Estrogen receptor-mediated neuroprotection: The role of the Alzheimer's disease-related gene *seladin-1*

Alessandro Peri
Mario Serio

Department of Clinical Physiopathology, Endocrine Unit, Center for Research, Transfer and High Education on Chronic, Inflammatory, Degenerative and Neoplastic Disorders for the Development of Novel Therapies (DENOThe), University of Florence, Florence, Italy

Abstract: Experimental evidence supports a protective role of estrogen in the brain. According to the fact that Alzheimer's disease (AD) is more common in postmenopausal women, estrogen treatment has been proposed. However, there is no general consensus on the beneficial effect of estrogen or selective estrogen receptor modulators in preventing or treating AD. It has to be said that several factors may markedly affect the efficacy of the treatment. A few years ago, the *seladin-1* gene (for selective Alzheimer's disease indicator-1) has been isolated and found to be down-regulated in brain regions affected by AD. *Seladin-1* has been found to be identical to the gene encoding the enzyme 3-beta-hydroxysterol delta-24-reductase, involved in the cholesterol biosynthetic pathway, which confers protection against β-amyloid-mediated toxicity and from oxidative stress, and is an effective inhibitor of caspase-3 activity, a key mediator of apoptosis. Interestingly, we found earlier that the expression of this gene is up-regulated by estrogen. Furthermore, our very recent data support the hypothesis that *seladin-1* is a mediator of the neuroprotective effects of estrogen. This review will summarize the current knowledge regarding the neuroprotective effects of *seladin-1* and the relationship between this protein and estrogen.

Keywords: *seladin-1*, DHCR24, estrogen, brain, Alzheimer's disease

Introduction

Epidemiological data, together with experimental and clinical evidence, appear to support a neuroprotective role of estrogen, Accordingly, hormonal therapy in Alzheimer's disease (AD), the most prevalent neurodegenerative disorder in the elderly, has been suggested. However, there is no general consensus on this issue, which will be briefly summarized in this review. A debated question concerns the factors that act as downstream mediators of estrogen receptor activation in the brain. The identification of the *seladin-1* gene and the finding that it protects the brain from toxic insults led us to hypothesize that this gene might represent the link between estrogen and neuroprotection. *Seladin-1* was found to be identical to the gene encoding the enzyme 3-beta-hydroxysterol delta-24-reductase involved in the cholesterol biosynthetic pathway. Cell cholesterol content appears to play an important role in protecting neuronal cells from toxic insults. This review will address the current knowledge on the neuroprotective effects of *seladin-1* and the relationship between this protein and estrogen.

Correspondence: Alessandro Peri
Endocrine Unit, Department of Clinical Physiopathology, University of Florence, Viale Pieraccini, 6, 50139 Florence, Italy
Tel + 39 55 4271 366
Fax + 39 55 4271 372
Email a.peri@dfc.unifi.it

Estrogen receptor-mediated neuroprotection

This topic has been extensively reviewed by many authors and the literature will be briefly summarized in this review. It is well known, based on *in vitro* evidence, that estrogen exerts neurotrophic and neuroprotective effects by stimulating the expression of neurotrophins and cell-survival factors, enhancing synaptic plasticity, and acting

as an antioxidant factor (Behl 2003; Maggi et al 2004; Turgeon et al 2006). In addition to the hypothalamus, which is the traditional site of estrogen action in the brain, both the estrogen receptor α (ERα) and β (ERβ) have been found in different brain areas such as the neocortex and the hippocampus, two areas highly involved in AD (Behl 2003). AD is the most prevalent form of late-life mental failure in humans (Selkoe 2001) and it has been calculated that every 72 seconds someone in the United States develops this disease. However, unfortunately there is still no reliable way of preventing or curing this disease. Experimental evidence supports a favorable estrogen effect in neurons, in agreement with the knowledge that AD is more common in women and that decreased estrogen levels after menopause are a risk factor for the disease (Paganini-Hill and Henderson 1994). Thus, estrogen therapy has been considered a rationale option for the treatment of this disease. To date, despite the lack of general consensus, several studies indicated that estrogen treatment may decrease the risk or delay the onset of AD in postmenopausal women (Fillit 2002). Conversely, the data from the Women's Health Initiative Memory Study (WHIMS) trial showed that hormone replacement therapy (HRT) has no benefit (Rapp et al 2003; Shumaker et al 2003). However, it has to be remembered that different factors may determine the efficacy of estrogen or HRT, such as age, the menopausal status, the route of administration and the dose, the starting cognitive function, and the presence of pre-existing risk factors (ie, smoking, apolipoprotein E genotype) (MacLusky 2004; Turgeon et al 2006). In particular, there seems to be a critical time for estrogen treatment. In fact, early and prolonged therapy has been found to produce the maximum benefit in terms of reduced risk for AD (Resnick and Henderson 2002; Zandi et al 2002). In addition, estrogen therapy is not the same as HRT and the type of progestogen used may determine the outcome of the therapeutic intervention (Schumacher et al 2007) (Figure 1).

