pear L, Wilkinson HA, Hayashi S, Pfaff DW, Ogawa S, Rohrer SP, Schaeffer JM, McEwen BS, Alves SE. Immunolocalization of estrogen receptor β in the mouse brain: Comparison with estrogen receptor α. Endocrinology 144:2055–2067, 2003.
49. Dubal DB, Wise PM. Neuroprotective effects of estradiol in middle-aged female rats. Endocrinology 142:43–48, 2001.
50. Shepherd JE. Effects of estrogen on cognition, mood, and degenerative brain diseases. J Am Pharm Assoc 41:221–228, 2001.
51. Chakraborty TR, Ng L, Gore AC. Age-related changes in estrogen receptor beta in rat hypothalamus: A quantitative analysis. Endocrinology 144:4164–4171, 2003.
52. Shughrue PJ, Lane MV, Merchenthaler I. Comparative distribution of estrogen receptor-alpha and -beta mRNA in the rat central nervous system. J Comp Neurol 388:507–525, 1997.
53. Salama J, Chakraborty TR, Ng L, Gore AC. Effects of polychlorinated biphenyls (PCBs) on female reproductive development and estrogen receptor β expression. Environ Health Perspect 111:1278–1282, 2003.
54. Orikasa C, McEwen BS, Hayashi H, Sakuma Y, Hayashi S. Estrogen receptor alpha, but not beta, is expressed in the interneurons of the hippocampus in prepubertal rats: an in situ hybridization study. Dev Brain Res 120:245–254, 2000.
55. Platania P, Laureanti F, Bellomo M, Giuffrida R, Giuffrida-Stella AM, Catania MV, Sortino MA. Differential expression of estrogen receptors alpha and beta in the spinal cord during postnatal development: localization in glial cells. Neuroendocrinol 77:334–340, 2003.
56. Shughrue PJ, Scrimo PJ, Merchenthaler I. Evidence for the colocalization of estrogen receptor-β mRNA and estrogen receptor-α immunoreactivity in neurons of the rat forebrain. Endocrinology 139:5267–5270, 1998.
57. Greco B, Allegretto EA, Tetel MJ, Blaustein JD. Coexpression of ERβ with ERα and progestin receptor proteins in the female rat forebrain: Effects of estradiol treatment. Endocrinology 142:5172–5181, 2001.
58. Kuiper GGJM, Lemmen JG, Carlsson B, Corton JC, Safe SH, Van der Saag PT, Van der Burg B, Gustafson J-A. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. Endocrinology 139:4252–4263, 1998.
59. Wise PM, Camp P. Changes in concentrations of estradiol nuclear receptors in the preoptic area, medial basal hypothalamus, amygdala, and pituitary gland of middle-aged and old cycling rats. Endocrinology 114:92–98, 1984.
60. Rubin BS, Fox TO, Bridges RS. Estrogen binding in nuclear and cytosolic extracts from brain and pituitary of middle-aged female rats. Brain Res 383:60–67, 1986.
61. Brown TJ, MacLusky NJ, Shanabrough M, Naftolin F. Comparison of age- and sex-related changes in cell nuclear estrogen-binding capacity and progestin receptor induction in the rat brain. Endocrinology 126:2965–2972, 1990.
62. Funabashi T, Kimura F. Effects of estrogen and estrogen receptor messenger RNA levels in young and middle-aged female rats: Comparison of medial preoptic area and mediobasal hypothalamus. Acta Biol Hung 45:2–4, 1994.
63. Miller MA, Kolb PE, Planas B, Raskind MA. Estrogen receptor and neurotensin/neuromedin-N gene expression in the preoptic area are unaltered with age in Fischer 344 female rats. Endocrinology 135:1986–1995, 1994.
64. Funabashi T, Kleopoulos SP, Brooks PJ, Kimura F, Pfaff DW, Shinohara K, Mobbs CV. Changes in estrogenic regulation of estrogen receptor α mRNA and progesterone receptor mRNA in the female rat hypothalamus during aging: an in situ hybridization study. Neurosci Res 38:85–92, 2000.
