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Selective Estrogen Receptor Modulators: Tissue Actions and Potential for CNS Protection

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ABSTRACT

Significant physiologic changes occur during menopause. Evidence exists to suggest that estrogen may be neuroprotective under specific conditions. However, there are limitations in the neuroprotection afforded by standard hormone therapy. Accordingly, alternative agents with selected estrogenic effects may hold even greater promise rather than conventional hormone replacement therapy for the prevention and treatment of CNS injury. Recently, a variety of selective estrogen receptor modulators (SERMs) have been developed to retain the favorable and minimize the adverse side effects of estrogens. This review focuses on the CNS and known neuroprotective effects of two specific SERMs, raloxifene and arzoxifene. Recent studies hint that raloxifene and arzoxifene are neuroprotective and may preserve some elements of cognitive function. However, the mechanism of action is not well described and it is unclear if the beneficial effects of SERMs rely on activation of estrogen receptors.

INTRODUCTION

Menopause often signals the beginning of significant changes in a woman's health status. With growing life expectancy, women now spend a substantial number of years in a postmenopausal physiology associated with low levels of ovarian steroids. Since there is much evidence that female sex steroids protect women from atherosclerosis-related ischemic stroke and other CNS pathologies, potential benefits of hormone replacement therapy (HRT) are of increasing interest. 17β estradiol, the principal human estrogen, has been

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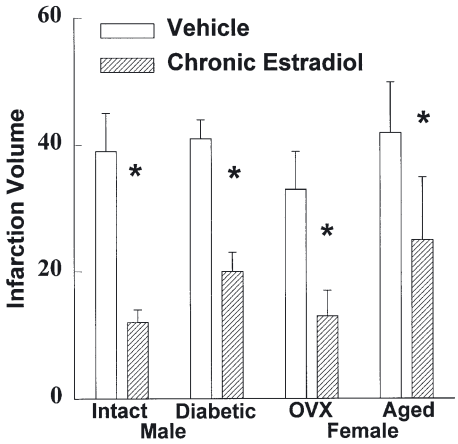

Fig. 1. Striatal infarction volume expressed as % of ipsilateral structure following MCAO in chronic estrogen-treated diabetic male (116,117), ovariectomized (98) and reproductively senescent (2) rats. Data are means ± S.E.M. (*$p \leq 0.05$).

widely shown to reduce brain injury in numerous animal models of ischemic and traumatic CNS injury (for reviews, see refs. 44,63). Early intriguing observations that estradiol is neuroprotective have been confirmed repeatedly, in diverse injury models, different animal strains, and even in the presence of genetic risk factors for CNS pathology such as hypertension (1) and diabetes (126,133). Both chronic estradiol treatment via subcutaneous implants or acute treatment with a one-time injection are efficacious in rodents. For example, steroid treatment prior to experimental middle cerebral artery occlusion reduces tissue damage in young ovariectomized (OVX) female, male, diabetic, and reproductively senescent female breeder rats (Fig. 1). A steadily enlarging literature emphasizes that estradiol likely acts via multiple cellular and molecular mechanisms. These mechanisms include preservation of cerebral blood flow, direct anti-oxidant activity by suppression of reactive oxygen species and preservation of endogenous anti-oxidants. They also include reduction of excitotoxicity, induction of neuroprotective gene products such as bcl2, suppression of glial cytokine production and reactive gliosis, anti-apoptotic action with maintenance of neuronal integrity, and enhancement of synaptic plasticity (for reviews see refs. 44,56,63,81,122,141).

However, there appears to be an increasing number of limitations in the neuroprotection offered by standard hormone therapy and whether the numerous effects of estradiol on the CNS translates into clinical benefit remains to be determined. For example, the therapeutic dose-response relationship may be narrow when prolonged treatment is employed in rat (107). In addition, clinical data obtained in HRT-treated women with developing CNS or cerebrovascular pathology have yet to confirm the promising results observed in animal studies. Recent data from the Women's Estrogen for Stroke Trial (WEST) indicate that 17β estradiol did not prevent cerebrovascular events in women with established cerebrovascular disease nor did it improve outcome from stroke (134). Estrogen replacement therapy using conjugated equine estrogen for 1 year also failed to slow disease progression or improve global cognitive functioning in postmenopausal women with mild to moderate Alzheimer's disease (87). Accordingly, alternative agents with selected estrogenic effects may hold even greater promise relative to conventional HRT for the prevention and treatment of CNS injury. The purpose of this paper is to review the

effect of selective estrogen receptor modulators (SERMs) in systems known to be affected by estrogen, and to discuss evidence suggesting that SERMs are neuroprotective.

SELECTIVE ESTROGEN RECEPTOR MODULATORS

In recent years several compounds possessing anti-estrogenic properties have been identified and have become important therapeutic adjuncts for estrogen responsive mammary tumors. Generally, these agents can be classified as either mixed estrogen agonists and antagonists or as pure anti-estrogens. However, pure anti-estrogens display none of estrogen's beneficial effects. Furthermore, administration of some mixed function anti-estrogens can produce untoward effects particularly on endometrial tissue. Subsequently, a variety of SERMs have been developed to retain the favorable, and minimize or eliminate the adverse effects of estrogen. Because these model agents should be estrogenic in relation to brain, bone, and lipids, yet display no effect on uterus or breast cancer cells, they are often referred to as "ideal" SERMs (32). Furthermore, these ideal SERMS should induce minimal side effects and not trigger drug resistance with prolonged use (112).