With regard to the ER involved in neuroprotection, the observations from ERα (ERKO) and ERβ (βERKO) mice

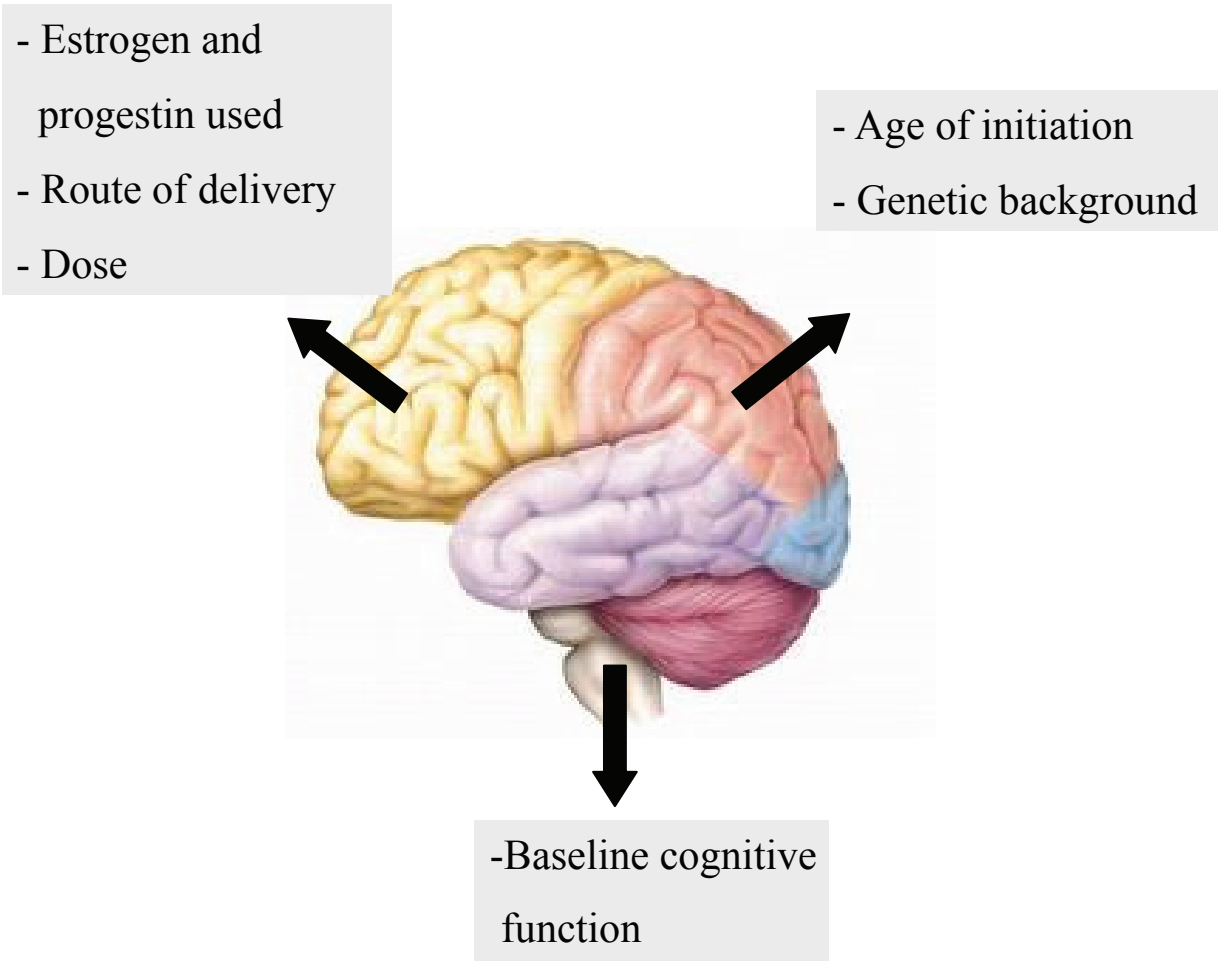

Figure 1 Factors affecting the response of the brain to hormone replacement therapy.

