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Review

Reprint of: From the 90's to now: A brief historical perspective on more than *two* decades of estrogen neuroprotection

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ABSTRACT

Historical perspective abstract: **From the 90's to now: a historical perspective on more than *two* decades of estrogen neuroprotection:** In the early 90's, estrogens were known to exert organizational and activational effects on reproductive tissues and sexual behavior. As well, the role of sex and gonadal hormones in altering the risk for developing Alzheimer's Disease (AD) was only beginning to be elucidated. Preliminary investigations suggested that estrogen-containing therapies typically given for the management of disruptive menopausal symptoms could reduce AD risk, attenuate disease-associated cognitive deficits, and modulate brain substrates known to be dysregulated by the condition, such as the cholingeric system. The findings from our seminal paper demonstrating cognitive benefits and cholinergic impacts with exogenous estrogen treatment in a rodent model of surgical hormone depletion provided initial support for use of estrogen-containing therapies as a treatment for age-related brain disorders. We then went on to demonstrate neuroprotective actions of estrogen in several other *in vivo* and *in vitro* models of neurological challenge, including stroke and AD. Further, our findings of the chemical structure requirements for estrogen's neuroprotective effects identified a novel approach for optimizing future estrogen-containing hormone therapy options. These early efforts laid the groundwork for later, large-scale clinical investigations into the potential of estrogen-based menopausal hormone therapies for the prevention of a variety of age-related disorders. Although findings of these studies were equivocal, the neuroprotective actions of estrogen, and specifically 17β-estradiol, identified by early investigations, remain well-documented. Future development of interventions that optimize cognitive aging are crucial and, with proper understanding of the factors that influence the realization of beneficial impacts, estrogen-containing treatments may still be among these.

Original article abstract: **Ovarian steroid deprivation results in a reversible learning impairment and compromised cholinergic function in female Sprague-Dawley rats:** We hypothesized that estradiol (E2) serves as a neurotrophomodulatory substance for basal forebrain cholinergic neurons thought to be involved in learning and memory. Learning/memory was assessed using the two-way active avoidance paradigm and the Morris water task. Female Sprague-Dawley rats were either ovariectomized (OVX) or OVX for 3 weeks, followed by s.c. implantation of a Silastic pellet containing 17-βE2 (E2 pellet), resulting in a replacement of E2 to physiological levels. Ovary-intact (INTACT) animals served as our positive control. Active avoidance behavior and choline acetyltransferase (ChAT) activity in the frontal cortex and hippocampus were assessed at 5 and 28 weeks postovariectomy while performance on the Morris water task and high-affinity choline uptake (HACU) were measured only at the 5-week time point. At the 5-week time point, E2 replacement caused a significant elevation in the level of active avoidance performance relative to OVX animals. At the 28-week time point, OVX animals demonstrated a significantly lower number of avoidances relative to controls (61%) whereas E2-pellet animals not only demonstrated superior performance relative to OVX animals but also showed an accelerated rate of learning. Morris water task performance, on the other hand, was not significantly affected by estrogenic milieu despite a trend towards better performance in the E2-pellet group. Neurochemical analyses revealed that 5 weeks of ovariectomy was sufficient to reduce HACU in both the frontal cortex and hippocampus by 24 and 34%, respectively, while E2 replacement was successful in elevating HACU relative to OVX animals in both regions. ChAT activity was decreased in the hippocampus but not the frontal cortex of 5-week OVX

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animals. E2 replacement resulted in a reversal of this effect. At the 28-week time period, an unexpected decrease in ChAT activity was observed across all treatment groups. Interestingly, E2-pellet animals demonstrated the least severe decline in ChAT. This phenomenon was most evident in the frontal cortex where ChAT decreased by 61 and 56% in INTACT and OVX animals, respectively, whereas the decline in E2-pellet animals was only 16% over the same time period, suggesting a previously unreported cyto-protective effect of E2. Taken together, these findings demonstrate important effects of estrogens on cholinergic neurons and support the potential use of estrogen therapy in treatment of dementias in postmenopausal women. © 1994.