At the present time, SERMs belong to 4 structural classifications: triphenlyethylenes, benzothiophenes, benzopyrans, and naphthalenes. Tamoxifen, a triphenylethylene, is the prototypical first generation SERM. Several triphenylethylene derivatives of tamoxifen or its metabolic product 4-hydroxytamoxifen exist including: toremifene, droloxifene, clomiphene, and idoxifene. Tamoxifen, which has been widely employed in the treatment of breast cancer, has adverse effects on coagulation (32,40,42) and has been linked to an increased risk for endometrial cancer in women (8,59). Subsequently, newer SERMs have been developed to maximize the beneficial effects on bone preservation, serum cholesterol and lipids, while lacking effects on the uterus and potentially reducing the risk of breast cancer. The most widely studied of these agents is the benzothiophene, raloxifene (Evista). Other benzothiophene SERMs include raloxifene analogs such as LY117018 (6-hydroxy-2-(4-hydroxyphenyl) benzothiophene), and most recently arzoxifene (LY353381). Benzopyrans (levormeloxifene, acolbifene), and the naphthalenes (trioxifene and lasofoxifene) have also been recently formulated. Figure 2 shows examples of the chemical structures of each class of SERM.

Some of these newer SERMs, including arzoxifene, acolbifene (EM-652) and lasoxifene (CP336156), display increased bioavailability and/or potency and are currently in preclinical testing and/or in limited phase 2 clinical trials. However, most information related to the actions of SERMs has been obtained in studies using tamoxifen and raloxifene, since these two SERMs are currently in clinical use. Both tamoxifen and raloxifene are currently approved for chronic clinical use (tamoxifen for prevention and treatment of breast cancer in high-risk populations, and raloxifene for prevention and treatment of postmenopausal osteoporosis).

MECHANISM OF ACTION

Estrogen and SERMS most likely trigger multiple molecular/cellular signaling pathways that are still not completely characterized (Fig. 3). Estrogen binds to its cognate re-

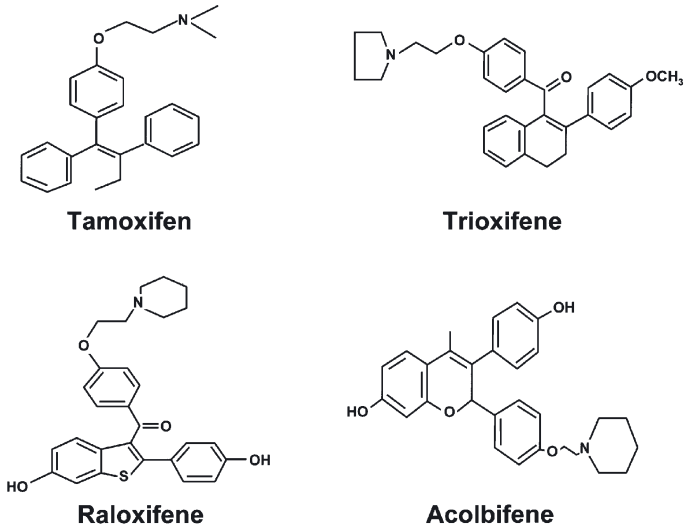

Fig. 2. Chemical structures of triphenylethylene (tamoxifen), benzothiophene (raloxifene), benzopyran (acolbifene) and naphthlene (trioxifene) classes of SERMs.

ceptor (ER), leading to conformational changes in the hormone-receptor complex. This leads to formation of receptor-ligand dimers that bind to a palindromic DNA sequence, the estrogen response element (ERE), on the promoter region to regulate estrogen-responsive gene transcription. Other proteins (co-activators or co-repressors) contribute to the conformational specificity of these complexes and their ability to regulate gene transcription. The anti-estrogenic actions of raloxifene and tamoxifen result, at least in part, from a competitive inhibition of estrogen binding to the ER, thus preventing the conformational changes necessary for the hormone-receptor complex to activate transcription of ERE-containing genes (76,151).

Composed of 6 functional domains, the ER has at least two, possibly three, sites necessary for activation following estrogen binding (86,130). Activating functional site-1 (AF-1) located at the amino terminus has been identified as a ligand-independent site, while AF-2 at the carboxy terminus is a key element necessary for ligand binding and subsequent transcription (32). The third activation site, labeled AF2a, has been identified near the AF-2 region (92). The structural variation in currently available SERMS is thought to uniquely modify the three dimensional receptor pocket conformation and alter the activation of either AF-1 or AF-2. It has been demonstrated that the alkyl aminoethoxy side chain of raloxifene blocks AF-2 leading to subsequent suppression of estrogen-responsive genes or co-regulatory proteins (18).

Estrogens and SERMs may achieve tissue specificity, by inducing different conformational changes at known receptor sub-types, by influencing dimerization, by complexing with cofactors and/or through genomic or non-genomic mechanisms (70,80,85,138). The recent identification of an additional ERE protein, the AP-1 site (activator protein-1) and its interaction with the ER_α or ER_β has shed some light on the mechanism underlying the differential effects of SERMS (61,96). Both tamoxifen and raloxifene interact with the AP-1 element associated with ER_β to stimulate transcription. However, transcription is in-