suggest a critical role for ERα. In fact, whereas 17β-estradiol exerted a protective effect in the brain of ovariectomized βERKO mice, it did not in ERKO mice (Dubal et al 2001). This finding appears in agreement with the reported decreased expression of ERα in hippocampal neurons of AD patients (Hu et al 2003). However, a possible role of ERβ in neuroprotection has been postulated, based on the evidence that βERKO mice undergo increased neuronal loss throughout life compared to wild-type controls (Wang et al 2001). It has to be said that, in addition to classical nuclear ERs, more recent findings suggest that the brain contains a plethora of ERs, such as ERγ and a variety of nuclear as well as cytoplasmic and plasma membrane receptors (Hawkins et al 2000; Toran-Allerand 2004; Vasudevan and Pfaff 2007; Arbogast 2008).

The neuroprotective role of the selective estrogen receptor modulators (SERMs) has been less extensively investigated. Nonetheless, a neuroprotective effect of tamoxifen and raloxifene has been observed (Dhandapani and Brann 2002) and a beneficial role of tamoxifen and raloxifene against β-amyloid toxicity has been demonstrated in rat neurons (O'Neill et al 2004a, 2004b). There is increasing evidence that SERMs may also be neurotrophic, by increasing for instance synaptic density and stimulating neurite outgrowth (Dhandapani and Brann 2002). Data regarding the clinical use of SERMs in AD are very limited, so far. However, the Multiple Outcomes of Raloxifene Evaluation trial evaluated the cognitive function in more than 5000 women with osteoporosis assigned to receive raloxifene (60 mg or 120 mg) or placebo daily for three years. Compared to those taking placebo, women receiving 120 mg/day of raloxifene had a 33% lower risk of mild cognitive impairment and somewhat lower risks of Alzheimer's disease and any cognitive impairment (Yaffe et al 2005).

In summary, the basic science strongly supports a neuroprotective role of estrogen/SERMs. Although there is no clear cut evidence yet that these molecules can decrease the risk or ameliorate the clinical course of AD, it is conceivable that there might be a proper space for a hormonal-based intervention in this disease. Undoubtedly, a more profound knowledge of the molecular mechanisms by which ERs activation determines neuroprotective effects may further support this conclusion.

The identification and characterization of *seladin-1*

The *seladin-1* gene was first identified by Greeve and colleagues (2000), who used a differential mRNA display approach to identify genes that were differentially expressed in selective vulnerable brain regions in AD, such as the hippocampus, the amygdala, the inferior temporal cortex, and the enthorhinal cortex (Selkoe 2001). Among the >30 genes differentially expressed in AD vulnerable brain regions versus unaffected areas, Greeve and colleagues (2000) identified a novel cDNA showing a markedly reduced expression in the inferior temporal cortex of AD patients compared to the frontal cortex, obtained shortly postmortem. Conversely, this cDNA was evenly expressed in the brain of unaffected individuals. This gene was named *seladin-1* from selective Alzheimer's disease indicator-1 and its full-length cDNA was cloned from a human brain cDNA library (GenBank accession number AF261758). The *seladin-1* gene spans 46.4 kb, maps to chromosome 1p31.1–p33, and comprises nine exons and eight introns; it encodes an open reading frame of 516 amino acid residues. To localize the cellular distribution of *seladin-1*, human H4 neuroglioma cells were transfected with a *seladin-1*-green fluorescent protein (GFP) fusion construct. *Seladin-1* appeared to be mainly located in the endoplasmic reticulum and, although to a lesser extent, in the Golgi apparatus. A subsequent study demonstrated that the down-regulation of *seladin-1* expression in vulnerable AD brain areas is paralleled by an increase in the amount of hyperhosphorylated tau, a protein component of neurofibrillary tangles (Iivonen et al 2002). Apart the brain, *seladin-1* mRNA has been also detected in many different human organs and the highest levels of expression have been found in the adrenal gland, the liver, the lung and the prostate.