65. Wilson ME, Rosewell KL, Kashon ML, Shughrue PJ, Merchenthaler I, Wise PM. Age differentially influences estrogen receptor-α (ERα) and estrogen receptor-β (ERβ) gene expression in specific regions of the rat brain. Mech Ageing Dev 123:593–601, 2002.
66. Adams MM, Flagg RA, Gore AC. Perinatal changes in hypothalamic NMDA receptors and their relationship to GnRH neurons. Endocrinology 140:2288–2296, 1999.
67. Madeira MD, Andrade JP, Paula-Barbosa MM. Hypertrophy of the ageing rat medial preoptic nucleus. J Neurocytol 29:173–197, 2000.
68. Lauber AA, Romano GJ, Mobbs CV, Pfaff DW. Estradiol regulation of estrogen receptor messenger ribonucleic acid in rat mediobassal hypothalamus: An in situ hybridization study. J Neuroendocrinol 2:605–611, 1990.
69. Simerly RB, Young BJ. Regulation of estrogen receptor messenger ribonucleic acid in rat hypothalamus by sex steroid hormones. Mol Endocrinol 5:424–432, 1991.
70. Yuri K, Kawata M. The effect of estrogen on the estrogen receptor-immunoreactive cells in the rat medial preoptic nucleus. Brain Res 548:50–54, 1991.
71. Meredith JM, Auger CJ, Blaustein JD. Down-regulation of estrogen receptor immunoreactivity by 17β-estradiol in the guinea pig forebrain. J Neuroendocrinol 6:639–648, 1994.
72. Butcher RL, Collins WE, Fugo NW. Plasma concentration of LH, FSH, prolactin, progesterone, and estradiol-17β throughout the 4-day estrous cycle of the rat. Endocrinology 94:1704–1708, 1974.
73. Huang HH, Steger RW, Bruni JF, Meites J. Patterns of sex steroid and gonadotropin secretion in aging female rats. Endocrinology 103:1855–1859, 1978.
74. Wise PM. Alterations in the proestrous pattern of median eminence LHRH, serum LH, FSH, estradiol and progesterone concentrations in middle-aged rats. Life Sci 12:165–173, 1982.
75. Conneely OM, Mulac-Jericevic B, Lydon JP. Progesterone-dependent regulation of female reproductive activity by two distinct progesterone receptor isoforms. Steroids 68:771–778, 2003.
76. Blaustein JD, Turcotte JC. Estradiol-induced progestin receptor immunoreactivity is found only in estrogen receptor-immunoreactive cells in guinea pig brain. Neuroendocrinol 49:454–461, 1989.
77. Warembourg M, Logeat M, Milgrom E. Immunocytochemical localization of progesterone receptor in the guinea pig central nervous system. Brain Res 384:121–131, 1986.
78. Blaustein JD, King JC, Toft DO, Turcotte JC. Immunocytochemical localization of estrogen-induced progestin receptors in guinea pig brain. Brain Res 474:1–15, 1988.
79. Shughrue PJ, Lane MV, Merchenthaler I. Regulation of progesterone receptor messenger ribonucleic acid in the rat medial preoptic nucleus by estrogenic and antiestrogenic compounds: An in situ hybridization study. Endocrinology 138:5476–5484, 1997.
80. Wise PM, Parsons B. Nuclear estradiol and cytosol progestin receptor concentrations in the brain and the pituitary gland and sexual behavior in ovariectomized estradiol-treated middle-aged rats. Endocrinology 115:810–816, 1984.
81. Rubin BS, Bridges RS. Alterations in luteinizing hormone-releasing hormone release from the mediobasal hypothalamus of ovariectomized, steroid-primed middle-aged rats as measured by push–pull perfusion. Neuroendocrinology 49:225–232, 1989.