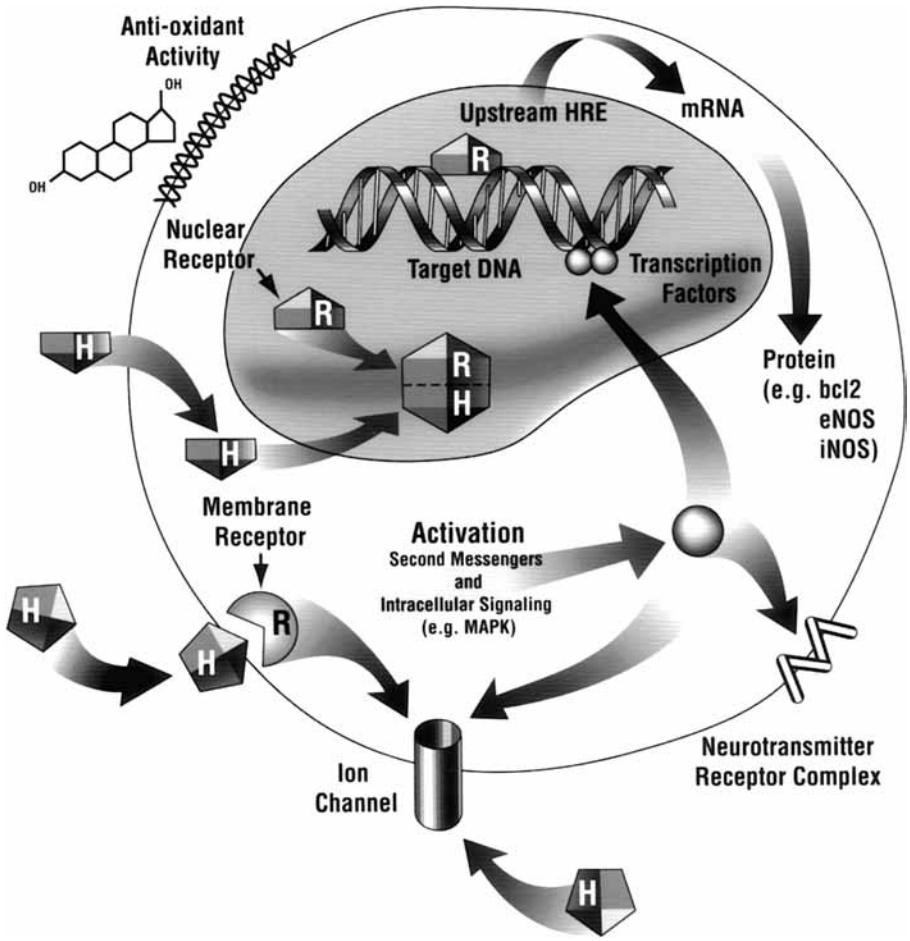

Fig. 3. Endogenous and exogenous estrogens act via multiple signaling pathways and neuroprotective mechanisms in target cells. These mechanisms include estrogen receptor-initiated transcriptional pathways in which the hormone binds to a nuclear receptor (RH) that binds to a hormone responsive element (HRE) and alters transcription of target genes. Examples of such genes include some members of the bcl2 family, endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthase (iNOS). Signaling may also occur through putative membrane receptors, with or without engaging kinase cascades such as mitogen-activated protein kinase (MAPK) or directly through ion channels or neurotransmitter complexes. Reproduced with permission from ref. 63.

hibited in the presence of ER_α (94). These data suggest that administration of tamoxifen and raloxifene are mediated by the ERE as well as the estrogen receptor sub-type (58).

TISSUE ACTIONS OF SERMs

By definition SERMs exert estrogenic effects on some tissues, while acting as anti-estrogens in other tissues. SERM targets include any tissues that possess estrogen receptors and may include breast, uterus, bone, liver, vasculature, and brain.

Breast

Solid data exist to indicate that both tamoxifen and raloxifene inhibit MCF-7 breast cancer cell proliferation *in vitro* (13,118), and inhibit mammary tumor cell xenografts in athymic mice (43). Tamoxifen decreases the risk of breast cancer in postmenopausal women at increased risk for breast cancer and is also effective in treating and reducing the recurrence of breast cancers (39,40). Raloxifene was associated with a decreased risk of breast cancer in postmenopausal women with osteoporosis (24). More recently, a third generation SERM, acolbifene, has been shown to rapidly arrest the growth of several neoplastic mammary cell lines including MCF-7, AR-75-1 and T-47D breast cancer cells *in vitro* (70). Data from a recent Phase 1 clinical trial suggest that arzoxifene effectively stabilizes the progression of metastatic breast carcinoma (88). Although, arzoxifene was cross-resistant with tamoxifen for treatment of tamoxifen-induced MCF7 tumors, it was highly effective in the reduction of T47D:17 tumor cell proliferation in the same mouse athymic model. Taken together, these data suggest that SERMs with the appropriate tissue selectivity profile may be valuable in the prevention or treatment of breast cancer.

Uterus

Tamoxifen causes estrogen-like stimulation of the uterus and is associated with endometrial hyperplasia. In addition, with chronic use, it increases the risk for development of endometrial cancer. In contrast to their similar effects on breast cancer cells as ER antagonists, tamoxifen and raloxifene differ in their profile in the uterus. Ethynyl estradiol and tamoxifen stimulate the endometrium and produce increases in uterine weight in rats, whereas raloxifene and the pure anti-estrogen ICI-164–384 [N-n-butyl-N-methyl-11-(3,17β-dihydroxyyoestra-1,3,5(10)-trien-7-α-yl) undecamide] do not (15). Both estrogen and tamoxifen stimulate endothelial cell height and eosinophil infiltration, but raloxifene does not (8). Histologic data confirm that, in contrast to women taking conjugated equine estrogens or tamoxifen, women taking raloxifene exhibit no uterine stimulation (23,29,34, 49). Treatment with tamoxifen increased uterine carcinomas after 1 year of exposure (38), whereas uterine cancer was not increased in women taking raloxifene for up to 4 years (20,24). Similar to raloxifene, arzoxifene has been reported to have negligible effects on uterine epithelia and does not increase uterine weight (109) in ovariectomized rat. In addition, women taking arzoxifene as part of a phase 1 study demonstrated marginal uterine growth as measured by changes in endometrial thickening (88).

Bone

Some of the most powerful actions of SERMs are on the skeletal system. Tamoxifen, raloxifine, arzoxifene, aclobifene, and lasofoxifene all preserve bone mass in ovariectomized rat, an important function of native estrogens (68,70,73,110). A decreased rate of bone reabsorption occurs with tamoxifen treated estrogen-deficient states. Tamoxifen reduces bone reabsorption in ovariectomized rats (129) and preserves bone, as measured by bone mineral density, in healthy postmenopausal women and in breast cancer patients (57,128). However, in premenopausal women, who have higher circulating estrogen levels, tamoxifen engenders loss of bone density (54,102). This result serves as evidence for a direct estrogen-antagonist effect. The cellular and molecular mechanisms underlying the agonist and partial agonist activities continue to be explored (see ref. 76 for review).