With regard to its biological effects, *seladin-1* was found to confer resistance against β-amyloid and oxidative stress-induced apoptosis and to effectively inhibit the activation of caspase-3, a key mediator of the apoptotic process. However, another study indicated that this multifaced protein may have a more complex role in cell apoptosis. In fact, following oncogenic and oxidative stress, *seladin-1* was found to bind P53 amino terminus domain and to displace E3 ubiquitin ligase Mdm2 from P53, thus resulting in P53 accumulation (Wu et al 2004).

Seladin-1 was found to have also an enzymatic activity involved in cholesterol biosynthesis, which was found to be markedly reduced in desmosterolosis, a rare autosomal recessive disorder characterized by multiple congenital anomalies (Fitzpatrick et al 1998). In fact, patients with desmosterolosis have elevated plasma levels of the cholesterol precursor desmosterol and this abnormality suggested a deficiency of the enzyme 3-beta-hydroxysterol delta-24-reductase (DHCR24), which catalyzes the reduction of the Δ^{24} double

bond in desmosterol to produce cholesterol. Waterham and colleagues (2001) were able to identify the human *DHCR24* cDNA, which appeared identical to *seladin-1*. *DHCR24* activity was confirmed *in vitro* by enzymatic assay following heterologous expression of the *DHCR24* cDNA in *Saccharomyces cerevisiae*. Conversely, in constructs containing mutant *DHCR24* alleles from patients with desmosterolosis the conversion from desmosterol into cholesterol was nil or markedly reduced.

Desmosterolosis belongs to a group of several inherited disorders, linked to enzyme defects in the cholesterol biosynthetic pathway at the post-squalene level, which have been described in recent years (Herman 2003). These genetic diseases are characterized by major developmental malformations and in most cases determine severe neuropsycological alterations, suggesting an important role of cholesterol in brain homeostasis. The role of cholesterol in facilitating the onset and progression of AD is a debated and unsolved question. In fact, on one hand cholesterol may be viewed as a toxic factor. It has been reported that elevated cholesterol levels increase β-amyloid formation in *in vitro* systems and in most animal models of AD (Yanagisawa 2002; Puglielli et al 2003). The identification of the ε4 allelic variant of the apolipoprotein E as a major genetic risk factor for AD is also consistent with a role of cholesterol in the pathogenesis of this disease. Accordingly, epidemiological studies suggested that statin therapy may provide protection against AD, although the clinical benefit of statins might be also due to their cholesterol-independent effects on cerebral circulation and inflammation (Reiss et al 2004). This hypothesis appears to be substantiated by the fact that the majority of the commercially available statins does not cross the blood–brain barrier. On the other hand, it has to be considered that the central nervous system (CNS) is a very unique organ with regard to cholesterol metabolism: in fact, although the CNS accounts for only 2% of the entire body mass, it contains about 25% of the total amount of unesterified cholesterol in the entire body. In addition, most of the CNS cholesterol is produced via local *de novo* synthesis. Keeping this in mind, it is not surprising that several studies pointed out the fact that the intracellular content of cholesterol, particularly the amount contained in the cell membrane, should be addressed much more than the plasma levels (Yanagisawa 2002). In this new scenario, an appropriate amount of membrane cholesterol would create a barrier against toxic insults, whereas a cholesterol-depleted membrane would ease the interaction with toxic factors such as β-amyloid, which may generate for instance an anomalous number of calcium channels leading to the accumulation of toxic levels of calcium (Arispe and Doh 2002). In addition, in membranes from AD patients or in rodent hippocampal neurons with a moderate reduction of membrane cholesterol the interaction between the amyloidogenic enzyme β-secretase and amyloid precursor protein (APP) appeared to be facilitated, thus leading to elevated production of β-amyloid (Abad-Rodriguez et al 2004). These results suggest that loss of neuronal membrane cholesterol contributes both to increased membrane interaction with β-amyloid and to excessive amyloidogenesis in AD. Thus, the reduced expression of seladin-1 in AD vulnerable regions is in keeping with the "membrane integrity" theory.

Mice with a targeted disruption of the *DHCR24* gene have been generated (Wechsler et al 2003). As expected, plasma and tissues of DHCR24$^{-/-}$ mice contained virtually no cholesterol, whereas desmosterol accumulation was observed. These animals were around 25% smaller in size than *DHCR24*$^{+/+}$ and *DHCR24*$^{+/-}$ littermates at birth. In contrast to initial observations, it was subsequently demonstrated that these animals show a lethal dermopathy at birth and die within a few hours (Mirza et al 2006). This finding is in agreement with the severe phenotype observed in humans affected by desmosterolosis.