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Prior to the early 1990's, the notion that estrogens exert organizational and activational effects on reproductive tissues and sexual behavior had been well studied (Arnold, 2009; Wilson and Davies, 2007). The site of these effects was the estrogen receptor (ER), first discovered in uterine tissue (Gorski, 1994; Jensen et al., 2010) and cloned in 1986 (Greene et al., 1986). ER-alpha (ERα) was the first nuclear ER that demonstrated binding specificity for estradiol (17βE2) and was thought to be the sole ER with which all estrogens interacted. Although additional receptors would be identified in the coming years, distribution of the then only known nuclear ER was mapped to several tissues including uterus, ovaries, and brain, including hypothalamus; interestingly, ERα also appeared in brain regions associated with cognitive functions such as the cortex and hippocampus (Handa et al., 1994; Miranda and Toran-Allerand, 1992). Realization of ERs in these extrahypothalamic brain regions prompted the investigation of the impact of sex hormone depletion and replacement on substrates that controlled non-reproductive, higher-order cognitive functions.

At the time of original publication, Alzheimer's disease (AD) was, and still is, a major mental health concern in the United States. With the impending retirement of the first wave of Baby Boomers and the 'greying' of the adult population, concerns regarding costs associated with the medical treatment of a large, and growing, population of at-risk adults were increasing. In the early 1990's, the role of sex and gonadal hormones in risk for developing AD was only beginning to be elucidated. Preliminary case reports and small scale clinical investigations suggested that treatment with estrogen-containing therapies typically given for the management of disruptive menopausal symptoms could reduce AD risk and attenuate disease-associated cognitive deficits (Fillit et al., 1986; Henderson et al., 1994). As well, emerging findings identified that a mechanism by which estrogen imparted its beneficial mnemonic actions in AD patients may be due to its ability to impact the cholinergic system (Luine et al., 1975; Luine, 1985), a system known to be related to learning and memory performance (Flicker et al., 1983). The cholinergic system is associated with sex-specific, age-related functional changes (Luine et al., 1986), is dysregulated in AD (Francis et al., 1999), and is the therapeutic target of the few pharmacological interventions available for the relief of dementia-related symptoms (Giacobini, 1998). However, the memory-enhancing effects of estrogen-containing treatments, the neurochemical basis for these beneficial cognitive impacts, and their therapeutic potential for the treatment of AD-like dementia, were not well characterized at this point.

Using a preclinical animal model to address this important issue (Singh et al., 1994), we administered a subcutaneous silastic capsule containing either cholesterol or 17βE2 to young adult, female rats depleted of endogenous circulating sex hormones via the surgical removal of the ovaries (ovariectomy, OVX). A positive control group of ovary-intact, untreated female rats was included. Behavioral findings indicated that 17βE2 treatment reversed OVX-induced cognitive deficits to at least the level of ovary-intact control rats. Indeed, non-spatial active avoidance learning and memory performance was markedly improved by both short-term (2 weeks) and long-term (25 weeks) estrogen treatment, as 17βE2-treated animals had a higher number of total avoidances and required fewer days to criterion than OVX animals. As well, although findings were not statistically significant, short-term 17βE2 treatment tended to improve spatial memory on the Morris water maze, as evidenced by an increase in time spent in the goal quadrant and a higher number of crossings over the platform area, further supporting the beneficial impact of estrogen on cognitive outcomes.

To relate cognitive impacts of estrogen treatment to neurobiological substrates associated with learning and memory, we assessed high affinity choline uptake (HACU), the process by which neurons take up choline for acetylcholine synthesis, and activity of choline acetyltransferase (ChAT), the enzyme involved in the synthesis of acetylcholine from choline and an acetyl group from acetylcoenzyme A (Jope, 1979; Oda, 1999), in two cognitively-relevant brain regions. The favorable mnemonic impacts of estrogen treatment were associated with modulation of the cholinergic system in a region- and treatment duration-dependent manner. Specifically, surgical hormone removal significantly reduced levels of HACU relative to ovary-intact animals in both frontal cortex and hippocampus. As compared to OVX animals, short-term 17βE2 treatment reversed these reductions in both regions. As well, short-term 17βE2 treatment protected against the OVX-induced reduction in hippocampal ChAT activity. Interestingly, frontal cortex ChAT activity declined to a similar extent in both ovary-intact and OVX groups after 28, but not 5 week time points, possibly representing age-related changes in cholinergic function in this area. Indeed ovary-intact and ovariectomized groups showed declines in ChAT activity of 61% and 56%, respectively. Yet, long-term 17βE2 treatment attenuated the rate of change in ChAT activity in these animals. Thus, data supported the ability of estrogen-containing therapies to alleviate cognitive and cholinergic dysfunction associated with surgical hormone depletion and lay the groundwork for use of estrogen-containing therapies as beneficial therapeutic interventions for the treatment of AD and potentially other brain diseases as well.