Raloxifene, currently approved for the prevention and treatment of osteoporosis in post menopausal women, produces even stronger beneficial effects on bone than tamoxifen. In fact, raloxifene preserves bone in a manner similar to estrogens (31,60) and reduces vertebral fractures in postmenopausal women (35). In preclinical studies, both raloxifene and tamoxifen produce similar anti-osteopenic effects on the distal femur metaphysis in ovariectomized rats, with maximal activity comparable to orally available ethynyl estradiol (15). Most preclinical studies employ ovariectomized animals to examine SERM effectiveness in bone mass preservation. However, a recent report described the arrest of age-related changes in trabecular density, bone strength, and the occurrence of osteoporetic fractures in aged male rats (68) following chronic treatment with lasofoxifene. The results of this study provide the basis for yet another potential clinical use for SERMS. Two of the more recent SERMs, arzoxifene and acolbifene, display a more robust effect than either tamoxifen or raloxifene in the prevention of bone demineralization. Both agents strongly preserve both bone density and trabecular bone volume (70,110) following ovariectomy.

Cardiovascular Effects

SERMS preserve estrogen's beneficial effect on circulating cholesterol and low density lipoprotein levels (LDL). Several studies show tamoxifen (21,73,76) and raloxifene display estrogenic effects on lipid metabolism. Raloxifene lowers serum cholesterol in ovariectomized rat by approximately 60–70% and has a similar, but less robust, effect in healthy postmenopausal women (15,31,67,135). A recent 2-year placebo-controlled study of healthy postmenopausal women demonstrated that raloxifene reduces several known plasma risk factors for cardiovascular disease relative to controls, including cholesterol, LDL and fibrinogen levels (132). Promising pre-clinical data suggest that arzoxifene is even more effective than raloxifene in the long-term reduction of plasma lipids (110).

Estrogen-like favorable effects on cardiovascular risk have also been shown with raloxifene treatment and include: improving vascular endothelial function in animal models (37,103) and postmenopausal women (110), lowering circulating levels of vascular adhesion molecules (12) and of homocysteine (136). Unlike estrogen, raloxifene does not increase serum levels of the systemic inflammatory marker, C-reactive protein (136), nor does it increase matrix metaloproteinase-9, an enzyme thought to be involved in atherosclerotic plaque rupture (9). Together, these effects form the basis for speculation that raloxifene and other SERMs may preserve cardiovascular health in postmenopausal women.

Nevertheless, hormone replacement therapy (HRT) on cardiovascular events in postmenopausal women remains in challenge. In contrast to data from observational studies, the Heart and Estrogen/Progestin Replacement Study (HERS) failed to show significant benefit of HRT on the risk of coronary events in postmenopausal women with established coronary artery disease, and suggested an early, transient increase in risk (62). Likewise, the Women's Estrogen for Stroke Trial (WEST) failed to show benefit of 17β-estradiol treatment on prevention of recurrent stroke in postmenopausal women, who had previously sustained stroke or transient ischemic attack (134). Whether SERMs reduce risk of coronary heart disease (CHD) events in postmenopausal women has not yet been determined. Some, but not all, reports suggest that tamoxifen used to treat women with breast cancer is also associated with a lower risk of cardiovascular events. The effect of raloxifene on risk of CHD has been studied in the Multiple Outcomes of Raloxifene Evalu-

ation (MORE) osteoporosis treatment trial. Unlike HERS, there was no evidence that raloxifene altered primary or secondary prevention of CHD (6). At 4 years, the risk of cardiovascular events in the placebo and the raloxifene groups was not significantly different in the total study cohort. However, the risk was significantly reduced by 40% among women at high-risk for cardiovascular events (6). Also among these high-risk women, raloxifene was associated with a 62% decrease in the likelihood of fatal or nonfatal stroke (6). As MORE was not designed to determine the effect of raloxifene on cardiovascular outcomes, further studies are required for confirm these findings.

Hormone replacement therapy and SERMs may also alter vasomotor function. HRT is frequently prescribed for the symptomatic treatment of hot flashes, but the underlying mechanism by which estrogen depletion modulates vasomotor function remains unclear. Women who report the occurrence of hot flashes in the year preceding cessation of menstrual flow have lower plasma estrogen and lower follicle stimulating hormone levels than those without vasomotor symptoms (95). A recent study demonstrated that estrogen replacement following natural or surgical menopause enhances endothelium-dependent arterial vasodilation (77). Furthermore, frequent episodes of transient vasodilation are a common side effect of anti-estrogen therapy for breast cancer. Like tamoxifen (39), raloxifene has been shown to trigger, hot flashes (29), but they are qualitatively milder (110). In the MORE trial, few women elected to discontinue treatment as a result of hot flashes (7).

RALOXIFENE AND ARZOXIFENE PROFILES IN BRAIN

Raloxifene

Raloxifene's unique pharmacological profile distinguishes it from both estrogen and tamoxifen. Structurally a benzothiophene, it possesses phenolic groups and an aminoethoxy side chain (18). Raloxifene's phenolic groups are known to interact with the identical amino acids to which estrogen binds, possibly conferring its high affinity for both ER_α and ER_β (18,48,49). The estrogen receptor binding characteristics of raloxifene closely mimic those of 17β estradiol (49). Approximately 60% of orally administered raloxifene is absorbed from the gastrointestinal tract. The reported bioavailability is 2% (53) and the plasma half-life is 27.7 h (53).