Seladin-1 as a new effector of ER-mediated neuroprotection

As mentioned previously, there is experimental evidence that estrogen and SERMs may confer neuroprotection. However, in most of the studies, that have been performed so far, animal models have been used. In other cases human cells, yet transformed or of neoplastic origin, have been used. Recently, we addressed the neuroprotective effects of estrogen/SERMs using a unique human cell model, ie, GnRH-secreting neuroblast long-term cell cultures from human fetal (8–12 weeks of gestational age) olfactory epithelium. These fetal neuroepithelial cells (FNC) were established, cloned and propagated previously by Vannelli and colleagues (1995) at the Department of Anatomy, Histology, and Forensic Medicine of the University of Florence, Italy. They show unique features, because they express both neuronal and olfactory markers that are typical of maturing olfactory receptor neurons (Vannelli et al 1995). FNC cells are electrically excitable and following exposure to a number of different aromatic chemicals show a specific increase in intracellular cAMP, indicating some degree of functional maturity. Thus, FNC cells appear to originate from the stem cell compartment that generates mature olfactory receptor neurons. In addition, they express both

ERα and β (Barni et al 1999). Therefore, they represented a suitable human *in vitro* model, that could be of help in i) providing further information on the role of estrogen in neurons, and in ii) answering the question whether *seladin-1* might be an effector of ERs activation.

Our findings confirmed the protective role of estrogen/SERMs in the brain. In fact, we observed that, whereas in the absence of estrogen pre-incubation β-amyloid (10 and 100 nM) and H_2O_2 (200 μM) significantly reduced cell viability, the pre-treatment with 17β-estradiol (100 pM–100 nM) effectively counteracted β-amyloid- or oxidative stress-induced toxicity (Benvenuti et al 2005). In agreement with estrogen, also the SERM tamoxifen (100 pM–100 nM) effectively protected FNC cells from the toxic effects of β-amyloid, whereas partially different results were observed using raloxifene. In fact, cell viability after exposure to β-amyloid was preserved at low concentrations of raloxifene (100 pM and 1 nM). Conversely, 10 and 100 nM did not exert protective effects (Figure 2). In addition, we demonstrated that the protective action of estrogen in FNC cells was associated to a counteracting effect against β-amyloid-induced apoptosis, as demonstrated by the strong inhibition of the activation of caspase-3.

Finally, in order to answer the question whether estrogen and/or SERMs have an effect on *seladin-1* expression, we evaluated the expression of *seladin-1* mRNA in FNC cells, treated or not with 17β-estradiol, tamoxifen or raloxifene, by quantitative real-time RT-PCR based on TaqMan technologies. We found that FNC cells constitutively express *seladin-1* (112 ± 2.26 fg/μg total RNA, mean ± SE). Noticeably, 17β-estradiol (10 pM–100 nM) significantly increased the amount of *seladin-1* mRNA. 1 nM tamoxifen or raloxifene determined a similar increase of *seladin-1* mRNA, compared to an equal concentration of

