Estradiol Exerts Neuroprotective Actions Against Ischemic Brain Injury

Insights Derived from Animal Models

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Over the last 100 yr, the life-span of women has increased from 50 yr to over 80 yr, but the age of the menopause has remained unchanged, at 51 yr. Menopause is one of the most permanent physiologic changes that a woman will experience and is marked by a dramatic decrease in circulating levels of ovarian estrogens. Because the timing of menopause has remained fixed in the face of an increasing life-span, more women will live a greater proportion of their lives in a hypoestrogenic state. We appreciate more and more that the actions of ovarian steroid hormones are complex, and possibly exert opposing actions in different contexts. I review here the results of my laboratory’s recent studies that clearly establish that low physiologic levels of estradiol replacement can exert profound neuroprotective actions when administered prior to an ischemic strokelike injury.

Key Words: Neuroprotection; estrogen receptors; apoptosis; menopause; ischemia; stroke.

Introduction

During most of the existence of the human species, the average life expectancy for women was between 20 and 40 yr. Today, the average life-span of women is 83 yr. In evolutionary terms, the rate of the increase in life expectancy that has occurred over the last century is nothing less than astounding. During this period, the age of the menopause has remained unchanged and continues today to occur at approx 50 yr of age. Hence, today most women will spend a third of their lives in the postmenopausal state, when estrogen secretion by the ovary is virtually nil. We once thought that ovarian steroids influenced predominantly reproductive targets, such as the hypothalamus, anterior pituitary, mammary glands, ovaries, and organs of the reproductive tract. More recently, we know that these hormones are pleiotropic and act beyond the scope of their reproductive functions to influence many nonreproductive organs, including regions of the brain such as the hippocampus, cerebral cortex, and striatum, areas that influence learning, memory, and balance; bone and mineral metabolism; the heart and vascular system; and the immune system. Because all of these physiologic systems depend upon estrogens to maintain normal function, it is not surprising that the absence of these hormones in postmenopausal women who do not take estrogen replacement therapy impacts the health of older women.

Many clinical studies show that estradiol, the major estrogen synthesized by the ovary, protects against osteoporosis, cardiovascular and neurologic diseases, and brain injury sustained after a cerebrovascular stroke or other insults. Yet, other studies suggest otherwise: they report a lack of protection or increased health risks of hormone replacement therapy in the occurrence of cerebrovascular stroke (1), cardiovascular disease (2), Alzheimer disease (3), and invasive breast cancer (4). We will review on our work that clearly demonstrates that low physiological levels of estradiol replacement exert profound protective actions both in vivo and in vitro, and our work that establishes the multiple mechanisms that estradiol utilizes to achieve these neuroprotective effects. Other more extensive reviews (see refs. 5–9) clearly show that several laboratories have contributed to our understanding of these novel nonreproductive actions of estrogens in maintaining normal brain function.

Estradiol Protects Against Permanent Middle Cerebral Artery Occlusion

Our initial work assessed whether physiologic levels of estradiol prevent brain injury in an in vivo model of permanent, focal ischemia (10). Rats were ovariectomized (OVX) and implanted, immediately or at the onset of middle cerebral artery occlusion (MCAO), with Silastic capsules that produced physiologic low or physiologic high 17β-estradiol levels in serum (10 or 60 pg/mL, respectively). One week after ovariectomy, we occluded the middle cerebral artery using the methods of Longa et al. (11). This method decreases
cerebral blood flow to the cerebral cortex by approx 50% (10). Estradiol pretreatment significantly reduced overall infarct volume compared to oil-pretreated controls in both young and middle-aged rats (Fig. 1). We were surprised to find that estradiol exerted equivalent protection in middle-aged rats (12), since we have previously reported that responsiveness of the hypothalamus to estradiol is severely compromised, regardless of the end point measured, when animals reach this age (13). The fact that estradiol continues to protect even as rats age suggests that estradiol’s mechanisms of action in this brain region may require different factors than those in the hypothalamus, and that this constellation of factors may be preserved in a brain region–specific manner. This protective effect was regionally specific to the cortex, since no protection was observed in the striatum (data not shown). When estradiol replacement was delayed until the time of the MCAO, we could not detect any protection (data not shown). The lack of protection when estradiol is administered acutely contrasts with the findings of Green and Simpkins (6) and Hurn and Macrae (7), who found that higher, more pharmacologic levels of estrogen replacement can effectively protect against brain injury even when administered at the time of ischemia or even after the injury is induced. Our findings that estradiol pretreatment reduces injury demonstrates that physiologic levels of estradiol can protect against neurodegeneration.

**Estradiol Alters Expression of Multiple Genes That May Influence Ability of Cells to Survive Injury**

Using our model of permanent cerebral artery occlusion and low physiologic levels of estradiol replacement, we began to elucidate potential mechanisms of estradiol action in injury. Bcl-2 is a protooncogene that promotes cell survival in a variety of tissues including the brain. Since it was known that estradiol promotes cell survival via Bcl-2 in non-neural tissues, we tested the hypothesis that estradiol decreases cell death by influencing bcl-2 expression in ischemic brain injury. Furthermore, since estradiol may protect the brain through estrogen receptor (ER)–mediated mechanisms, we examined expression of both receptor subtypes, ERα and ERβ in the normal and injured brain. We analyzed gene expression by using reverse transcriptase polymerase chain reaction in microdissected regions of the cerebral cortex obtained from injured and sham female rats treated with estradiol or oil. Our data demonstrate that estradiol pretreatment prevents the injury-induced downregulation of bcl-2 gene expression. This effect was specific to bcl-2; expression of other members of the Bcl-2 family (bax, bcl-xL, bcl-xS, bad, or bim) was unaffected by estradiol treatment (Fig. 2).

Using the same microdissected tissue punches, we found that ERs were differentially modulated in injury (14). We discovered that the expression of ERα was dramatically