Recent evidence indicates that in addition to blocking activation of the ERE, raloxifene may use DNA response elements/pathways that are distinct from those used by estrogen to regulate gene transcription. Transforming growth factor β_3 ($TGF\beta_3$) is a non-ERE-containing gene that is abundant in bone matrix protein. Its expression is potently stimulated, *in vitro*, by raloxifene and estrogen metabolites through the ER (150). In an *in vitro*, cell-based, transcriptional reporter assay, raloxifene induced $TGF\beta_3$ expression through actions at a novel DNA response element. Thus, some of the agonist activities of raloxifene on bone, serum cholesterol and the central nervous system (CNS) may be mediated through transcriptional activation triggered by response elements distinct from the ERE.

Due to the multiplicity of mechanisms by which estrogens and SERMs affect cellular function, it is likely that different cell populations may respond differently. This is particularly important in attempts to clarify the effects of SERMs in the brain. The brain, which consists of numerous interconnected phenotypically different cell populations, requires a systematic region and cell group specific evaluation of SERM pharmacology.

Brain penetration

The ability of raloxifene to penetrate the blood brain barrier is not well established. While the amounts of raloxifene entering the brain are small, relative to the pituitary, in vivo assays confirm that these concentrations are sufficient to produce pharmacological effects in animals. In fact, 7 days of treatment with raloxifene enhanced ER_β mRNA levels in the amygdala of ovariectomized rats. This demonstrates that the drug crosses the blood brain barrier in sufficient quantities to alter estrogen receptor expression (153). Furthermore, it has recently been observed that raloxifene alters brain activation patterns detected by functional magnetic resonance imaging during memory task in postmenopausal women (89). This result suggests that raloxifene enters the brain in relevant quantities and exerts a measurable effect in humans as well.

Neurotransmitter receptor effects

It is likely that estrogens can modulate regional neurotransmitter receptor binding and density in brain with potential effects on cognition, behavior and learning. Estrogen depletion and repletion reportedly affects amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), N-methyl-D-aspartate (NMDA) and 5-hydroxytrympamine-2α ($5\text{-}HT_{2A}$) receptor binding in rodent brain, particularly in frontal cortex, striatum and nucleus accumbens (25–28). Minimal data are available regarding raloxifene's regional estrogenic effect on brain neurotransmitter receptors or behavioral outcomes. However, a series of recent studies by Cyr and colleagues (25–28) have elucidated raloxifene's effects on receptors located in rodent brain primarily in cortex, striatum and nucleus accumbens. These regions of the brain are associated with cognition, emotion and memory and may be affected both in Alzheimer's disease and in clinical depression in humans. Across these studies it is apparent that raloxifene produces an effect similar to that seen with estrogen. Audioradiographic binding studies of [3H]AMPA confirmed that raloxifene or chronic estradiol treatment depresses binding at the receptor in a comparable manner (28). Follow-up studies by Cyr and associates (28) examining the effect of estradiol or raloxifene treatment on L-[^{3}H]glutamate and NMDA receptor binding following ovariectomy extended these earlier findings. Treatment with estrogen or raloxifene triggered parallel drops in glutamate binding in the cingulate cortex, lowered NMDA receptor specific binding in striatum and nucleus accumbens, but preserved NMDA specific binding in the hippocampus (28). Further, raloxifene mimics the effect of estrogen on $5\text{-}HT_{2A}$ receptors after ovariectomy. Raloxifene increases mRNA for $5\text{-}HT_{2A}$ receptors, and preserves receptor density in cingulate and frontal cortex, as well as in striatum and nucleus accumbens (26). Compared to tamoxifene, brains of raloxifene-treated animals displayed better preservation of receptor expression and mRNA (26). Taken together these data indicate that raloxifene serves primarily as an estrogen agonist in several brain regions.

Estrogen treatment of ovariectomized rats increases the transcription of genes and proteins expressed in the brain, including trkA (tyrosine receptor kinase A), brain-derived neurotrophic factor (BDNF), and choline acetyltransferase activity (ChAT). These factors are thought to play an important role in the regulation of growth and integrity of neurons, including those that synthesize acetylcholine. Cholinergic transmission in the hippocampus and cortex plays an important role in cognition and degeneration of cholinergic neurons and is an important feature of the neurodegenerative processes in Alzheimer's disease. Raloxifene functions as an estrogen agonist on these genes and proteins, sug-

gesting that raloxifene may mimic some of estrogen's putative trophic effects in the CNS. For example, choline acetyltransferase is a rate-limiting enzyme involved in the synthesis of acetylcholine. Ovariectomy decreases ChAT activity while estrogen enhances ChAT expression within basal forebrain cholinergic neurons, resulting in an increase in cholinergic activity (75). This increase in rat brain ChAT is dose- and treatment duration-dependent (47). In ovariectomized rats (2 to 3 months old), hippocampal, but not hypothalamic, ChAT activity levels decrease significantly (up to 50%) compared to intact estrogen-competent controls. Treatment with estradiol benzoate for 3 or 10 days restores enzyme activity in the hippocampus to levels seen in intact females. Similarly, 3 to 10 days of raloxifene treatment restores hippocampal ChAT levels without changing hypothalamic ChAT (145,146). The ED50 for raloxifene is about 2.4 mg/kg and plateaus at around 5 mg/kg, about 10 times the effective dose required in rats to preserve bone (108). The difference in potency may reflect the relative brain penetration into basal forebrain that is well protected by the blood brain barrier. Importantly, the combination of raloxifene and estradiol benzoate also restores hippocampal ChAT activity to levels in ovary-intact controls.