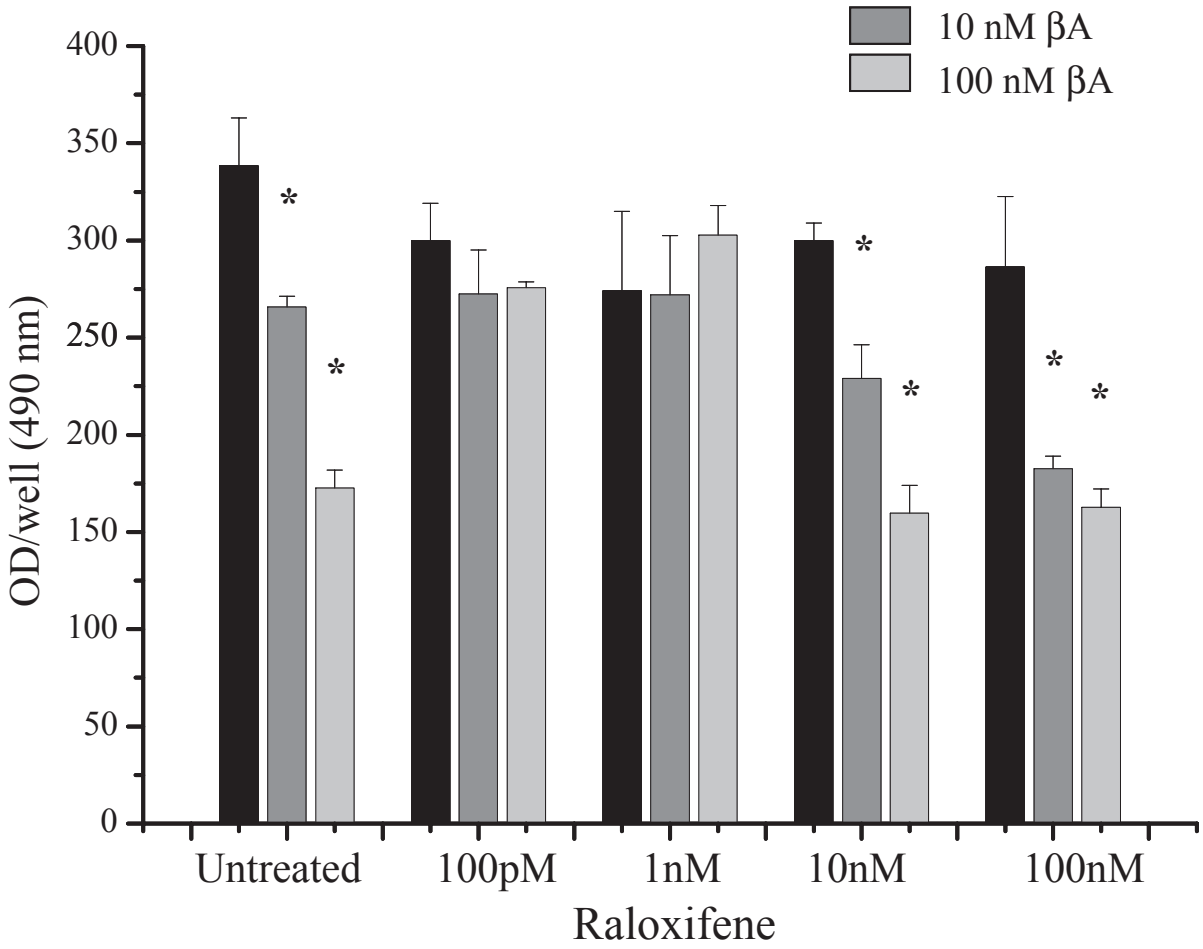

Figure 2 Effect of different concentrations of raloxifene on β-amyloid (βA) (10 and 100 nM) toxicity, as assessed by MTS assay (Promega Corp., Madison, WI).
Notes: *$p < 0.05$ versus control cells not exposed to β-amyloid (black bars). Modified with permission from Benvenuti S, Luciani P, Vannelli GB, et al 2005. Estrogen and SERMs exert neuroprotective effects and stimulate the expression of seladin-1, a recently discovered anti-apoptotic gene, in human neuroblast long-term cell cultures. *J Clin Endocrinol Metab*, 90:1775–82.

17β-estradiol. However, higher concentrations of raloxifene (10–100 nM) determined a marked reduction of *seladin-1* expression, in keeping with the observed lack of a neuroprotective effect at these concentrations (Figure 3). The effect of a selective ERα (propylpyrazole-triol, PPT) or a selective ERβ (diarylpropionitrile, DPN) agonist was also tested. We found that PPT determined a significant increase of the amount of *seladin-1* mRNA at a concentration (10 nM) which has been reported to induce evident transcriptional activity (Harrington et al 2003), whereas DPN produced a weaker effect. These additional findings suggested a predominant role of ERα in mediating the stimulatory effect of estrogen on *seladin-1* expression. In conclusion, this study led us to hypothesize that *seladin-1* might be a mediator of the neuroprotective effects of estrogen/SERMs. In particular, the parallelism between the concentrations of raloxifene that conferred neuroprotection on one hand, and stimulated *seladin-1* expression on the other hand, was highly predictive that this was true.

This hypothesis was supported by very recent additional findings. In fact, we demonstrated that, upon silencing *seladin-1* expression by small interfering RNA methodology, the protective effect against β-amyloid and oxidative stress toxicity exerted by 17β-estradiol was lost. Furthermore, a computer-assisted analysis revealed the presence of half-palindromic estrogen responsive elements (EREs) upstream the coding region of the *seladin-1* gene. A region spanning around 1500 bp was cloned in a luciferase reporter vector, which was transiently co-transfected with the estrogen receptor α in CHO cells. The exposure to 17β-estradiol, as well as to raloxifene and tamoxifen increased luciferase activity, thus suggesting a functional role for the half EREs of the *seladin-1* gene (Luciani et al 2008). Admittedly, these data provide a direct

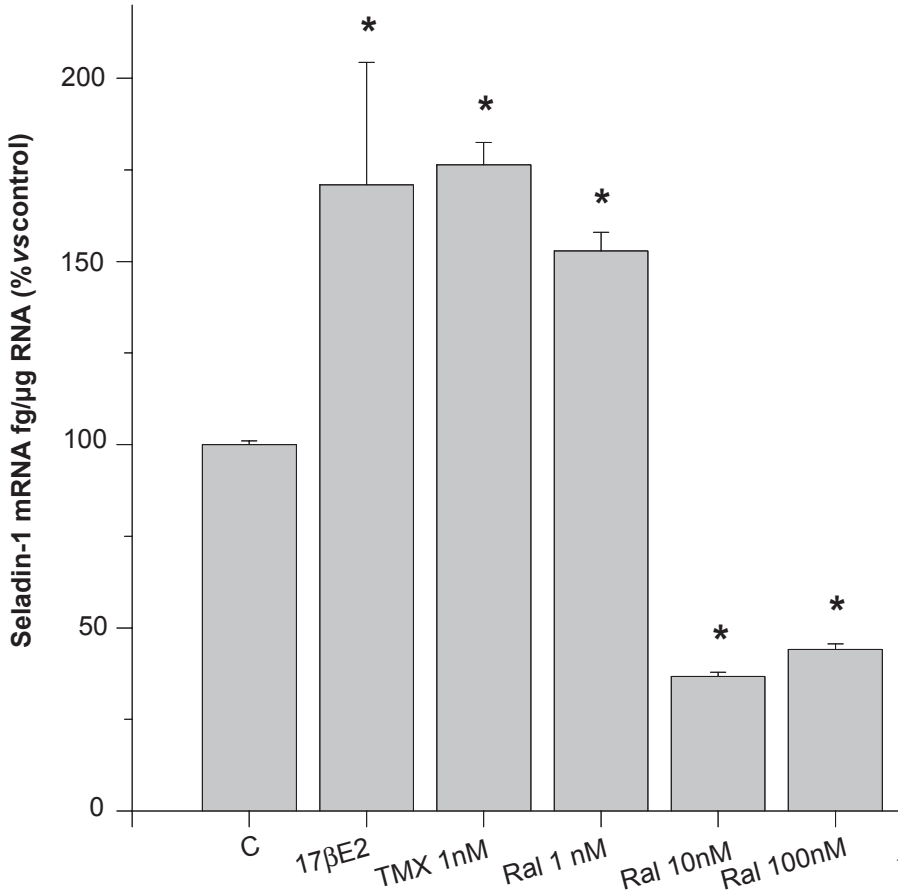

Figure 3 Amount of seladin-1 mRNA, assessed by real-time RT-PCR, in untreated control FNC cells (C), in cells treated with 1 nM 17β-estradiol ($17\beta E_2$), 1 nM tamoxifen (TMX) or raloxifene (Ral) (1–100 nM).

Notes: *$p < 0.05$ vs control cells (C) value (112 ± 2.26 fg/µg total RNA, mean ± SE), considered as 100%. Modified with permission from Benvenuti S, Luciani P, Vannelli GB, et al 2005. Estrogen and SERMs exert neuroprotective effects and stimulate the expression of seladin-1, a recently discovered anti-apoptotic gene, in human neuroblast long-term cell cultures. *J Clin Endocrinol Metab*, 90:1775–82.

demonstration that *seladin-1* is a fundamental mediator of the neuroprotective effects of estrogen.

Conclusions

ER-mediated neuroprotection has been assessed by a number of studies and justifies the attempts that have been proposed to prevent of treat AD with estrogen or SERMs. The partially disappointing results from previous clinical trials may have been determined by several confounding factors, such as the rather advanced age of the patients, the cognitive function at baseline, the presence of pre-existing risk factors and the progestogen used. Our data reinforce the role of estrogen as a protective factor for the brain and address *seladin-1* as a mediator of this effect. Thus, we feel that further trials involving estrogen or SERMs in neuroprotection may be encouraged, provided that the patients are carefully selected and the treatment regimen is appropriately chosen.

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