Hypothalamic pituitary gonadal axis effects

Estrogen is an important regulator of the hypothalamic-pituitary-gonadal axis. The pituitary is outside the blood brain barrier and accumulates high levels of radiolabeled raloxifene. Furthermore, raloxifene blocks estrogen-induced increases in the weight of the pituitary gland (22) in ovariectomized rat, suggesting anti-estrogen actions. Estrogen stimulates growth hormone (GH) synthesis and release from isolated pituitary cells (78,114) while raloxifene antagonizes this estrogen-stimulated GH release from somatotrophs (78,114). Recent data support the finding that raloxifene has strong estrogenic effects in the female neonatal rat brain. In the neonatal rat brain raloxifene acts like estrogen to foster hypothalamic differentiation (101). Female neonatal rats treated with raloxifene 1 to 5 days after birth displayed a fall in serum gonadatrophins and a rise in prolactin concentrations. This was accompanied by a loss of positive feedback between estradiol and lutenizing hormone (LH) identical to animals treated with estrogen postnatally (100). The effects of raloxifene on serum LH in adults are somewhat less clear. In vitro, short-term (4 h) exposure to estrogen suppressed gonadotrophin releasing hormone (GnRH-)-stimulated release of LH in rat anterior pituitary cells, whereas long-term exposure (24 h) increased LH release. *In vitro*, raloxifene, alone, had no effect on the GnRH-stimulated release, but blocked both the effects of short- and long-term estrogen on release (5,93,94). *In vivo*, raloxifene appears to block estrogen's feedback inhibition of LH release, however, this is not a consistent finding. For example, in ovariectomized female rats LH levels rise and estrogen suppresses LH through feedback inhibition. Similarly, in ovariectomized rats treated with estrogen and raloxifene, estrogen feedback inhibition of LH was antagonized, but this was time-of-day dependent (i.e., it was observed during the morning, but not during the afternoon) (22). Importantly, while raloxifene antagonizes estrogen's actions on the pituitary in animal models, its effects remain to be established in women (see ref. 33 for effects in males).

In the hypothalamic-pituitary-gonadal axis, raloxifene appears to display a combination of estrogen agonist and antagonist effects. Antagonist activity in hypothalamus is suggested by the finding that ventromedial nucleus hypothalamic implants of crystalline raloxifene fail to elicit lordosis behavior. In addition the implants reduce lordosis quotients by about 50% in estrogen/progesterone primed OVX female rats, similar to effects

produced by tamoxifen (83). Furthermore, the raloxifene analog, LY117018, upregulates hypothalamic ER mRNA levels (14) indicative of agonist-like effects on ER in hypothalamus. In contrast to estrogen, raloxifene does not stimulate expression of the progesterone receptor in hypothalamic tissue (14). The neuroactive compound allopregnanolone, normally present in rat hypothalamus, hippocampus and pituitary, is reduced by ovariectomy and restored by 17β estradiol or LY117018. However, co-administration of 17β estradiol and LY117018 reversed the return of normal allopregnanolone levels in rats treated with estradiol or LY117018 alone (45,41). These dual effects of the drug illustrate raloxifene's central nervous system actions may depend on basal tissue estrogen levels. Furthermore, raloxifene may demonstrate estrogen agonistic properties at the level of the pituitary since ovariectomized, hypophysectomized animals display loss of the drug's bone preservation effects and blunt the hypocholesteremic effect (16,108). Therefore, raloxifene may involve direct effects at the level of the pituitary, on pituitary-hypothalamic feedback systems or a combination of both.

Cognitive function

The hippocampus, which is implicated in learning and memory, responds to estrogen by increasing dendritic spine densities in the CA1 region (80,142–144) and modulates excitatory and inhibitory input to pyramidal hippocampal cells (144). LY117018 does not produce similar increases in spine density nor does it block estrogen's effects in CA1 (80,81). Interestingly, however, recent *in vitro* tests demonstrate that PC12 cells stimulated with NGF express both ER_α and ER_β and respond to estrogen with an increase in the number of cells that only express ER_α. Raloxifene promoted much more neurite outgrowth than estradiol, and co-administration of estrogen plus raloxifene resulted in a significant increase in the average length of neurites relative to that observed with either estrogen or raloxifene alone (91). The finding that raloxifene stimulates neurite outgrowth in cells that express both ER_α and ER_β, but not ER_β alone, suggests that there may be a critical role for the ER_α in mediating the nerve-growth effects. Alternatively, the presence of both receptor subtypes in the same cell may be important to mediate activity. The additive effects of raloxifene and estradiol on neurite outgrowth suggest the possibility that they may be acting through distinct pathways.

Cognitive function in postmenopausal women

Estrogens have been proposed to improve cognitive function, including verbal memory and memory retrieval (80,116,117,140). Furthermore, conjugated estrogens have been shown to have a neurotrophic effect on neurons in rat hippocampus, basal forebrain, and parietal and frontal cortex, areas associated with learning and memory (10,11). However, randomized controlled trials in healthy postmenopausal women have not consistently demonstrated such an effect (72,147,148). Raloxifene mimics estrogen in the cholinergic system and increases BDNF and nerve growth factor receptors in a manner similar to estrogens and may very well influence cognitive status. To date, few studies have addressed raloxifene's effects on cognition in women. However, based on the results of the MORE trial (7478 postmenopausal women), raloxifene apparently displays a relatively minor effects related to cognition and mood (147). The women, randomly assigned to placebo or raloxifene (60 and 120 mg), were treated for 3 years and were tested for cognitive function at initiation of treatment, at 6 months, and 1, 2, and 3 years. No significant

differences were observed between groups. However, the investigators noted slight positive trends in measures of verbal memory and attention (147). These results were consistent with a similar earlier study in postmenopausal women taking raloxifene that produced a small, but significant, improvement in verbal memory scores (90). Improvements in cognitive function with raloxifene were also reported in 2 smaller studies, one enrolling older postmenopausal women without Alzheimer's disease (131) and the other involving postmenopausal women with Alzheimer's disease (50).

Nerve growth factors and neurotrophic factors

TrkA is one of the two components of the nerve growth factor NGF receptor. NGF contributes to neuronal integrity and is thought to be a significant factor maintaining survival of cholinergic neurons. The estrogen-induced increase in ChAT activity described above is associated with a decrease in hippocampal NGF synthesis and release that, with time, reduces NGF receptors on cholinergic neurons and ChAT activity back to basal levels (46,47). These and other effects are consistent with the proposal that estrogen provides trophic support to cholinergic neurons through the trkA-NGF pathway (82). It has been suggested that estrogens regulate neuronal growth by co-opting NGF's growth stimulatory pathway, and this may be the mechanism underlying estrogen's effects on neuronal growth *in vivo* and *in vitro* (125). Regardless of which pathway is involved, estrogens reverse the decrease in trkA mRNA levels in the rat nucleus basalis of Meynert following ovariectomy (82). Similar to estrogen, LY117018 upregulates estrogen deficient rat hippocampal, hypothalamic and to a lesser extent, cortical trkA mRNA (N. Ostrowski, personal communication). Speculation that the benzothiophene SERMs may be potent stimulators of the NGF pathway is consistent with observations that raloxifene induces neurite outgrowth more effectively than estradiol in NGF-responsive PC12 cells (91).

BDNF, a neurotrophin associated with neuronal plasticity and memory, binds to estrogen receptor-ligand complexes (121) in rat cerebral cortex and olfactory bulb. Further, estrogen depletion is associated with significant reduction of BDNF mRNA in rat cortex and hippocampus (115). In the cortical projection fields of basal forebrain neurons in ovariectomized rats raloxifene produces an estrogen-like elevation of BDNF mRNA, detected by *in situ* hybridization histochemistry. A similar increase is observed when estrogen plus raloxifene are combined (Fig. 4), indicating that raloxifene functions as a full estrogen agonist on BDNF induction.

Neuroprotective effects

Several lines of evidence support the hypothesis that estrogen administration following experimental stroke or global ischemia (1,64,79,97,137) is neuroprotective. In contrast, results of the recent WEST trial indicate that unopposed estrogen does not improve outcome in women started on estrogen following stroke or transient ischemic attack (134). Accumulating data reveal that raloxifene elicits both estrogen antagonist and estrogen agonist activity in brain. However, few studies address the effects of raloxifene relative to neuroprotection. Data from two studies of 1-methyl-4-phenyl-1,2,3,6-tetrahydro-pyridine (MPTP) lesioned male mice provide evidence that raloxifene has distinct neuroprotective actions similar to those of estradiol and progesterone (19,55) following a cytoxic brain insult. MPTP, a dopaminergic neurotoxin, elicits dopamine depletion, mimicking the deficit associated with Parkinson's disease (19). Five days of raloxifene pretreatment pre-

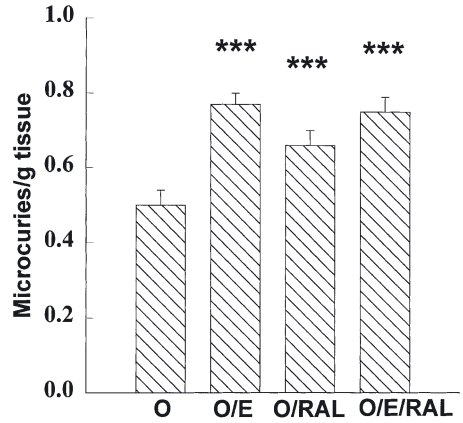

Fig. 4. Ovariectomized rats were injected with 1 mg/kg of raloxifene, 0.3 mg/kg of estradiol benzoate or both for 14 days. The brains were harvested and processed for *in situ* hybridization histochemistry using a ^{14}C-labeled riboprobe complementary to BDNF mRNA. Cortical BDNF gene expression was increased in all groups relative to the untreated ovariectomized rats ($n = 7$). Data are means ± S.E.M. (***$p \leq 0.001$ compared to OVX animals).

vented striatal dopamine depletion (55) and degeneration of dopaminergic uptake sites (19). In addition, Callier and associates showed that the protective action of raloxifene is dose-dependent as 5 mg/kg, but not 1 mg/kg, was effective (19). In contrast, raloxifene failed to reverse delayed neuronal cell death in the CA1 region of the hippocampus, even at doses of 10 or 20 mg/kg in a gerbil model of transient global ischemia (30). Based on the results of these available reports it is likely that neuroprotective benefits of raloxifene are highly dependent on target cells, selected brain regions or type and duration of neuronal damage. Table 1 summarizes the beneficial effects observed with raloxifene.

Arzoxifene (LY353381)

Arzoxifene exhibits estrogenic effects resembling those of raloxifene, but has better bioavailability (109). A methoxy side group replaces a 4′-hydroxyl group of raloxifene, enhancing its oral availability (123). The methoxy substitute not only improves the oral availability, but may also permit arzoxifene's metabolism to its powerful precursor, des-

TABLE 1. Beneficial effects of raloxifene

Central Nervous System Effects	General Effects
• Modulation of AMPA, NMDA, 5-HT receptor binding and density (25–28)	• Inhibition of breast cancer cell growth (24,38,39,118)
• Restoration of hippocampal ChAT levels after ovariectomy (145,146)	• Preservation of bone mass and density (15,31,560)
• Up-regulation of hypothalamic ER mRNA (13)	• Reduction in vertebral fracture (35)
• Amplification of neurite outgrowth when co-administered with estrogen (91)	• Reduction of plasma cholesterol, LDL and fibrinogen (15,67,135,136)
• Modest improvement in verbal memory in postmenopausal women (90,147)	• Reduction of plasma adhesion molecules (12)
• Positive effects on neuronal growth factors (91)	• Improvement of vascular endothelial health (37,103,111)
• Prevention of striatal dopamine depletion following experimental cytoxic brain injury (19,55)	

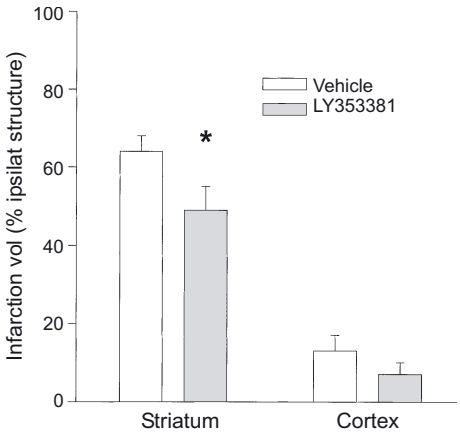

Fig. 5. Striatal and cortical infarction volume expressed as % of the ipsilateral structure in arzoxifene (n = 16) or vehicle-treated (n = 14) ovariectomized rats. Data are means ± S.E.M. (*$p \leq 0.05$). Reproduced with permission from ref. 105.

methylarzoxifene (71,123); in humans it has an average half-life of approximately 31 to 46 h (34,88) irrespective of dose. Available data indicate that arzoxifene more potently protects bone from osteoporosis than raloxifene (109,110), yet has virtually no effect on uterine growth (109,123). In a phase I clinical trial of women with metastatic breast cancer, arzoxifene reportedly provoked few toxic side effects and had no adverse effects on liver or kidney function (88). However, even at the lowest dose (10 mg/day) arzoxifene produced hot flashes. This effect is consistent with the reports of mild hot flashes in women treated with raloxifene (51,84) and likely represents anti-estrogenic effects on vasomotion. The pharmacokinetics of arzoxifene in a small sample of healthy women (33) is reportedly linear over dose and time. Currently, arzoxifene is in preclinical testing and in ongoing phase II clinical trials; only one study is published on the effects of arzoxifene on the CNS.

Unlike with raloxifene, no data are currently published regarding the actions of arzoxifene on neurotransmitter/receptors, synaptogenesis or neurotropic factors. However, one recent pre-clinical study suggests that pretreatment with 10 mg/kg of azoxifene provides some degree of neuroprotection in an ovariectomized rat focal stroke model (105). Five to eight days pretreatment with oral arzoxifene significantly reduced ischemic infarction volume in the caudoputamen during early reperfusion (Fig. 5). However, this protection did not extend to the cortex. End-ischemic regional cerebral blood flow was not altered by arzoxifene as evaluated by quantitative autoradiography (105). Interestingly, chronic pretreatment with estrogen in this same model reduced infarct in both cortex and caudoputamen (1). These data indicate that arzoxifene crosses the blood brain barrier in sufficiently high concentrations to confer protection from cerebral ischemia. However, differential neuroprotective potential may exist between arzoxifene and estrogen possibly via interaction with estrogen receptor subtypes in the cortex.

SUMMARY

The available data do not permit definitive conclusions, but suggest that SERMs are neuroprotective and may preserve some elements of cognitive function. However, there is

a paucity of data regarding the mechanism of action of SERMs. It remains to be elucidated whether their neuroprotective effects require direct activation of estrogen receptors. Evidence exists that raloxifene and arzoxifene cross the blood brain barrier in sufficient amounts to achieve a biological effect. However, the therapeutic benefit of SERMs in the brain could be either estrogenic or anti-estrogenic and is likely dependent on the targeted cell type and brain region.

Raloxifene increases neurotransmitter receptor binding in brain areas associated with cognition and memory and increases transcription and protein levels of neurotrophic factors such as TrkA, BDNF, and ChAT in rats. It has little effect on hippocampal synaptic density *in vivo*, but strongly provokes neurite outgrowth in PC12 cells. It is unclear whether this effect on neurite outgrowth in culture is clinically significant, but suggests that SERMs can activate the NGF pathways. Clinically, raloxifene appears to produce a beneficial effect on cognitive function as it elicits a modest improvement in verbal memory and attention in postmenopausal women with and without Alzheimer's disease.

Few studies address the neuroprotective actions of SERMs following brain injury. Raloxifene protects the striatum after cytotoxic insult akin to the damage associated with Parkinson's disease, but has no effect after a global ischemic insult. Conversely, arzoxifene reduces caudoputamen infarct size following experimental focal stroke. These divergent results likely illustrate that beneficial effects of SERMs following CNS injury are highly dependent on the type, location and duration of the insult.

FUTURE DIRECTIONS IN SERM DEVELOPMENT

As the number of postmenopausal women increases globally, the prevalence of diseases of aging associated with menopause and estrogen deficiency will continue to rise, as will the medical need for effective strategies to prevent and treat these diseases. Selective estrogen receptor modulators offer the possibility of providing the benefits of estrogens to postmenopausal health with fewer risks. Several compounds with the pharmacological profile of a SERM are currently in widespread clinical use, either as fertility drugs or for the prevention and treatment of breast cancer. The clinical indication for most of these, including tamoxifen, toremifene, and clomiphene, is related solely to their ability to antagonize estrogen activity and its receptor. To date, raloxifene is the only SERM to be used clinically as an estrogen agonist for the treatment and prevention of osteoporosis. However, the clinical indications of SERMs are likely to expand in the future.

Future research involving SERMs will likely focus on maximizing the efficacy profile across the spectrum of postmenopausal diseases while optimizing their safety and tolerability. For example, a SERM that retains efficacy in the bone and serum lipids, does not increase the risk of breast or endometrial cancer, and is devoid of side effects associated with current SERMs (mainly venous thromboembolism and hot flashes) would be desirable. The "ideal" SERM has been described as one that is not associated with an increased risk of events that are serious or affect quality of life with prolonged therapy, and yet has beneficial effects on outcomes associated with osteoporosis, cardiovascular disease, breast cancer, cognitive function, and menopausal symptoms (3). Whether a single SERM with this pharmacological profile can be identified remains uncertain. Future research should enhance our understanding of the structure, function, mechanisms and tissue distribution of different ER subtypes, and provide a fuller understanding of how

SERMs interact with and modulate the function of the ER subtypes. With this knowledge, it may be possible to develop future SERMs with receptor and/or tissue selectivity that are optimized for one or more disease states associated with postmenopausal women, and yet retain the optimal safety profile described above. Such developments could open the door for more individualized SERM prevention or treatment regimens for postmenopausal women to address their specific health concerns.

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